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Determination of cadmium and zinc levels and oxidative status in cadmium treated developing chick embryonic liver

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Abstract: In the present study Chick embryos of *Bobcock* strain were treated with different concentrations (0.04, 0.05and 0.06 mg/egg) of $CdCl_2$ on the day 10 (d10), day 11 (d11), day 12(d12) of embryonic development. In this study metals like Cd, Zn were determined with ICP-OES in liver tissue of control and treated groups at different time intervals. The levels of (GSH) and activity levels of antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) were measured in liver tissue after different time intervals (24 h, 48h and 72 h) of $CdCl_2$ exposure. Significant induction was observed in GST activity in liver tissue after 24 h, 48 h and 72 h. However, the GPx, GR, SOD, CAT and GSH levels were decreased in dose and time dependent manner. In this study Cd retention in the liver increased with dose level of Cd and this inturn caused induction in the levels of Zinc in liver tissue.

Keywords: Antioxidant enzymes, Chick embryonic liver, Cadmium, ICP-OES, Zinc

I. INTRODUCTION

Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, and fertilizer industries, and cigarette smoke [1]. Cd shows different mechanisms of toxicity under different experimental conditions and in various species [2-6]. Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues.

The effects of Cd on antioxidative capacity are dual: on one hand, Cd can induce oxidative stress via the inhibition of antioxidants, and on the other it activates several antioxidative components as a result of a disturbed redox balance to consecutively induce signal transduction cascade. The mechanism of cadmium-mediated acute hepatotoxicity has been the subject of numerous investigations and sufficient evidence has emerged to reveal reasonable mechanisms for the toxic process, although some unexplained aspects still persist. Acute hepatotoxicity involves two pathways: one for the initial injury produced by direct effects of cadmium and the other for the subsequent injury produced by inflammation. Primary injury appears to be caused by the binding of Cd^{2+} to sulfhydryl groups on critical molecules in mitochondria. Thiol group inactivation causes stress, mitochondrial permeability transition and mitochondrial dysfunction. Secondary injury from acute cadmium exposure is assumed to originate from the activation of Kupffer cells and a cascade of events involving several types of liver cells and a large number of inflammatory and cytotoxic mediators [7].

As Cd shows a high affinity for thiols, the major thiol antioxidant, glutathione (GSH) that is highly abundant in cells, is a primary target for free Cd-ions. Therefore Cd-induced depletion of the reduced GSH pool [8] results in a disturbance of the redox balance leading to an oxidative environment. Under natural conditions ROS are produced in organelles with a highly oxidizing metabolic rate or those possessing electron transport chains, such as peroxisomes and mitochondria. Because Cd is a non redox-active, non-essential element, it cannot induce ROS production directly.

The cellular redox status and antioxidant defense mechanisms are more sensitive and lower in the embryo compared to adults [9-13]. Antioxidant defense mechanisms against free radical-induced oxidative damage include the following (i) catalytic removal of free radicals and reactive species by factors such as CAT, SOD, peroxidase and thiol-specific antioxidants; (ii) binding of proteins (e.g., transferrin, metallothionein, haptoglobins, ceruloplasmin) to pro-oxidant metal ions, such as iron and copper; (iii) protection against macromolecular damage by proteins such as stress or heat shock proteins; and (iv) reduction of free radicals by electron donors, such as GSH, vitamin E, vitamin C, bilirubin and uric acid [14-20]. CAT, in animals, is a heme-containing enzyme that converts H_2O_2 to water and O_2 and these enzymes are largely localized in subcellular organelles such as peroxisomes [21].

Upon absorption in the blood, cadmium binds to albumin and is transported to the liver. Cadmium-induced liver damage increases hepatic enzymes [22]. Metallothionein (MT), a low molecular weight metal-binding protein, binds cadmium where it is either stored in this conjugated form in the liver or transported to the kidney. Once filtered through the renal glomerulus, the cadmium-MT complex is reabsorbed in the proximal

tubules and degraded to release free cadmium. It is this reactive cadmium ion that contributes to renal tubular toxicity while accumulating in the cortex of the kidney [23].

Cadmium can compete with heavy metals such as Zinc for their binding sites on various proteins and thus modulate their function. Zinc-binding sites such as zinc finger motifs of transcription factors and signal transducers like PKC, or of metallo-enzymes of SOD, are all targets for cadmium occupation leading to non-physiological regulation of the protein activities. Cadmium is believed to replace zinc in its binding site on Zn/Cu SOD, inhibiting the enzyme leading to an increased production of ROS [24]. In a model that describes the cadmium induced activation of the PKC-mediated signal transduction, Cd^{2+} substitutes for Zn^{2+} in the regulatory domain of the enzyme thus exposing the putative protein–protein interaction site [25].

In the present study, the influence of Cd on GSH, on the antioxidant defense system (AOS), its retention in liver and its effect on Zinc levels in the liver of 13th day chick embryo was analysed. After 24, 48 and 72 hours of Cadmium exposure, the activities of enzymatic (SOD, CAT, GSH, GPx, GR and GST) components of the system were determined.

