

Original Research Article

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## Oxidative Stress Mediated Dose-Dependent Pathophysiological Alterations in Liver, Kidney, Heart and Intestine of Rats Exposed to Different Levels of Cadmium Chloride

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### ABSTRACT

Present study was carried out to evaluate toxic effect of cadmium chloride at 15, 50 and 100 ppm in drinking water for 28 days on liver, kidney, heart and intestine in male rats. Twenty-four male albino rats (270 to 340 g, 4 weeks of age) were randomly divided in to 4 groups having 6 animals in each group. The animals of control group received distilled water throughout the experimental period, while rats of other three groups received either 15, 50 or 100 ppm of CdCl<sub>2</sub> in drinking water for consecutive 28 days. Haematological and biochemical parameters, histopathological evaluation and status of oxidative stress markers were observed at the end of study. Alterations in ALT, AST, AKP and BUN of rats of all three toxic groups were dose dependant. SOD activity (U/mL) was significantly increased ( $p < 0.05$ ) in serum and liver while decreased in kidney and intestine of all toxic groups as compared to control. Activity of catalase in blood (molar/min) was significantly decreased ( $p < 0.05$ ) in all groups as compared to control. Catalase activity (U/mg protein) in liver, heart and intestine were significantly decreased as compared to control in dose dependant way. However, significant decrease in catalase activity was observed in kidney at high level of exposure to cadmium. The level of GSH ( $\mu\text{g}/\text{mg}$  of tissue) was significantly decreased in tissues at higher level of exposure. Histopathological analysis revealed that liver, kidney, heart and intestine showed changes in dose dependent manner. In conclusion, cadmium at 100 ppm caused marked alterations to multiple organs through oxidative damage in rats following continuous exposure for 28 days.

#### Keywords

Cadmium chloride,  
Subacute toxicity,  
Male rats, Oxidative  
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### Introduction

Cadmium (Cd) is listed in top hazardous substances which are used in nickel- cadmium batteries, pigments and plastic stabilizers. Major occupational exposures to Cd occur in nonferrous metal smelters, production and processing of Cd alloys and compounds

(WHO, 1992; Fay and Mumtaz, 1996). Cigarette smoke is also the source of Cd exposure to humans (Zalups and Ahmad, 2003). It is well known that long-term exposure to Cd causes various toxic effects in various organs such as heart, kidneys, liver, brain, lung, bones, haemopoietic organs, endocrine and reproductive organs (Fowler,

2009; Satarug *et al.*, 2010; Cuypers *et al.*, 2010). There is a positive correlation between Cd exposure and an augmented risk for cardiovascular diseases (Everett and Frithsen, 2008; Peters *et al.*, 2010).

Cd may be deposited in the heart muscle and produce cardiotoxicity at as low as 0.1  $\mu\text{M}$  concentration (Limaye and Shaikh, 1999). Cadmium enhances the production of free radicals and interferes with the antioxidant defence system which in turn leads to a cadmium induced alterations in the structural integrity of lipids and secondarily affects the membrane bound enzymes (Shukla *et al.*, 1996).

Cd accumulates mainly in the liver and to a lesser extent in the kidney and other tissues. In all tissues, Cd induces and binds to metallothionein (MT) and is stored as a nontoxic CdMT complex (Webb, 1986). CdMT is translocated from liver to kidneys due to normal turnover of hepatocytes as well as hepatic injury (Dudley *et al.*, 1985; Chan *et al.*, 1993).

Investigations also demonstrated that cadmium exposure increased oxidative stress, endocrine disruption and increased apoptosis in rabbit, dog and calf stallion (Waalkes, 2000; Siu *et al.*, 2009).

Various studies demonstrated specific organ toxicity due to exposure to particular level of cadmium in rodent. The effect of cadmium exposure at different levels on liver, kidney, heart and intestine has not been studied with special reference to oxidative damage.

Thus, the present study was carried out to evaluate the sub-acute toxic effect of cadmium at low (15 ppm), medium (50 ppm) and high (100 ppm) level exposure on different organs in rats with special attention to oxidative stress mediated changes.

## Materials and Methods

### Chemicals

Cadmium chloride was purchased from Himedia, Mumbai (Lot No: 0000298204). The chemicals like  $\text{KH}_2\text{PO}_4$  (Lot No: I12A/3212/0907/53) and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (Lot No: K14A/0514/2104/31) were purchased from S.D. fine Chemicals, Mumbai. Other chemicals like RBC lysis buffer (Lot No: RNBG5300), Pyrogallol (Lot No: 1002139642), dTNB (Lot No: SHBG1688V) and Bradford reagent (Lot No: SLBV5669) of analytical grade were purchased from Sigma Aldrich, USA. EDTA (Lot No: 61803701001730) and  $\text{H}_2\text{O}_2$  (Lot No: CE6C660325) were purchased from Merck Ltd., Mumbai.

### Experiment animals and design

The study was conducted on 24 albino rats (270-340 g weight, 4 weeks of age). The rats were acquired from registered breeder. The experimental was approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, Gujarat (JAU/JVC/IAEC/SA/32/18).

The rats were maintained in standard polypropylene cages with stainless steel top grill and Corn Cobb was used as bedding material. During whole study period, feed and water were supplied *ad libitum*. Rats were accommodated in cool environment ( $23^\circ$  to  $27^\circ\text{C}$ ) with relative humidity ranged between 42 to 55% along with 12 hours light-dark cycle. The rats were randomly divided to four groups (six rats in each group). The first group was received the *ad libitum* drinking water for a period of 28 days and it served as a control. The rats of group II, III and IV were exposed to cadmium chloride at 15, 50 and 100 ppm

respectively through drinking water for the period of 28 days. All animals were given feed (VRK Nutritional Solutions, Maharashtra) *ad libitum* throughout the study period.

