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Research Article

ANTIOXIDANT ROLE OF ZINC AND CALCIUM SUPPLEMENTATION AGAINST CADMIUM INDUCED OXIDATIVE STRESS IN FRESH WATER TELEOST *OREOCHROMIS MOSSAMBICUS* (TILAPIA)

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A B S T R A C T

Cadmium (Cd) has the ability to accumulate in the tissues and disturb antioxidant defense system. In the present study, Fishes were exposed to 1/10th of LC_{50} / 48 hr. of Cd for 7, 15 and 30days to know the antioxidant role of zinc and calcium on cadmium toxicity. After 15d Cd exposure, fish were divided into three groups. 1st group was supplemented with Zinc (1 ppm), 2nd group received Calcium (1ppm) and 3rd group was supplemented with combination of both zinc and calcium at the above said doses for 7, 15 and 30days. After specific time intervals fish were sacrificed and selected tissues such as liver, kidney, brain, gill and muscle were isolated for the assay of oxidative stress enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). Simultaneously lipid peroxidation (LPO) levels were also measured. A significant elevation in LPO levels with decreased activity levels of CAT, SOD, GPx and GST were observed during Cd exposure. With zinc and calcium supplementation, a significant reversal in the activity levels of above said enzymes was observed. Our findings clearly envisage that the zinc and / or calcium supplementation is very effective in reducing the Cd toxicity.

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INTRODUCTION

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the few decades (Dirilgen, 2001; Vutukuru, 2005; Kwong *et al.*, 2011). Natural aquatic resources are extensively contaminated with heavy metals like lead (Pb), cadmium (Cd), nickel (Ni) and copper (Cu) released from domestic, industrial and other man made activities. Among the heavy metals, Cd is one of the most toxic, non-essential heavy metal; known for its corrosive nature and is widely used in paints and dyes, cement and phosphate fertilizers (Jarrup, 2003). Cd occurs naturally in the environment in significant amounts but its release in the recent past is steadily increasing due to human activities causing pollution at considerably toxic amounts was reported by earlier workers in various aquatic ecosystems (Usha Rani, 2000; Siraj Bhasha and Usha Rani, 2003; Kiran *et al.,* 2006; Bhavani *et al*., 2009; Jia *et al.,* 2011; Kim *et al.,* 2011; Obaiah and Usha Rani, 2012; 2013; 2014; 2015; 2016).

Cd has an extremely long half-life (20-30 Years) in the human body (Flora *et al.,* 2008) and is highly cumulative, especially in the liver and kidney (Hijova and Nistiar, 2005; Mahtap and Ethem, 2006; Nordberg *et al.,* 2007; Tim *et al.,* 2008).

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It is a ubiquitous toxic metal and induces oxidative damage by disturbing the prooxidant – antioxidant balance in the tissues (Ognjanovic *et al.,* 2008). Cd inhibits oxidative stress enzymes which protect tissues by either binding to sulfhydryl (-SH) groups essential for the enzymes, replace the bivalent metals like zinc (Zn), copper (Cu), selenium (Se), iron (Fe) and manganese (Mn) required for the enzymes (Eriyamremu *et al.,* 2008).

Cd like many other heavy metals is antagonistic to essential trace elements like Zn, Fe, Cu, Ca etc., (Sobha *et al*., 2007) and competes with these trace elements for binding sites as transport and storage proteins, metalloenzymes and receptors.
Zn is a ubiquitous essential trace element with numerous functions in biological systems. It occurs in all living cells as a constituent of metallo enzymes involved in major metabolic pathways. It plays a catalytic, inhibitory or accessory role in the regulatory enzymes such as kinases or phosphatases. Zn controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immune competence and plays a significant role in homeostasis of hormones (Brando *et al.,* 1995). It has been noted that Zn is a constituent of several enzymes (more than 300 enzymes) in the body and can prevent cell damage through activation of the antioxidant defense system (Ozturk *et al.,* 2003; Prasad *et al.,* 2004; Ozdemir and Inanc, 2005; Bashandy *et al.,* 2006; Amara *et al.,* 2008).