II. MATERIALS AND METHODS

2.1 Chemicals

Cadmium chloride (analytical standard), supplied by SD fine chemicals Ltd (India), was used for the study. Saline was used for preparing suspensions of Cd (volume of injection was 100 µl per egg). Bovine serum albumin (BSA), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2, 4 dinitrobenzene (CDNB) were obtained from Sigma Chemical Company, USA. Standard solutions of Cadmium and Zinc metals were prepared by dilution of 1000 ppm certified solution. Argon gas has been 99.99% purity. All other chemicals used were of analytical grade obtained from local firms.

2.2 Egg procurement, *in ovo* injections and incubation

Fertile *Bobcock* strain eggs procured from the Sri Venkateswara Veterinary University, Tirupati and Sri Balaji hatcheries, Chittoor, Andhra Pradesh, India were administered with 40, 50 and 60 µg of Cd/egg, separately with saline as vehicle. The injection volume was 100µl/egg according to the method of Blankenship 2003 [26]. The eggs were incubated with their broad ends up in an incubator. The eggs were rotated for every 1 hr and were examined through the Candler every day for the proper growth and viability. During all experiments, the live embryos were maintained at 37.5±0.5˚C and a humidity of 70–75%, except for brief intervals (60-120 seconds) required during the different treatment conditions. During this interval embryos experienced ambient room temperature (29-30˚C).

2.3 Egg treatment and tissue processing

The egg shell was opened at the blunt end at the top to obtain access to the air cell, where the respective test substance (100 µl) was injected directly on to the inner shell membrane. Covering the hole by wax could ensure the embryos vitality for the remaining time until dissection and tissue collection. Chick embryonic liver was collected on d13 after 24hr (d12), 48hr (d11) & 72hr (d10) initial administration of the test substance. The liver of 13th day was dissected out, weighed and washed using chilled saline solution. Tissue was minced and homogenized (10% w/v) in appropriate buffer (pH7.4) and centrifuged (3000g for 10min). The resulting clear supernatant was used for following antioxidant assays.

2.4 Estimation of Protein, GSH and antioxidant enzymes

The protein was determined according to the method of Lowry 1951 [27] using BSA as standard [28]. The estimation of total GSH was carried out by the method of Griffith, 1980 [29]. SOD activity was determined according to the method of Misra and Fridovich, 1972 [30] at room temperature. CAT activity was measured in the PMF by the method of Aebi, 1974 [31]. GR activity was determined by a slightly modified method of Carlberg and Mannervik, 1985 [32]. at room temperature. GST activity was assayed by the conventional method of Habig et al., 1974 [33]. GPx assay was carried out by monitoring the oxidation of NADPH in a recycling assay as described by Wendel, 1981[34].

2.5 Cadmium and Zinc estimation

Tissue was homogenized with 1N Nitric acid. After digestion with 1N Nitric acid samples were brought to a constant volume. The digested samples of tissue were analysed for Cd, Zn against suitable standards in linear range by inductively coupled plasma-optical emission spectrometer (ICP/OES) Optima 2100-DV-Perkin Elmer which is a fast multi-element technique with a dynamic linear range and moderate-low detection limits. The absorption wavelength was 228.802 nm for Cd and 206.200 nm for Zn.

2.6 Statistical analysis

All the data were expressed as mean ± standard deviation (Mean±SD). Statistical significance between more than two groups was tested using one way ANOVA. Values were considered significant at $p<0.05$.

III. RESULTS

3.1 Effect of Cadmium on antioxidant enzymes

3.1.1 Glutathione s-transferases (GSTs)

The levels of GST measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 1**;** TABLE 1. The GST activity was significantly ($p<0.05$) increased in treated liver in a dose dependent manner. The maximum percentage of induction was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 1.30, 1.32 and 1.33 fold increase in the induction of GST activity in 24, 48 and 72 hrs treatment, was observed respectively. In 0.05 mg $CdCl_2$ treated embryos 1.33, 1.33 and 1.34 fold increase in the induction of GST activity in 24, 48 and 72 hrs treatment, was observed. In 0.06 mg $CdCl_2$ treated embryos 1.34, 1.34 and 1.37 fold increase in the induction of GST activity in 24, 48 and 72 hrs treatment, was observed compared to controls.

3.1.2 Superoxide dismutase (SOD)

The levels of SOD measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 2; TABLE 2. The SOD activity was significantly ($p<0.05$) decreased in treated liver in a dose dependent manner. The maximum decreased activity of SOD was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 0.73, 0.64 and 0.45 fold decrease in the SOD activity in 24, 48 and 72 hrs treatment, was observed. In 0.05 mg $CdCl_2$ treated embryos 0.53, 0.19 and 0.09 fold decrease was observed. In 0.06 mg $CdCl_2$ treated embryos 0.18, 0.09 and 0.06 fold decrease in the SOD activity in 24, 48 and 72 hrs treatment was observed compared to controls.