### **Collection of samples**

Blood sample from each rat was collected from retro-orbital plexus under light anaesthesia on day 29 for evaluation of haematological, biochemical and oxidative stress parameters. All rats were humanely sacrificed at the end of study to observe gross pathological changes in organs and the tissues of major organs like liver, kidney, heart and intestine were collected in 10% formalin for histopathological examination. The tissue samples of liver, kidney, heart and intestine were collected and homogenized in 10% phosphate buffer (7.5 pH; PBS:  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ) and centrifuged at 12000g for 5 minutes and resulted supernatant was used for estimation of various antioxidant enzymes except for SOD in which Tris-EDTA buffer (8.2-8.5 pH) was used and centrifuged at 12000g for 40 minutes. Weight of liver, kidney, heart, spleen and lung were recorded using analytical balance (Model: Sartorius, BSA-423SCW) to calculate the relative organ body weight ratio.

### **Body weight and feed consumption**

During experimental period, body weight of each rat was observed daily. The feed offered to each group was accurately recorded daily in the morning. The residual feed given day before was accounted. Based on these data, amount of feed consumed by rats of each group was calculated.

### **Haematological and biochemical evaluation**

Haematological parameters like hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count

(TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were estimated by using automated haematology analyzer (Abacus Junior Vet 5, Diatron, Hungary) at Department of Veterinary Pathology, Junagadh Agricultural University, Junagadh.

Biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), uric acid, total protein (TP), albumin and globulin were estimated by using standard kits (Diatek Health Care Pvt. Ltd, India) on semi-automatic biochemistry analyser (Diatek Health Care Pvt. Ltd, India).

### ***In vivo* antioxidant activity**

#### **Preparation of blood lysate for catalase and GSH**

Each blood sample (50  $\mu\text{L}$ ) was mixed with 450  $\mu\text{L}$  of RBC lysis buffer (Sigma Aldrich, Lot No. RNBG0536) and kept for 5 minutes for efficient lysis of erythrocytes. The resultant blood lysate was used for evaluation of catalase activity and glutathione (GSH) level.

#### **Preparation of tissue samples for SOD, catalase and GSH**

Samples of liver, kidney, heart and intestine (100 mg each) were collected from all rats and immediately stored in 1mL ice cold 0.1 M phosphate buffer saline (PBS:  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , pH: 7.5) for evaluation of catalase activity and GSH level, whereas samples of liver, kidney, intestine and heart 100 mg each were separately collected in Tris-EDTA buffer (pH: 8.5) for analysis of SOD activity. Manual homogenizer was used to

prepare the tissue homogenates which then centrifuged at 12000g at 4 °C for 10 minutes except for SOD in which centrifugation was done at 12000g for 40 minutes and supernatant was used for evaluation of catalase activity, GSH level and SOD activity. Protein estimation in liver, kidney, intestine and heart was carried out using the standard method (Bradford, 1976). These data were used to calculate catalase activity in liver, kidney, heart and intestine.

### **Evaluation of SOD activity in serum and tissues (Inhibition of pyrogallol autoxidation method)**

Serum (Cu-Zn) SOD activity in serum and tissue homogenates was determined using a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol (Marklund and Marklund, 1974).

The 2900 µL Tris-EDTA and 100µL pyrogallol (2mM for tissue sample; 20 mM for serum) were taken in the cuvette and scanned for 3 minutes at 420 nm wavelength as control reading in spectrophotometer (Fusiontek-UV2900). Then, 2890 µL of Tris-EDTA buffer (pH-8.5), 100 µL of pyrogallol and 10µL of tissue homogenate or 100 µL of serum were taken and scanned for 3 minutes at the same wavelength.

Absorbance per 2 minutes difference was determined. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50%. (Cu-Zn) SOD activity was expressed as units/mL for serum sample and for tissue sample as U/ mg of tissue.

The enzyme unit can be calculated by using the following equations:

$$\% \text{ of inhibition} = \frac{(A - B) \times 100}{B}$$

Enzyme unit (U) = (% of inhibition/50) \*common dilution factor (100) [50% inhibition = 1 U]

### **Evaluation of catalase activity in blood and tissue**

Catalase activity in blood and tissue sample was determined according to the method of Aebi *et al.* (1974). The 20 µL blood lysate (or 20µL of supernatant of tissue homogenate) was added to 1980 µL PBS (0.1 M PBS, pH 7.5) in a test tube. One mL of 30 mM H<sub>2</sub>O<sub>2</sub> was added to it and absorbance of reaction mixture was taken at 240 nm in a spectrophotometer for 1 minute, against blank having mixture of PBS and blood lysate or tissue homogenate only. Unit activity of catalase in blood was expressed in molar/minute. The activity of catalase from tissue samples was calculated using the molar extinction coefficient of 43.6 cm<sup>-1</sup>.

$$\text{mmoles of H}_2\text{O}_2 \text{ decomposed/minute/mg protein} = \frac{\Delta A/\text{min} \times 1000 \times 3}{43.6 \times \text{mg protein in sample}}$$

### **Estimation of GSH in blood and tissue samples**

The levels GSH in blood and tissue were estimated according to standard method (Ellman, 1959). Blood lysate (10 µL) was mixed with 2970 µL of PBS (0.1 M PBS, pH 7.5) in a test tube. dTNB (30 mM) (20 µL) was added into it and the mixture was allowed to stand for reaction up to 45 minutes. Then, absorbance was taken at 412 nm against blank having mixture of PBS and blood lysate only without dTNB using spectrophotometer. Concentration of GSH was expressed in molar.