Ca plays diverse role in the living organisms. In most of the vertebrates it is a major component of the skeleton but it also has vital functions in the body fluids and soft tissues (Bhavani *et al.,* 2012; Obaiah and Usha Rani, 2013). It acts as a cofactor in various enzymatic processes and couples stimulus excitation reactions, as in muscle contraction or the secretion of exocrine and endocrine glands. Both Ca and Cd are divalents and they use the same transitional channel interacting with each other antagonistically. Zohouri *et al.,* 2001 have been reported that high concentrations of Ca either in water or diet clearly envisages ameliorating effects on water borne Cd toxicity in fish *Oreochromis mossambicus.*

Hence, an attempt is made in the present investigation on the interactions of Zn and/or Ca against Cd induced oxidative stress in liver, kidney, gill, brain and muscle of teleostean fish *Oreochromis mossambicus.*

MATERIALS AND METHODS

Chemicals

Cadmium as cadmium chloride ($CdCl_2$), zinc as zinc chloride ($ZnCl_2$) and calcium as calcium chloride ($CaCl_2$) were purchased from Merck (Dormstadt, Germany). The other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, Mo, USA), SD Fine Chemicals. The chemicals used for this study were of the highest purity.

Maintenance of animals (fish)

Fish *O. mossambicus* (*Tilapia*) weighing 10 ± 2 gm were collected from the local fresh water ponds and acclimatized to laboratory conditions for a week in separate troughs. The laboratory temperature was maintained at $28^0C \pm 2^0C$. The fish were feed *ad libitum* with ground nut cake and water was renewed for every 24 hrs with routine changing of troughs leaving no fecal matter.

Experimental design

Fish were divided into four groups, the first group as control and other groups as experimental. The experimental groups were exposed to sub lethal concentration of $CdCl_2$ i.e., 5 ppm ($1/10^{th}$ of LC_{50} / 48 hrs) daily for 7, 15 and 30 days (d) time periods. Then 15d Cd exposed animals were subjected to Zn and Ca supplementation (i.e., 1 ppm) individually and in combination for again 7, 15 and 30d long sojourn. After specific time intervals fish were sacrificed and tissues like liver, kidney, muscle, brain and gill were isolated for the assay of antioxidant enzymes.

Lipid peroxidation (LPO)

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

Superoxide dismutase (SOD) (E.C. 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 4^0C in cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min using UV-Spectrophotometer (Hitachi U-2000). Values are expressed in superoxide anion reduced / mg protein / min.

Catalase (CAT) (E.C. 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 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 4^0C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% ethylalcohol (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 and 50 µl of tissue extract was added 250 µl of 0.006 M H_2O_2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 seconds (s) 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.

Glutathione – S – transferase (GST) (E.C. 2.5.1.18)

GST activity was measured with its conventional substrate 1-chloro, 2, 4-dinitro benzene (CDNB) at 340 nm as per the method of Habig *et al*., (1974). The tissues were homogenized in 50mM Tris-Hcl buffer pH 7.4 containing 0.25 M sucrose and centrifuged at 4000 g for 15 min at 4^0C and the supernatant was again centrifuged at 16,000 g for 1 hour (hr) at 4^0C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 ml contained 2.4 ml of 0.3 M potassium phosphate buffer pH 6.9, 0.1 ml of 30 mM CDNB, 0.1 ml of 30 mM glutathione and the appropriate enzyme source. The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against reagent blank and the activity was expressed as µ moles of thioether formed / mg protein / min.

Glutathione peroxidase (GPx) (EC: 1.11.1.9)

GPx was determined by a modified method of Flohe and Gunzler, (1984) at 37^0C. 5% (W/V) of tissue homogenate was prepared in 50mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 g for 10 min at 4^0C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500 µl of phosphate buffer, 100µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100µl of GR (0.24 units). The 100µl of tissue extract was added to the reaction mixture and incubated at 37^0C for 10 min. Then 50 µl of 12 mM t-butyl hydroperoxide was added to 450 µl of tissue reaction mixture and measured at 340 nm for 180 s. The molar extinction

coefficient of 6.22 $X10^3$ M cm^{-1} was used to determine the activity. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

Estimation of protein content

Protein content of the tissues were estimated by the method of Lowry *et al.,* (1951). 1% (W/V) homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. To 0.5ml of homogenate, 1ml 10% TCA was added and the samples were centrifuged at 1000g for 15 min. Supernatant was discarded and the residues were dissolved in 1ml of 1N sodium hydroxide. To this 4ml of alkaline copper reagent was added followed by 0.4ml of folin-phenol reagent (1:1folin:H_2O). The color was measured at 600nm in a UV spectrophotometer (Hitachi U-2000) against reagent blank. The protein content of the tissues was calculated using a protein (BSA) standard graph.