3.1.3 Catalase (CAT)

The levels of CAT measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 3; TABLE 3. The CAT activity was significantly ($p<0.05$) decreased in treated liver in a dose dependent manner. The maximum decreased activity of CAT was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 0.62, 0.54 and 0.45 fold decrease in the CAT activity in 24, 48 and 72 hrs treatment, was observed. In 0.05 mg $CdCl_2$ treated embryos 0.54, 0.40 and 0.28 fold decrease was observed. In 0.06 mg $CdCl_2$ treated embryos 0.30, 0.18 and 0.10 fold decrease in the CAT activity in 24, 48 and 72 hrs treatment was observed compared to controls.

3.1.4 Glutathione peroxidase (GPx)

The levels of GPx measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 4; TABLE 4. The GPx activity was significantly ($p<0.05$) decreased in treated liver in a dose dependent manner. The maximum decreased activity of GPx was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 0.69, 0.64 and 0.61 fold decrease in the GPx activity in 24, 48 and 72 hrs treatment was observed. In 0.05 mg $CdCl_2$ treated embryos 0.61, 0.62 and 0.60 fold decrease was observed. In 0.06 mg $CdCl_2$ treated embryos 0.57, 0.38 and 0.33 fold decrease in the GPx activity in 24, 48 and 72 hrs treatment was observed compared to controls.

3.1.5 Glutathione reductase (GR)

The levels of GR measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 5**;** TABLE 5. The GR activity was significantly ($p<0.05$) decreased in treated liver in a dose dependent manner. The maximum decreased activity of GR was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 0.66, 0.57 and 0.46 fold decrease in the GR activity in 24, 48 and 72 hrs treatment was observed. In 0.05 mg $CdCl_2$ treated embryos 0.55, 0.47 and 0.34 fold decrease was observed. In 0.06 mg $CdCl_2$ treated embryos 0.37, 0.24 and 0.11 fold decrease in the GR activity in 24, 48 and 72 hrs treatment was observed compared to controls.

3.2 Effect of Cadmium on non-enzymatic antioxidant; Glutathione (GSH)

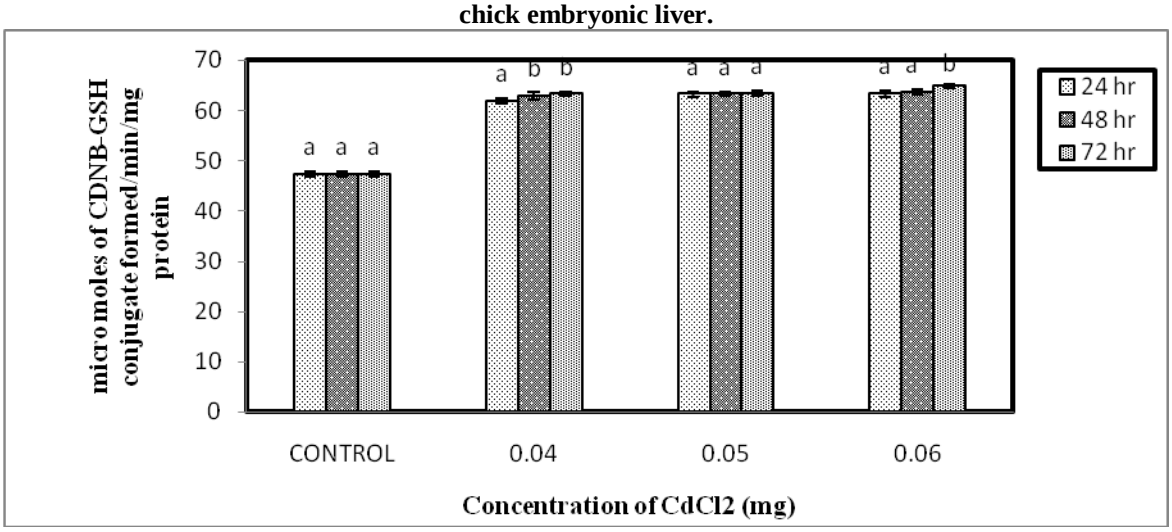
The levels of GSH measured in the Cadmium treated group in relation to the control group of 13th day old chick embryo were represented in Fig 6; TABLE 6. The GSH activity was significantly ($p<0.05$) decreased in treated liver in a dose dependent manner. The maximum decreased activity of GSH was observed in 0.06 mg $CdCl_2$ treatment compared to controls. In 0.04 mg $CdCl_2$ treated embryos 0.67, 0.57 and 0.47 fold decrease in the GSH activity in 24, 48 and 72 hrs treatment was observed. In 0.05 mg $CdCl_2$ treated embryos 0.54, 0.44 and 0.34 fold decrease was observed. In 0.06 mg $CdCl_2$ treated embryos 0.41, 0.30 and 0.20 fold decrease in the GSH activity in 24, 48 and 72 hrs treatment was observed compared to controls.