To estimate the levels GSH from tissue (liver, kidney, heart and intestine), 0.5 mL of tissue homogenate was taken and added with equal volume of 20% trichloroacetic acid (TCA)

containing 1 mM EDTA for precipitation of proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 minutes at 12000g. The supernatant (200 µL) was then transferred to a new set of test tubes and added with 1.8 mL of the Ellman's reagent (5, 5 - dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.1 M phosphate buffer with 1% of sodium citrate solution). All test tubes were made up to the volume of 2 mL. After completion of the total reaction, absorbance of each set of mixture was measured at 412 nm against blank having mixture of PBS and supernatant. Absorbance values were compared with a standard curve to know concentration of GSH. Various standards ranged from 1 to 6 µg/mL were prepared and used to get standard graph by using the values of absorbance which was used to know the concentration of GSH in tissue homogenate. The blood GSH level was expressed in molar and calculated using the formula given below.

$$\text{GSH (molar)} = \frac{\text{Absorbance(A)} \times \text{Total reaction volume}}{13600 \times \text{sample volume}}$$

### **Estimation of malondialdehyde (MDA) level from plasma (Lipid peroxidation)**

Lipid peroxidation was measured as a malondialdehyde (MDA) level using the standard kit (Sigma Aldrich, Germany) (Lot No: 3L08K07390).

### **Histopathology**

The formalin fixed tissues of liver, kidney, heart and intestine were embedded in paraffin and processed as per standard procedures. These tissue samples were sectioned at 6 – 8 µ thickness with semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with haematoxylin and eosin (H & E) stain (Luna, 1968). The H & E stained slides were observed under microscope and

microscopic pathological lesions were recorded.

### **Statistical analysis**

Numerical data obtained from this study have been expressed as mean ± standard error (SE). Data were analyzed statistically by ANOVA and mean of different treatment groups means were compared by Duncan's multiple range tests (DMRT) to observe difference among the treatments (Snedecor and Cochran, 1980).

## **Results and Discussion**

### **Feed consumption and body weight**

During 1<sup>st</sup> week, feed consumption was significantly decreased ( $P < 0.05$ ) in animals exposed to 50 and 100 ppm of cadmium as compared to those of control group (Figure 1). During the 2<sup>nd</sup> week of exposure, animal exposed to only 100 ppm cadmium showed significant reduced feed consumption. However, values of feed consumption during 3<sup>rd</sup> and 4<sup>th</sup> week in animals exposed to all 3 levels of Cd were not significantly differ as compared to control animals which might be due to acclimatization of body of animals with exposure to cadmium. The mean value of average body weight of animals of all 3 toxic groups were significantly decreased dose dependently ( $P < 0.05$ ) after 2 weeks of exposure to cadmium as compared to that of control group (Figure 2).

### **Haematological and biochemical parameters**

The mean value of Hb, PCV, TLC, MCV, MCHC and MCH were significantly decreased ( $P < 0.05$ ) in animals exposed to medium and high level of Cd as compared to those observed in animals of control group (Table 1). The mean values of AST and AKP were significantly increased in treatment

groups in dose dependent manner as compared to those of control group. The mean values of ALT were significantly lower ( $P < 0.05$ ) in 15 and 50 ppm groups but in high dose (100 ppm) group, it was significantly higher as compared to that of control group. The mean values of BUN were significantly affected in all 3 toxic groups. However, levels of uric acid were significantly higher in higher level of exposure as compared to control (Table 2).

### **Antioxidant enzymes**

The mean values of SOD activity in blood of animals exposed to different levels of Cd were similar but they were significantly higher ( $P < 0.05$ ) than that observed in control animals (Table 3). Mean values of SOD activity in kidney, intestine and heart significantly decreased ( $P < 0.05$ ) in all treatment groups (15, 50, 100 ppm) as compared to control groups. But in liver, SOD activity was significantly increased at higher level of exposure (50 and 100 ppm) as compared to control groups.

The mean values of catalase activity in blood of animals exposed to Cd were significantly decreased ( $P < 0.05$ ) according to level of exposure as compared to the control group (Table 4). The mean values of catalase activity in tissue samples of intestine and heart were significantly decreased as compared to control group. The kidney tissue catalase activity in animals exposed to only high level of cadmium (T4) was significantly decreased as compared to control group. The catalase activity of tissue samples of liver significant decreased in animals exposed to higher level of Cd (50 and 100 ppm).

The mean values of serum GSH levels in rats exposed to all 3 levels of Cd were significantly decreased as compared to that of control animals (Table 5). In liver and kidney levels of GSH were significantly decreased particularly on higher dose level. Levels of GSH in intestine of animals exposed to all 3

levels of exposure were significantly lower as compared to that of control animals. The level of GSH in heart of animals exposed to lowest level of Cd was significantly increased.

### **Malondialdehyde concentration (MDA)**

The mean value of plasma MDA concentration (nmol/ $\mu$ L) in rats exposed to 15, 50 and 100 ppm of Cd level were  $21.74 \pm 6.18$ ,  $26.74 \pm 5.84$  and  $34.20 \pm 13.60$ , respectively which were significantly higher ( $P < 0.05$ ) as compared to the value of  $17.94 \pm 2.42$  (nmol/ $\mu$ L) observed in control animals (Figure 3). The levels of plasma MDA were significantly increased dose dependently after exposure to all tested levels of cadmium.

### **Histopathological changes**

Histopathological evaluation of liver, kidney, heart and intestine were carried out to evaluate the toxic effect of cadmium chloride. In liver, the changes were vacuolar degeneration, widening of sinusoids at 15, 50 ppm level of exposure. But at 100 ppm level of exposure, the liver showed necrosis of hepatocytes, fragmentation of nuclei, pyknotic nuclei and widening of sinusoids (Figure 4). The kidney showed alterations in normal architecture with degenerative changes in glomeruli, proximate convoluted tubule, necrosis of glomeruli and denudation of bowmen capsule segments in a dose dependent manner (Figure 5). In intestine, Cd showed toxic effect in dose dependent manner with degenerative changes in villi, atrophy of intestinal glands, muscularis mucosa and lamina propria (Figure 6). Heart showed mild degenerative changes of cardiac muscle fibers at low level of exposure but perivascular fibrosis, hemorrhagic lesion and early focal necrosis at higher exposure level (Figure 7). Histopathological changes in all organs were more at 50 and 100 ppm exposure groups compared to low level of exposure to cadmium.