Data Analysis

The data was subjected to statistical analysis such as mean, standard deviation (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 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

RESULTS

The data on the alterations in the oxidative stress enzymes such as SOD, CAT, GST and GPx as well as LPO in Cd exposed fish liver, kidney, gill, brain and muscle both before and after supplementation with Zn and / or Ca were depicted in figures 1-5. The data obtained in the present study was statistically significant ($p<0.05$).

A significant increase in LPO was observed in all the test tissues exposed to Cd. Muscle tissue showed highest LPO levels (12.093± 0.176 µ moles of MDA formed / gm wet wt. of the tissue) in the present study followed by brain, gill, liver and kidney respectively in 30d Cd exposure. The increased levels of LPO were as follows:

Muscle > Brain > Gill > Liver > Kidney

However with Zn and / or Ca supplementation there was reversal in the Cd induced LPO. Maximum reduction was found in brain (5.077±0.106) under 30d Ca supplementation (Fig.1). The activity levels of SOD were significantly decreased in all the tissues of fish during 7, 15 and 30d Cd exposure. Maximum depletion of SOD activity was observed at 30d Cd exposure period in all the selected tissues over control (Fig.2). More or less similar activity was found in brain and muscle tissues (0.152±0.007 superoxide anion reduced / mg protein / min, 0.113±0.005 superoxide anion reduced / mg protein / min respectively). However, the data showed depletion in the levels of SOD activity and was in the following order:

Liver > Kidney > Gill > Brain > Muscle

When 15d Cd exposed fish were supplemented with Zn and / or Ca at the above said time intervals, the SOD activity levels significantly increased in all the test tissues.