Table 1: Levels of GST measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	47.261± 0.621[a]	47.261 ±0.621[a]	47.261±0.621[a]
0.04mg $CdCl_2$	61.903±0.457[a]	62.851± 0.558[b]	63.275 ± 0.697[b]
0.05mg $CdCl_2$	63.288 ±0.843[a]	63.721 ±0.529[a]	63.370 ±0.475[a]
0.06mg $CdCl_2$	63.398 ± 0.39[a]	63.721 ±0.533 [a]	64.763 ± 0.478[b]

Units: µmoles of CDNB-GSH conjugates formed per minute per mg protein. Each value represents the mean ± SD (n=6). Different letters are significantly different at the level of $p < 0.05$.

Fig.1. Levels of GST measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



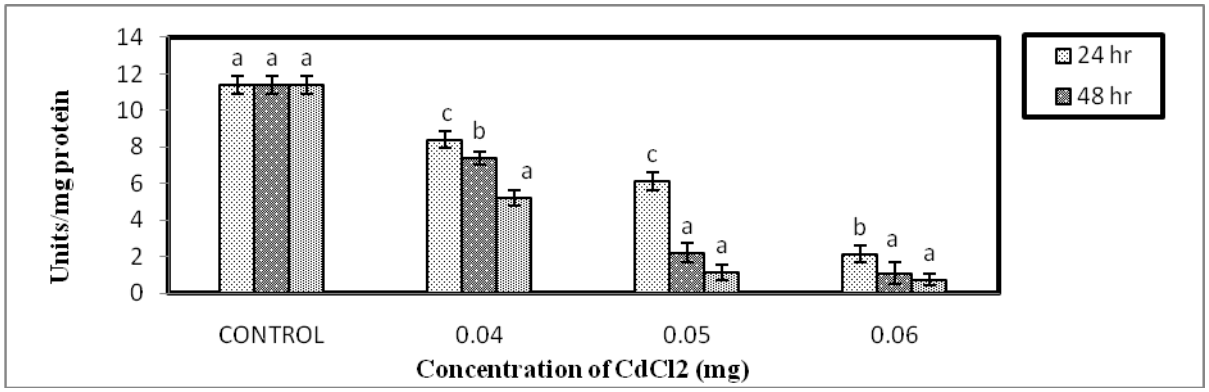
Data expressed as mean±SD, $p<0.05$.

Table 2: Levels of SOD measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	11.418±0.532[a]	11.418±0.532 [a]	11.418±0.532 [a]
0.04mg $CdCl_2$	8.41±0.511[c]	7.385±0.565 [b]	5.21±0.532 [a]
0.05mg $CdCl_2$	6.108±0.356 [c]	2.213±0.582 [b]	1.138±0.657 [a]
0.06mg $CdCl_2$	2.141±0.482 [b]	1.081±0.455 [a]	0.745±0.327 [a]

Units: The amount of enzyme required to inhibit 50% NBT (nitroblue tetrazolium) reduction/mg protein. Data are expressed as mean± SD; different letters are significantly different at the level of $p < 0.05$.

Fig.2. Levels of SOD measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



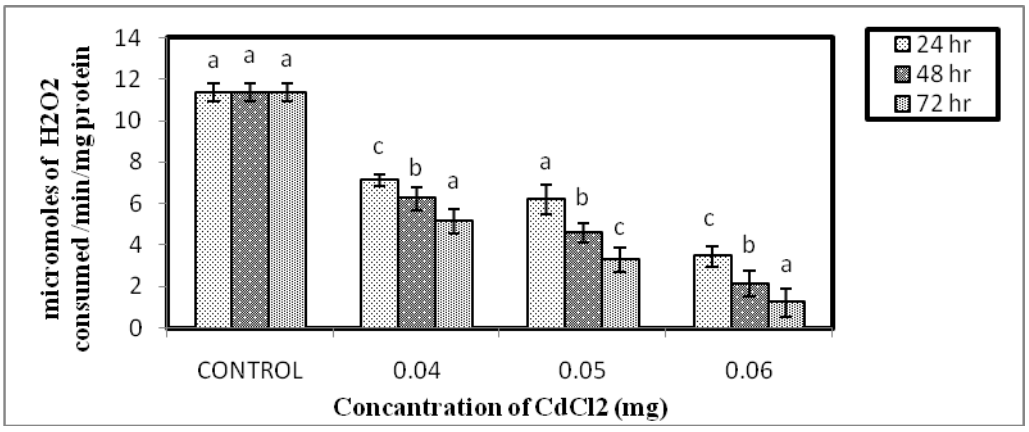
Data expressed as mean±SD, p<0.05.

Table 3: Levels of CAT measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	11.353±0.483[a]	11.353±0.483[a]	11.353±0.483[a]
0.04mg $CdCl_2$	7.126±0.300[c]	6.243±0.618[b]	5.148±0.667[a]
0.05mg $CdCl_2$	6.183±0.762[a]	4.58±0.512[b]	3.283±0.625[c]
0.06mg $CdCl_2$	3.445±0.557[c]	2.135±0.649[b]	1.218±0.742[a]

Units: µmoles of H_2O_2 used per minute per mg protein. Data expressed as mean± SD; different letters are significantly different at the level of $p < 0.05$.