Environmental toxicant like cadmium may produce a variety of clinical manifestations. In man and animals, several organ systems, including the urinary, hepatic, nervous, reproductive, digestive and immune system may get affected. The heavy metals have potential towards production of the highly reactive chemical entities such as free radicals which have ability to cause lipid peroxidation, DNA damage, oxidation of sulfhydryl groups of proteins, depletion of protein, and several other effects (Valko *et al.*, 2015)

In the present study, there was significant decrease in mean values of Hb, PCV, TLC, MCV, MCHC and MCH in all treatment groups as compared to those of control group. According to previous study, anaemia has been reported in rats exposed to cadmium (Yuan *et al.*, 2014). The anemia is result of accumulation of non-essential toxic metal in haematopoietic organs like kidney, liver and spleen (Gill and Epple, 1993). Moreover, the anemia is also resulted due the destruction of erythrocytes because of altered erythrocyte membrane permeability and fragility or due to the failure of iron uptake through intestine having mucosal lesions upon cadmium exposure (Pawaiya *et al.*, 1998; Horiguchi *et al.*, 2011). Histopathological examination of intestine confirmed that there was more damage to intestinal architecture due to direct toxic effect of cadmium in our study. High oxidative stress in red blood cells of cadmium exposed animals can account for the increase in metHb% produced through HbO<sub>2</sub>-autoxidation reactions (Waltkins *et al.*, 1985). Thus, the inactive components of Hb (SHb, metHb and HbCO) are unable to transport oxygen and interferes the normal physiological process of the body. In addition, the reduction in the blood parameters (PCV, RBC and Hb) may be attributed to hyperactivity of bone marrow that leading to production of red blood cells with impaired integrity that easily destructed in the circulation (Tung *et al.*, 1975).

Liver and kidneys are considered as the main targets of Cd induced toxicity (Tondon *et al.*, 2003). In the present study, the results showed increase in plasma AST activities which indicate an active transamination of amino acids and operation of keto acids which are probably fed into tricarboxylic acid cycle (TCA) for oxidation (Knox and Greengard, 1965). The decrease in ALT activities may be due to liver damage and disturbance in the synthesis of these enzymes (Rana *et al.*, 1996). As in our study, significant changes in alkaline phosphatase (AKP) in cadmium treated groups compared with that of the control group were also reported (Ashour, 2014). This elevation could be attributed to liver damage which included swelling and ruptured parenchymal cell, leukocyte infiltration and necrosis (Quig, 1998).

The enzymatic antioxidant defence system includes mainly SOD, CAT and GSH which protect cells against reactive oxygen species (ROS) induced toxicity and lipid peroxidation. SOD converts the superoxide anion radical to hydrogen peroxide and CAT cleaves this hydrogen peroxide into water and oxygen. But heavy metals like Cd has adverse effect on numerous tissues through oxidative stress by increasing lipid peroxidation and fluctuating the antioxidant status (Ognjanovic *et al.*, 2003; Sinha *et al.*, 2008; Kanter *et al.*, 2009). In our study, we observed a markedly decrease in SOD, CAT and GSH in majority of organs of rats exposed to Cd and along with a significant increase in lipid peroxidation as indicated by significant high MDA level (dose dependently). Interestingly, there was no significant alteration in level of GSH in blood, liver and kidney of animals exposed to the low level of Cd as compared to that of control group. The level of GSH in heart of animals exposed to lowest level of Cd was significantly increased. However, the GSH level in intestine at all tested levels of exposure was significantly lower as compared

to that of control animals, which indicates variation in level of GSH in different organs which probably depends on activity of SOD and catalase in particular condition. Intracellular accumulation of Cd induces oxidative stress leading to cellular damage via displacement of redox-active metals, depletion of redox scavengers, inhibition of anti-oxidant enzymes and inhibition of the electron

transport chain resulting in mitochondrial damage (Nair *et al.*, 2013; Adiele *et al.*, 2012; Patra *et al.*, 2011). Cadmium has been documented to impact the body system damage through inhibition of antioxidant enzymes and induce oxidative damage with ROS generation which destroy proteins, lipids and DNA by oxidation.

**Table.1** Haematological parameters in rats of different groups

Parameters	Treatment groups			
	Control	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
HB (g/dL)	15.35 ± 0.29 <sup>c</sup>	15.17 ± 0.30 <sup>c</sup>	14.27 ± 0.41 <sup>a</sup>	14.93 ± 0.28 <sup>b</sup>
PCV (%)	42.68 ± 1.13 <sup>c</sup>	42.09 ± 0.57 <sup>bc</sup>	40.71 ± 0.84 <sup>a</sup>	41.91 ± 0.69 <sup>b</sup>
TEC (10 <sup>6</sup> /l)	8.85 ± 0.23 <sup>b</sup>	8.72 ± 0.11 <sup>a</sup>	8.65 ± 0.18 <sup>a</sup>	8.92 ± 0.15 <sup>b</sup>
TLC (10 <sup>3</sup> /cmm)	12.16 ± 1.04 <sup>c</sup>	10.35 ± 0.48 <sup>b</sup>	10.30 ± 0.88 <sup>b</sup>	9.30 ± 0.78 <sup>a</sup>
MCV (fl)	48.67 ± 0.49 <sup>c</sup>	48.33 ± 0.33 <sup>c</sup>	47.00 ± 0.73 <sup>a</sup>	47.50 ± 0.67 <sup>b</sup>
MCHC (%)	36.02 ± 1.64 <sup>c</sup>	36.10 ± 2.93 <sup>c</sup>	35.05 ± 3.52 <sup>a</sup>	35.63 ± 1.95 <sup>b</sup>
MCH (pg)	17.50 ± 3.69 <sup>c</sup>	17.35 ± 5.86 <sup>c</sup>	16.48 ± 3.28 <sup>a</sup>	16.75 ± 4.85 <sup>b</sup>