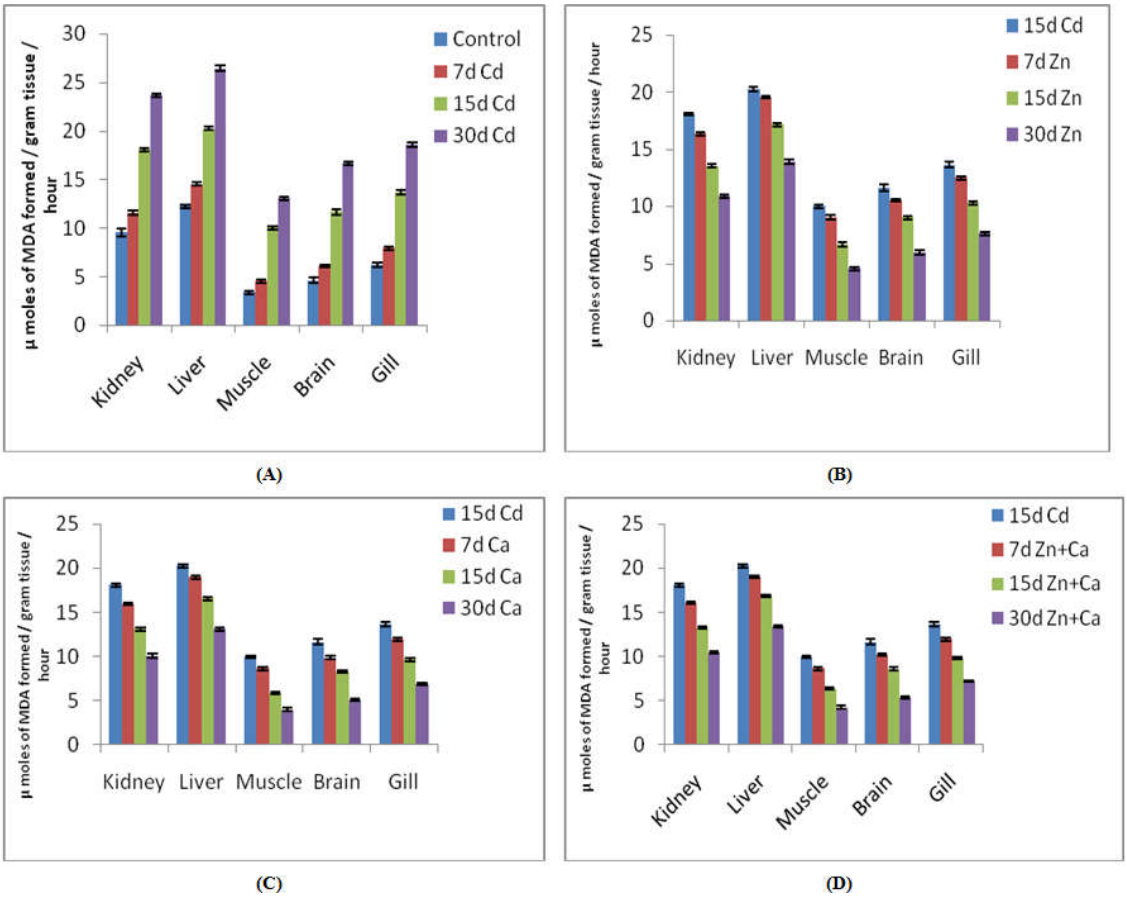

Fig 1 (A). Changes in LPO levels in different tissues of Cd exposed *O. mossambicus*. (B). Changes in LPO levels in different tissues of Cd exposed *O. mossambicus* under Zn supplementation. (C). Changes in LPO levels in different tissues of Cd exposed *O. mossambicus* under Ca supplementation. (D). Changes in LPO levels in different tissues of Cd exposed *O. mossambicus* under Zn+Ca supplementation. 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. 15d Cd treated rats considered as control for supplementation groups (B, C, D).

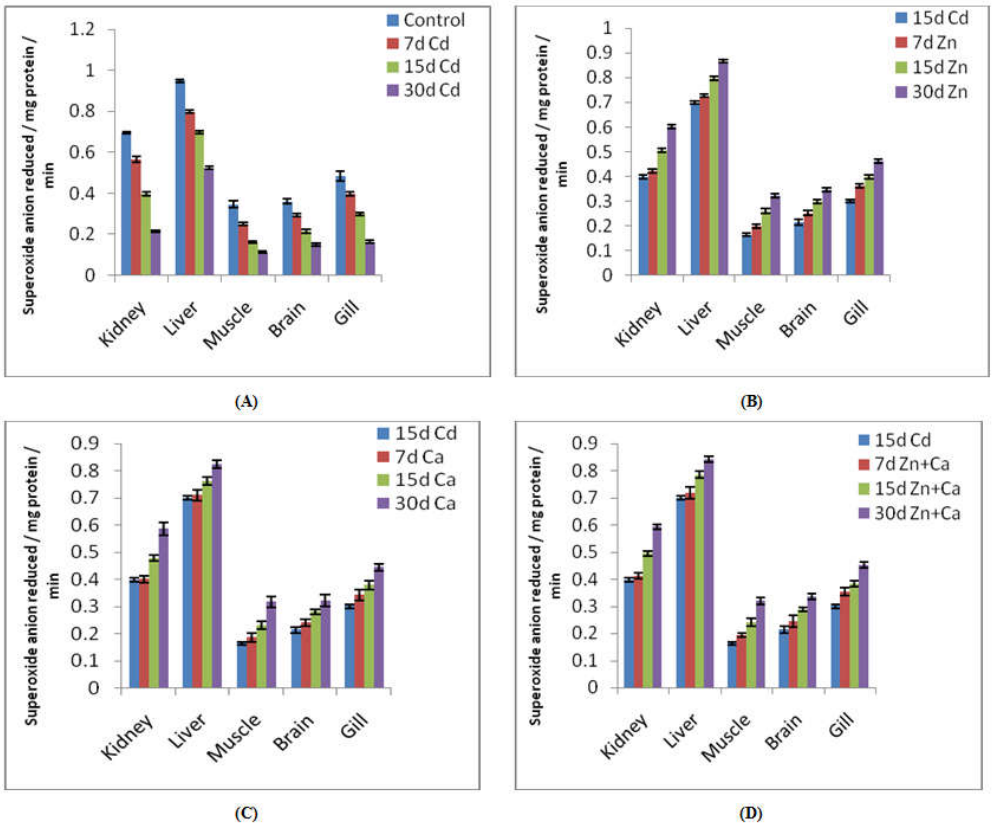

Fig 2 (A). Changes in SOD activity in different tissues of Cd exposed *O. mossambicus*. (B). Changes in SOD activity in different tissues of Cd exposed *O. mossambicus* under Zn supplementation. (C). Changes in SOD activity in different tissues of Cd exposed *O. mossambicus* under Ca supplementation. (D). Changes in SOD activity in different tissues of Cd exposed *O. mossambicus* under Zn+Ca supplementation. 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. 15d Cd treated rats considered as control for supplementation groups (B, C, D).