Fig.3. Levels of CAT measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



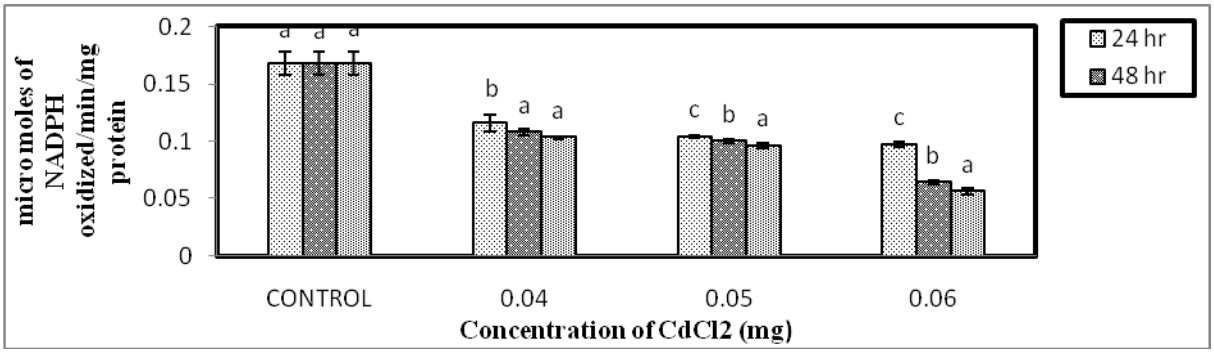
Data expressed as mean±SD, p<0.05.

Table 4: Levels of GPx measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	0.168±0.011[a]	0.168±0.011[a]	0.168±0.011[a]
0.04mg $CdCl_2$	0.116±0.078[b]	0.108±0.0021[a]	0.103±0.0024[a]
0.05mg $CdCl_2$	0.104±0.0033[c]	0.100±0.0025[b]	0.096±0.0023[a]
0.06mg $CdCl_2$	0.097±0.0015[c]	0.064±0.0026[b]	0.056±0.0034[a]

Units: µmoles of glutathione used per minute per g protein. Data expressed as mean± SD; different letters are significantly different at the level of $p < 0.05$.

Fig.4. Levels of GPx measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



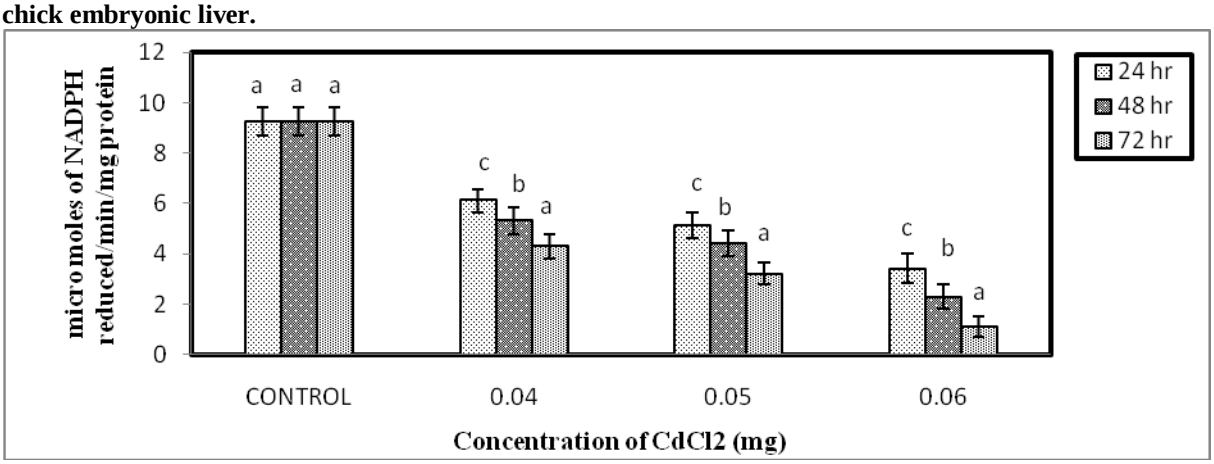
Data expressed as mean±SD, p<0.05.

Table 5: Levels of GR measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	9.238±0.620[a]	9.238±0.620[a]	9.238±0.620[a]
0.04mg $CdCl_2$	6.12±0.530[c]	5.306±0.587[b]	4.301±0.610[a]
0.05mg $CdCl_2$	5.131±0.599[c]	4.420±0.563[b]	3.215±0.517[a]
0.06mg $CdCl_2$	3.420±0.531[c]	2.298±0.487[b]	1.108±0.469[a]

Units: nanomoles NADPH oxidised /minute/ milligram protein. Data expressed as mean± SD; different letters are significantly different at the level of $p < 0.05$.