Value with different superscripts in a row were significantly different (P<0.05)

**Table.2** Biochemical parameters in rats of different groups

Parameters	Treatment groups			
	Control	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
ALT (IU/L)	59.40 ± 8.42 <sup>c</sup>	46.90 ± 2.62 <sup>a</sup>	54.00 ± 4.80 <sup>b</sup>	60.66 ± 8.39 <sup>c</sup>
AST (IU/L)	123.50±14.19 <sup>a</sup>	147.64±5.76 <sup>b</sup>	156.95±8.58 <sup>c</sup>	219.74±13.71 <sup>d</sup>
AKP (IU/L)	134.43±9.40 <sup>a</sup>	168.10±13.17 <sup>b</sup>	167.95±7.74 <sup>b</sup>	174.55±16.60 <sup>b</sup>
BUN (mg/dL)	24.94 ± 1.20 <sup>a</sup>	22.18 ± 0.84 <sup>a</sup>	22.52 ± 0.94 <sup>a</sup>	21.62±0.72 <sup>a</sup>
Uric acid (mg/dL)	0.63 ± 0.12 <sup>c</sup>	0.72 ± 0.10 <sup>a</sup>	0.62 ± 0.11 <sup>b</sup>	0.99 ± 0.10 <sup>a</sup>
Total protein (g/dL)	5.98 ± 0.13 <sup>b</sup>	5.80 ± 0.06 <sup>a</sup>	6.18 ± 0.07 <sup>c</sup>	5.99 ± 0.06 <sup>b</sup>
Albumin (g/dL)	3.20 ± 0.05 <sup>c</sup>	3.10 ± 0.03 <sup>a</sup>	3.21 ± 0.03 <sup>c</sup>	3.16 ± 0.02 <sup>b</sup>
Globulin (g/dL)	2.78 ± 0.08 <sup>b</sup>	2.70 ± 0.05 <sup>a</sup>	2.97 ± 0.06 <sup>d</sup>	2.83 ± 0.05 <sup>c</sup>

Value with different superscripts in a row were significantly different (P<0.05)

**Table.3** SOD activity in serum and different organs of rats under experiments

Parameters		Treatment groups			
		Control	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
SOD (U/mL)	Serum	4.18 ± 0.62 <sup>a</sup>	5.33 ± 0.48 <sup>b</sup>	5.74 ± 0.77 <sup>b</sup>	5.57 ± 0.93 <sup>b</sup>
SOD (U/mg of tissue)	Liver	0.73 ± 0.08 <sup>b</sup>	0.66 ± 0.06 <sup>a</sup>	0.85 ± 0.03 <sup>c</sup>	0.85 ± 0.04 <sup>c</sup>
	Kidney	0.70 ± 0.10 <sup>d</sup>	0.66 ± 0.05 <sup>c</sup>	0.53 ± 0.07 <sup>a</sup>	0.62 ± 0.05 <sup>b</sup>
	Intestine	0.38 ± 0.04 <sup>d</sup>	0.26 ± 0.06 <sup>c</sup>	0.17 ± 0.01 <sup>a</sup>	0.22 ± 0.02 <sup>b</sup>
	Heart	0.42 ± 0.11 <sup>b</sup>	0.36 ± 0.07 <sup>a</sup>	0.59 ± 0.11 <sup>c</sup>	0.33 ± 0.05 <sup>a</sup>

Value with different superscripts in a row were significantly different (P<0.05)

**Table.4** Catalase activity in blood and different organs of rats under experiments

Parameters		Treatment groups			
		Control	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
Blood catalase (molar/min)	Blood	11.70 ± 1.45 <sup>c</sup>	9.31 ± 1.84 <sup>b</sup>	7.42 ± 0.67 <sup>a</sup>	6.82 ± 0.51 <sup>a</sup>
Catalase (U/mg protein)	Liver	17.66 ± 5.49 <sup>b</sup>	22.40 ± 7.55 <sup>c</sup>	12.64 ± 1.42 <sup>a</sup>	14.48 ± 2.7 <sup>a</sup>
	Kidney	44.04 ± 8.02 <sup>b</sup>	40.80 ± 9.20 <sup>b</sup>	42.72 ± 5.33 <sup>b</sup>	35.15 ± 6.93 <sup>a</sup>
	Intestine	18.24 ± 3.16 <sup>d</sup>	13.90 ± 5.1 <sup>c</sup>	5.62 ± 1.81 <sup>b</sup>	2.44 ± 0.26 <sup>a</sup>
	Heart	6.58 ± 2.22 <sup>c</sup>	2.41 ± 0.35 <sup>b</sup>	1.87 ± 0.27 <sup>ab</sup>	1.45 ± 0.37 <sup>a</sup>

Note: Value with different superscripts in a row were significantly different (P<0.05)

**Table.5** GSH levels in blood and different organs of rats under experiments

Parameters		Treatment groups			
		C1	T2	T3	T4
GSH (molar)	Blood	11.09 ± 0.56 <sup>c</sup>	10.27 ± 1.24 <sup>b</sup>	8.86 ± 0.95 <sup>a</sup>	10.07 ± 0.46 <sup>b</sup>
GSH (µg/mg of tissue)	Liver	0.08 ± 0.01 <sup>b</sup>	0.09 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>a</sup>	0.04 ± 0.01 <sup>a</sup>
	Kidney	0.07 ± 0.02 <sup>b</sup>	0.07 ± 0.01 <sup>b</sup>	0.09 ± 0.04 <sup>b</sup>	0.04 ± 0.01 <sup>a</sup>
	Intestine	0.154 ± 0.53 <sup>b</sup>	0.027 ± 0.003 <sup>a</sup>	0.027 ± 0.001 <sup>a</sup>	0.033 ± 0.003 <sup>a</sup>
	Heart	0.01 ± 0.003 <sup>a</sup>	0.03 ± 0.006 <sup>d</sup>	0.02 ± 0.001 <sup>b</sup>	0.02 ± 0.002 <sup>c</sup>

Value with different superscripts in a row were significantly different (P<0.05)

Fig.1 The average feed consumption (g/Animal/Day) of experimental animals of different groups

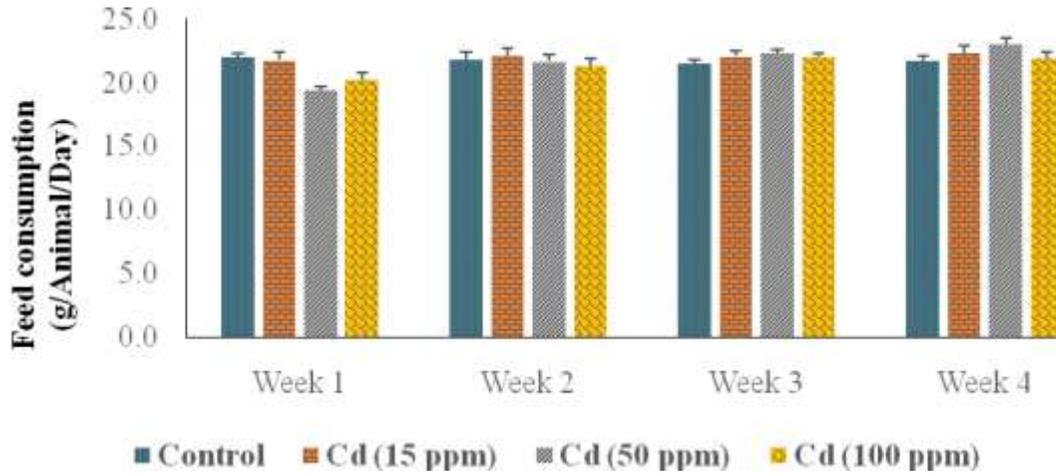


Fig.2 Body weight (g) of experimental animals of different groups

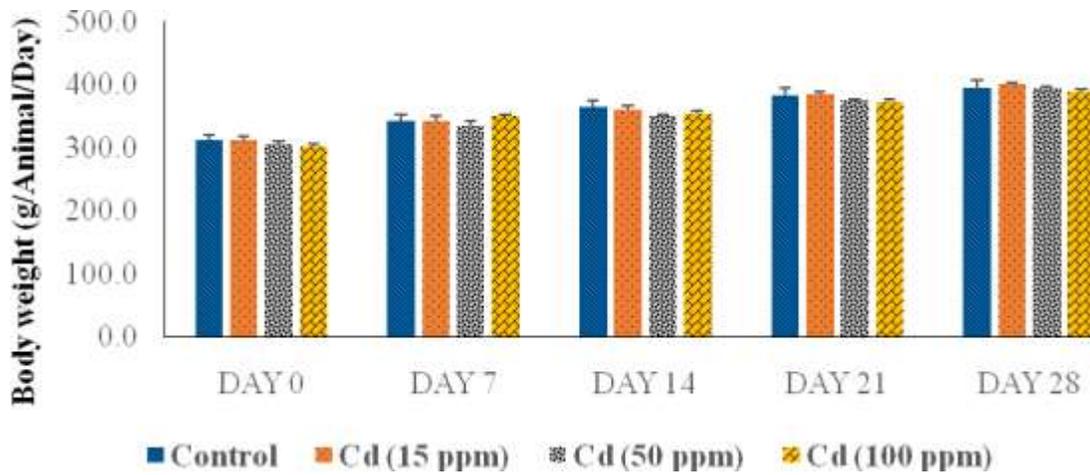
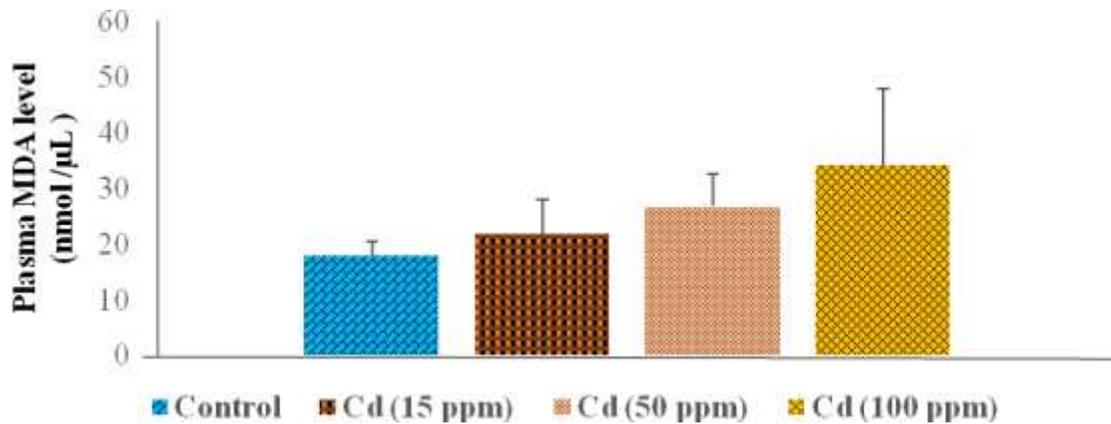
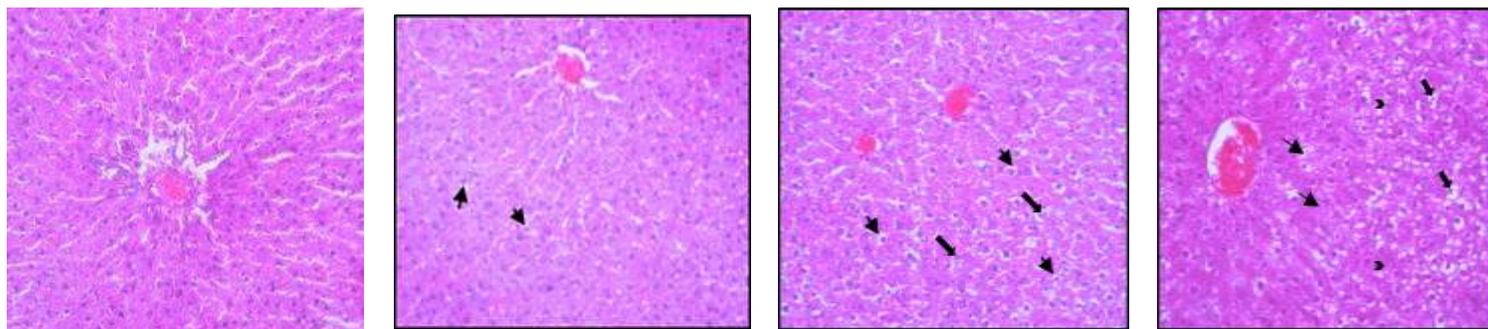