Fig.5. Levels of GR measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



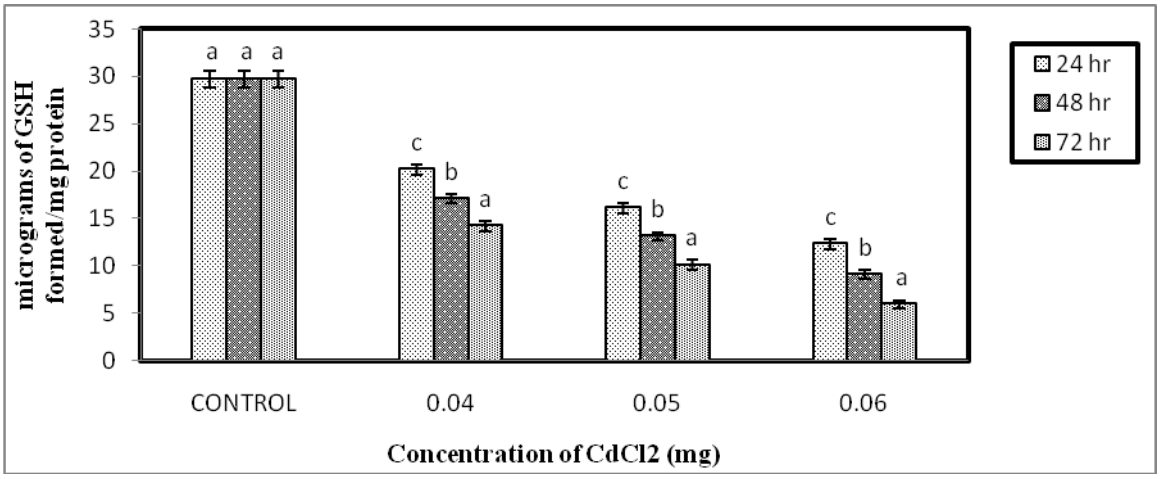
Data expressed as mean±SD, p<0.05.

Table 6: Levels of GSH measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.

Treatment	24 hrs	48 hrs	72 hrs
Control	29.751±0.980[a]	29.751±0.980[a]	29.751±0.980[a]
0.04mg $CdCl_2$	20.175±0.557[c]	17.131±0.567[b]	14.223±0.592[a]
0.05mg $CdCl_2$	16.121±0.575[c]	13.133±0.409[b]	10.143±0.505[a]
0.06mg $CdCl_2$	12.34±0.553[c]	9.141±0.633[b]	5.955±0.477[a]

Units: mg/g tissue. Data are expressed as mean± SD; different letters are significantly different at the level of $p < 0.05$.

Fig.6. Levels of GSH measured in the Cadmium treated group in relation to the control group of 13th day chick embryonic liver.



Data expressed as mean±SD, $p<0.05$.

3.3 Analysis of Cadmium and Zinc levels in liver tissue

The ICP OES was used for the determination of Cadmium and Zinc concentration in liver (13th day) of control and treated groups. Cadmium and Zinc concentrations in liver tissue of developing chick embryo treated with different concentrations of Cadmium at different time intervals were presented in TABLE 7, 8 & Fig 7, 8. In this study Cadmium and Zinc levels in liver were increased with increased concentrations of Cadmium exposure with increased time intervals.

Cadmium levels ranged from 0.107 – 0.793 ppm/500 mg wet tissue. The level of the cadmium in the control group was 0.005 ppm/500 mg tissue. In Liver tissue treated with 0.04 mg of $CdCl_2$ the retention of Cadmium concentration at 24, 48 and 72 hr was 0.107 ppm/500 mg wet tissue, 0.113 ppm/500 mg wet tissue and 0.196 ppm/500 mg wet tissue respectively (TABLE 7, Fig 7).

In Liver tissue treated with 0.05 mg of $CdCl_2$ the retention of Cadmium concentration at 24, 48 and 72 hr was 0.306 ppm/500 mg wet tissue, 0.393 ppm/500 mg wet tissue and 0.412 ppm/500 mg wet tissue respectively.

In Liver tissue treated with 0.06 mg of $CdCl_2$ the retention of Cadmium concentration at 24, 48 and 72 hr was 0.715 ppm/500 mg wet tissue, 0.764 ppm/500 mg wet tissue and 0.793 ppm/500 mg wet tissue respectively.

Zinc levels ranged from 0.826 – 0.973 ppm/500 mg wet tissue. The level of the Zinc in the control group was 0.804 ppm/500 mg tissue. In Liver tissue treated with 0.04 mg of $CdCl_2$ the Zinc concentration at 24, 48 and 72 hr was 0.826 ppm/500 mg wet tissue, 0.831 ppm/500 mg wet tissue and 0.854 ppm/500 mg wet tissue respectively (TABLE 8, Fig 8).

In Liver tissue treated with 0.05 mg of $CdCl_2$ the Zinc concentration at 24, 48 and 72 hr was 0.871 ppm/500 mg wet tissue, 0.905 ppm/500 mg wet tissue and 0.915 ppm/500 mg wet tissue respectively.