Fig.3 Plasma MDA levels (nmol/μL) of animals under experiment



**Fig.4** Microscopic changes in liver of rats of different treatment groups (H & E, x400)



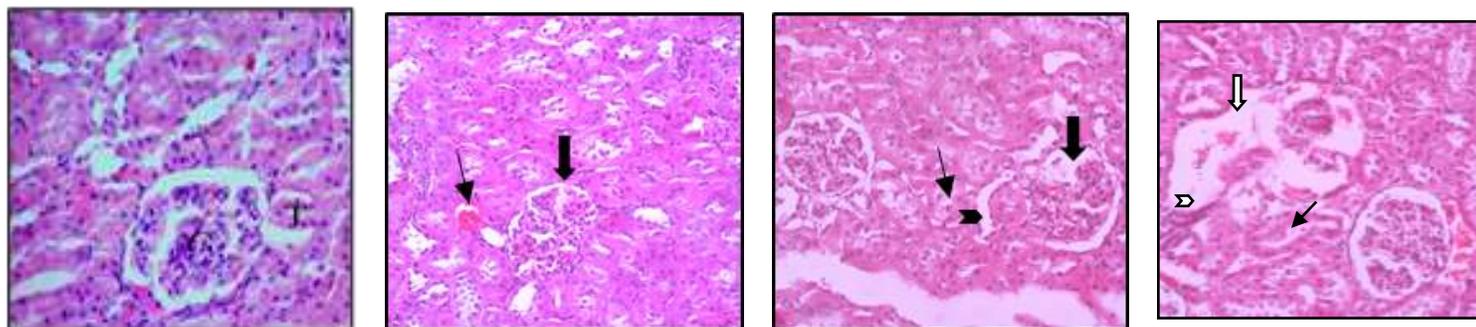
C1 group: Normal liver with hepatocytes (H), nuclei (N), Central vein (CV), sinusoids (S), Hepatic artery (HA) and bile duct (BD)

T1 group: Slight vacuolar degeneration of hepatocytes (thin arrow) with hemorrhage.

T2 group: Vacuolar degeneration (Thin arrow), widening of sinusoids, hemorrhage, pyknotic nucleus.

T3 group: Showing necrosis of hepatocytes (thin arrow), widening of sinusoids, fragmentation of nuclei and pyknotic nuclei.

**Fig.5** Microscopic changes in kidney of rats of different treatment groups (H & E, x400)



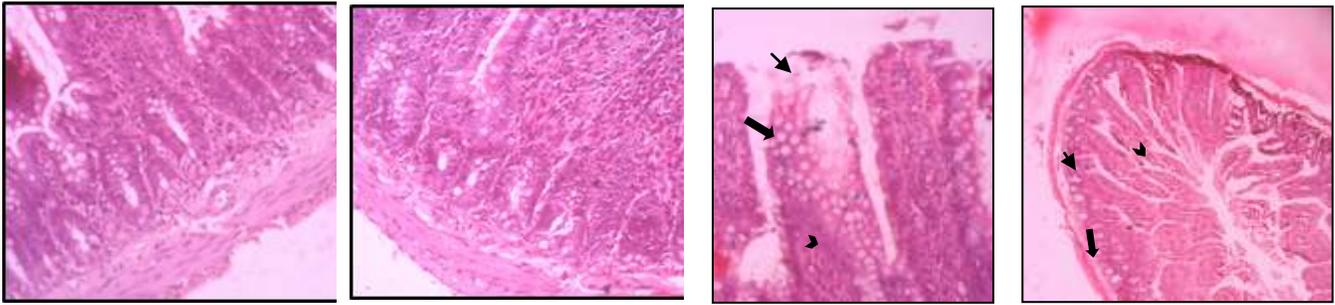
C1 group: Normal architecture of glomeruli (G) and renal tubules (T).

T1 group: Mild degenerative changes in renal tubular (thin arrow) with swelling of glomeruli (thick arrow).

T2 group: Degenerative changes in glomeruli (thick arrow) and proximate convoluted tubules (thin arrow). Denudation of bowmen capsule segment (arrow head)

T3 group: Necrosis of glomeruli and loss of structure glomeruli (arrow), necrotic changes in proximal (thick arrow) and distal convoluted tubules, Denudation of bowmen capsule segment (arrow head).

**Fig.6** Microscopic changes in intestine of rats of different treatment groups (H & E, x400)

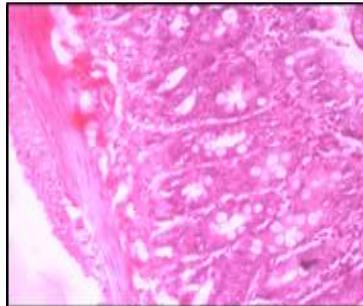


C1 group: Normal structure of intestine.

T1 group: With mild degenerative changes of villi.

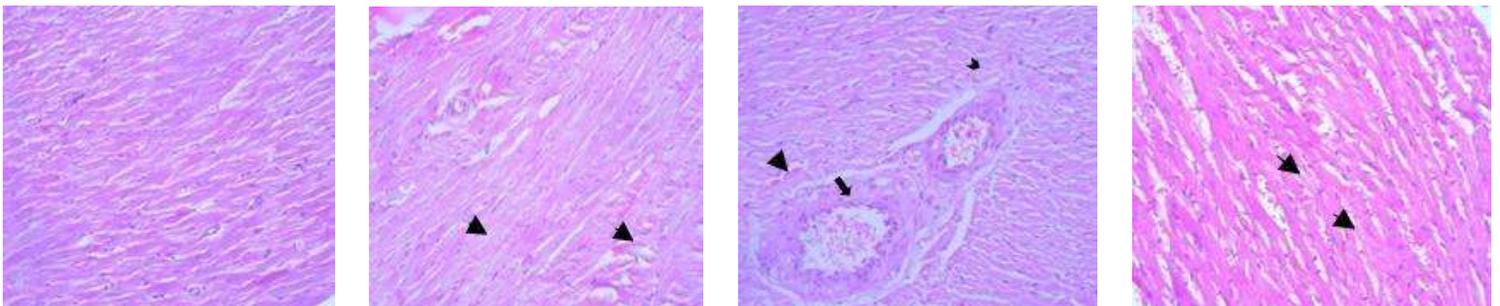
T2 group: Degenerative changes in villi (arrow head), increase in number of goblet cells (thick arrow), disruption of epithelial membrane (thin arrow).

T3 group: Atrophy of intestinal glands (thin arrow) and muscularis mucosa (thick arrow), breakage of villi (arrow head).



T3 group: Degeneration with loss of architecture of villi, atrophy of lamina propria and mucosa epithelium.

**Fig.7** Microscopic changes in heart of rats of different treatment groups (H & E, x400)



C1 group: Normal structure of cardiac muscle fibers.

T1 group: Mild degenerative changes of cardiac muscle fibers (thin arrow).

T2 group: Degenerative changes in cardiac muscle fibers (arrow head), perivascular fibrosis (thick arrow) and haemorrhagic lesions (thin arrow).

T3 group: Mild Early focal necrosis of cardiac muscle fibers (thin arrow)

Decreased activity of SOD in major organs may be due to either the antagonistic effect of cadmium with copper and zinc, which are important metals for the activity of SOD or inactivation of SOD by cadmium induced lipid peroxidation (Tang *et al.*, 1998). Whanger (1979) reported that the decrease in catalase activity by cadmium may be due to alterations in absorption of iron, an essential trace element required for the activity of catalase. Lipid peroxidation involves a broad spectrum of alterations in cells and the consequent degeneration of cell membranes. Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the functions of bio-membranes, which can lead to the development of many pathological processes (Shukla *et al.*, 2000). Glutathione is an important antioxidant plays a crucial role in preventing the damage to cellular components caused by species such as free radicals and peroxides (Kaur *et al.*, 2003). It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine. It was invented that there is a correlation between the histological observations and oxidative damage induced by CdCl<sub>2</sub>. Our results revealed that the mean level of MDA was increased significantly associated with a reduction in antioxidant defence systems that protect tissues against oxidative damage through a decrease in GSH, SOD and CAT levels in CdCl<sub>2</sub> treated group which were dose dependent manner in majority.

The CdCl<sub>2</sub> exposure affects the cardiovascular system via several mechanisms. It produces vascular endothelial dysfunction, reduces nitric oxide (NO) level (Nwokocha *et al.*, 2013) and oxidative stress that cause the induction of hypertension and further exacerbates the cardio toxicity (Kukongviriyapan *et al.*, 2014). Antioxidant defence system plays a pivotal role in organ

protection. Antioxidant enzymes, such as CAT, GPx, and SOD, mitigate the toxic effects of reactive oxygen species (ROS) (Halliwell, 1989). SOD also inhibits oxidative inactivation of nitric oxide, consequently preventing peroxynitrite formation and endothelial dysfunction (Fukai *et al.*, 2011). In our study, there was significantly decrease in levels of SOD and catalase in heart of animals exposed to medium and high level of cadmium but GSH level in heart tissue were significantly higher as compared to control which indicates protective effects to heart tissue in terms of conversion of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O which might be the reasons for lesser effect of cadmium on heart as compared to kidney, liver and intestine. Yanez *et al.* (1991) reported that Cd exposure in rats significantly induced the levels of glutathione in heart tissue. The glutathione production in heart showed defensive mechanism to combat against toxic effect of cadmium.

The histopathological analysis revealed dose dependent damage to liver, kidney and intestine. The changes in liver morphology were might be due to accumulation of Cd in hepatocytes and direct cytotoxic effect of Cd. Rikans and Yamano (2000) also reported that hepatic endothelial cells (EC) is the first cellular targets for Cd-induced hepatocellular injury and damaged EC produced local ischemia by blocking the capillary lumen and this leads a number of molecular and cellular events, which results in hepatocellular injury. In kidney and intestine the architectural alterations were caused by disturbance of membrane integrity and involvement of inflammatory mediators by Cd (Muller, 1986; Kayama *et al.*, 1995). The histopathological alterations in heart were mild but dose dependent. Mitra *et al.* (2012) also observed cadmium induced alteration in heart of rats. Yazihan *et al.* (2011) reported Cd induced caspase activated apoptosis with degenerative changes in cardiac muscle fibers. In general,

histopathological changes in liver, kidney, heart and intestine were more or remarkable at high level of exposure.

In conclusion, cadmium produced remarkable alterations in blood biochemistry along with ultrastructural changes in liver, kidney, heart and intestine which were either through direct toxicity to organs or oxidative stress in rats following sub-acute exposure at 100 ppm level. This level of cadmium exposure to rats produced classical oxidative stress mediated toxicity and which can be further used for related toxicological studies.

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