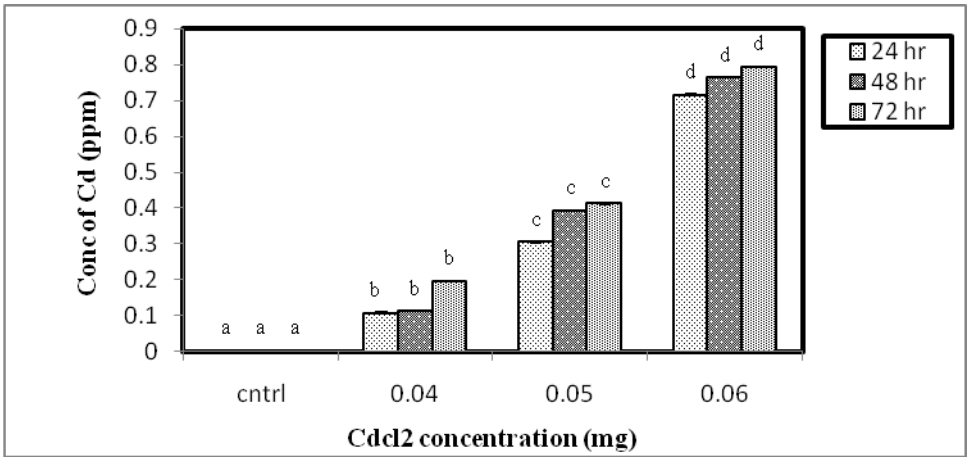
In Liver tissue treated with 0.06 mg of $CdCl_2$ the Zinc concentration at 24, 48 and 72 hr was 0.926 ppm/500 mg wet tissue, 0.954 ppm/500 mg wet tissue and 0.973 ppm/500 mg wet tissue respectively.

Table 7: Heavy metal (Cadmium) concentration in tissue (500 mg wet weight) of 13th-day old chick embryo liver exposed to different concentrations of Cadmium during embryogenesis at different time intervals (i.e., 24, 48 and 72 hr).

Dose	**24 hr**	**48 hr**	**72 hr**
Control	0.005 ± 0.000^{a}	0.005 ± 0.000^{a}	0.005 ± 0.000^{a}
0.04mg $CdCl_2$	0.107 ± 0.001^{b}	0.113 ± 0.001^{b}	0.196 ± 0.001^{b}
0.05mg $CdCl_2$	0.306 ± 0.002^{c}	0.393 ± 0.001^{c}	0.412 ± 0.001^{c}
0.06mg $CdCl_2$	0.715 ± 0.002^{d}	0.764 ± 0.001^{d}	0.793 ± 0.001^{d}

Units: ppm/500 mg wet tissue weight. Each value represents the mean±SD (n=6). $p < 0.05$ compared with control. (ppm - parts per million)

Fig.7. ICP-OES examination of Cadmium metal content in liver tissue of 13th-day old chicks exposed to different concentrations of Cadmium during embryogenesis at different time intervals (i.e., 24, 48 and 72 hr).



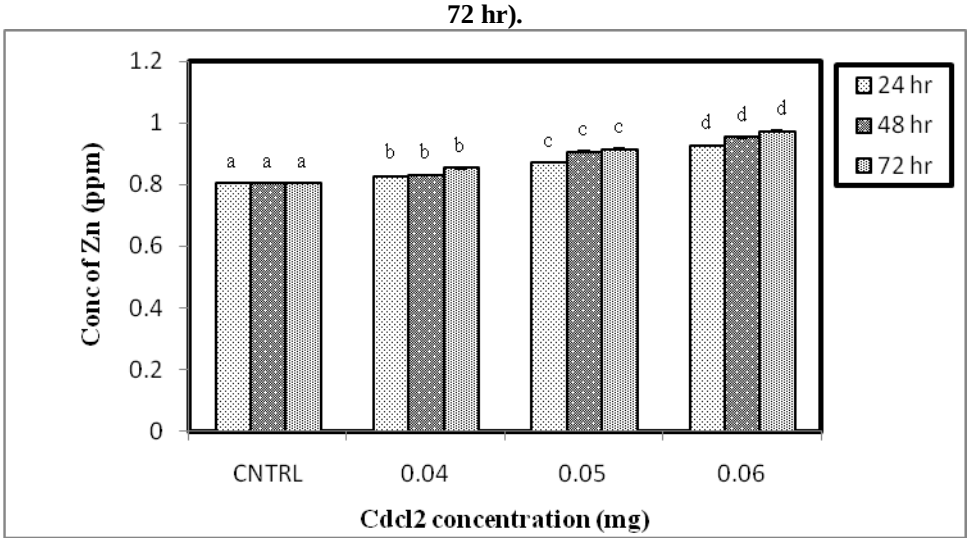
Data expressed as mean±SD, p<0.05.

Table 8: Zinc concentration in tissue (500 mg wet weight) of 13th-day old chick embryo liver exposed to different concentrations of Cadmium during embryogenesis at different time intervals (i.e., 24, 48 and 72 hr).

Treatment	24 hr	48 hr	72 hr
Control	0.804±0.001[a]	0.804±0.001[a]	0.804±0.001[a]
0.04mg $CdCl_2$	0.826±0.001[b]	0.831±0.001[b]	0.854±0.001[b]
0.05mg $CdCl_2$	0.871±0.001[c]	0.905±0.002[c]	0.915±0.002[c]
0.06mg $CdCl_2$	0.926±0.001[d]	0.954±0.002[d]	0.973±0.002[d]

Units: ppm/500 mg wet tissue weight. Each value represents the mean±SD (n=6). Different letters were significantly different at the level of $p < 0.05$. (ppm - parts per million)

Fig.8. ICP-OES examination of Zinc metal content in liver tissue of 13th-day old chicks exposed to different concentrations of Cadmium during embryogenesis at different time intervals (i.e., 24, 48 and 72 hr).



Data expressed as mean±SD, p<0.05.

IV. DISCUSSION

The results of the present investigation revealed that Cd induces significant alterations in the levels of certain enzymatic antioxidant enzymes status in liver of 13th day old chick embryo at all specific time intervals. A number of reports concerning the chemical toxicology of metals which are released in the environment by natural as well as anthropogenic sources have been increasing constantly. Cd has been recognized as one of the most toxic environmental and industrial pollutants. It is an ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant antioxidant balance in the tissues. Liver, kidney, lung, testes, and heart are the target organs following Cd exposure, with the severity of their intoxication dependent on the route, dose, and duration of the exposure to the metal [35, 36]. Cadmium found a variety of uses in industry, craft and agriculture

owing to their physical and chemical properties. The environmental burden of Cadmium has been rising substantially by smelter emission in air and waste sewage in water. Such environmental contamination of air, water, soil and food is a serious threat to all living kinds.

The damaging action of Cd on the antioxidant defense system in liver tissue during Cd exposure has been extensively studied. It has been reported that the sulfhydryl group of cysteine moiety of glutathione has a high affinity for metals such as Cd, forming thermo-dynamically stable mercaptide complexes which are inert and excreted via the bile [37, 38]. Exposure to Cd, originating from different sources-air, water, food-may produce effects in organs such as kidneys, liver, lungs, cardiovascular, immune and reproductive systems [39]. Moreover, Cd is identified as a human carcinogen. Due to complex interactions between Cd ions and metabolism, a diverse range of cellular responses is found in different organs after Cd-exposure.

The above discussion provides an insight into the role of reactive species in metal induced toxicity. The direct damage may involve conformational changes of bio-molecules or alter specific binding sites. On the other hand, indirect damage is a consequence of metal driven formation of reactive oxygen/nitrogen species involving superoxide, hydroxyl radicals or nitric oxide, hydrogen peroxide and/or endogenous oxidants.

Due to the ease of absorption, accumulation in tissues, and extremely long biological half-life in the body, cadmium is considered one of the most hazardous heavy metals. Due to the exceptionally long biological half-life (10-30 years) in the body, the toxicity of cadmium ions increases with advancing age and may persist after the end of exposure [40]. In animals exposed to cadmium compounds, the ions of this metal are particularly abundant in kidneys, heart and liver, and, to a lesser degree, in pancreas and brain [41]. This can induce irreversible damage to these organs in addition to anemia, osteoporosis and carcinogenic (for kidney, lung, prostate, testis and breast) and teratogenic lesions [42]. The effects at the cellular level are increased oxidative stress, damage to mitochondria, disturbances in trace element (e.g. calcium and zinc) and vitamin metabolism, and disruption of cell signaling pathways [43]. It is nutritionally nonessential and toxic, and it interacts with the metabolism of three essential metals: calcium, zinc, and iron.

In the present study ICP OES analysis was performed to test out the level of accumulated Cadmium and its effect on Zinc levels in the liver of treated as well as control groups. The result showed that maximum levels of Cadmium and Zinc accumulated in liver treated with 0.06 mg $CdCl_2$ at 72 hr. This result is also in accord with previous result, suggesting that liver is the main organ for the accumulation of heavy metals. We observed that the cadmium accumulation was continually increased in liver at a dose dependent manner and inturn caused the induction of Zinc levels in liver tissues. It can be inferred from above studies that Cd affects the homeostasis of essential metal like Zinc since it is an integral component of about 200 metalloenzymes including Cu/Zn Superoxide Dismutase which plays an important role in protection against oxidative damage. This in turn reflects its effect on antioxidant defense system. Cd also alters Zn distribution in body. Since the levels of Zinc increased under Cadmium exposure, excess accumulation of Zinc within cells may disrupt functions of biological molecules like Protein, enzymes and DNA, thus leading to toxic consequences. Thus the Cd and Zinc concentration in the liver increased with dose level of Cd.

V. CONCLUSION

From the present study it can be concluded that Cd accumulation in liver of 13^{th} day old chick embryo is associated with marked alterations in non enzymatic and enzymatic (SOD, CAT, GSH, GR, GPx and GST) components of AOS and in Zinc levels.

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