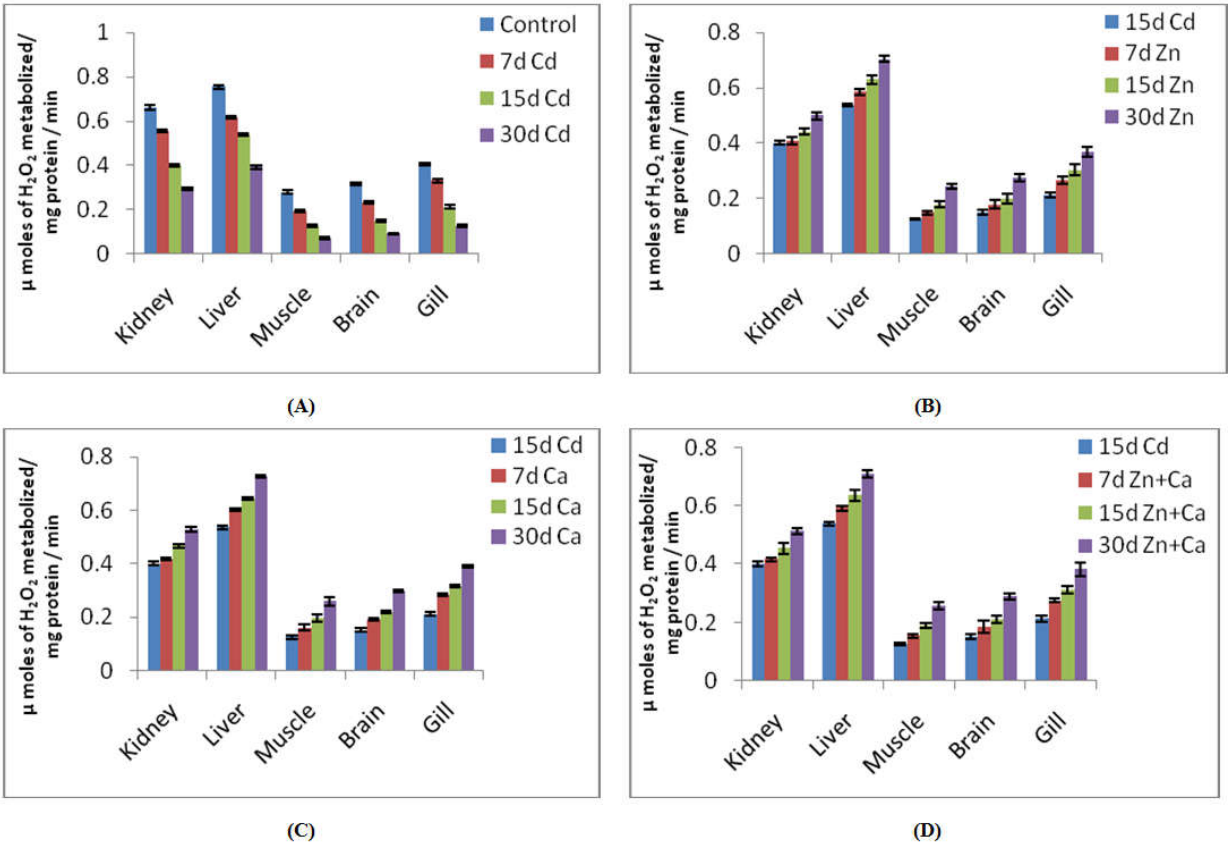

Fig 3 (A). Changes in CAT activity in different tissues of Cd exposed *O. mossambicus*. (B). Changes in CAT activity in different tissues of Cd exposed *O. mossambicus* under Zn supplementation. (C). Changes in CAT activity in different tissues of Cd exposed *O. mossambicus* under Ca supplementation. (D). Changes in CAT activity in different tissues of Cd exposed *O. mossambicus* under Zn+Ca supplementation. 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. 15d Cd treated rats considered as control for supplementation groups (B, C, D).

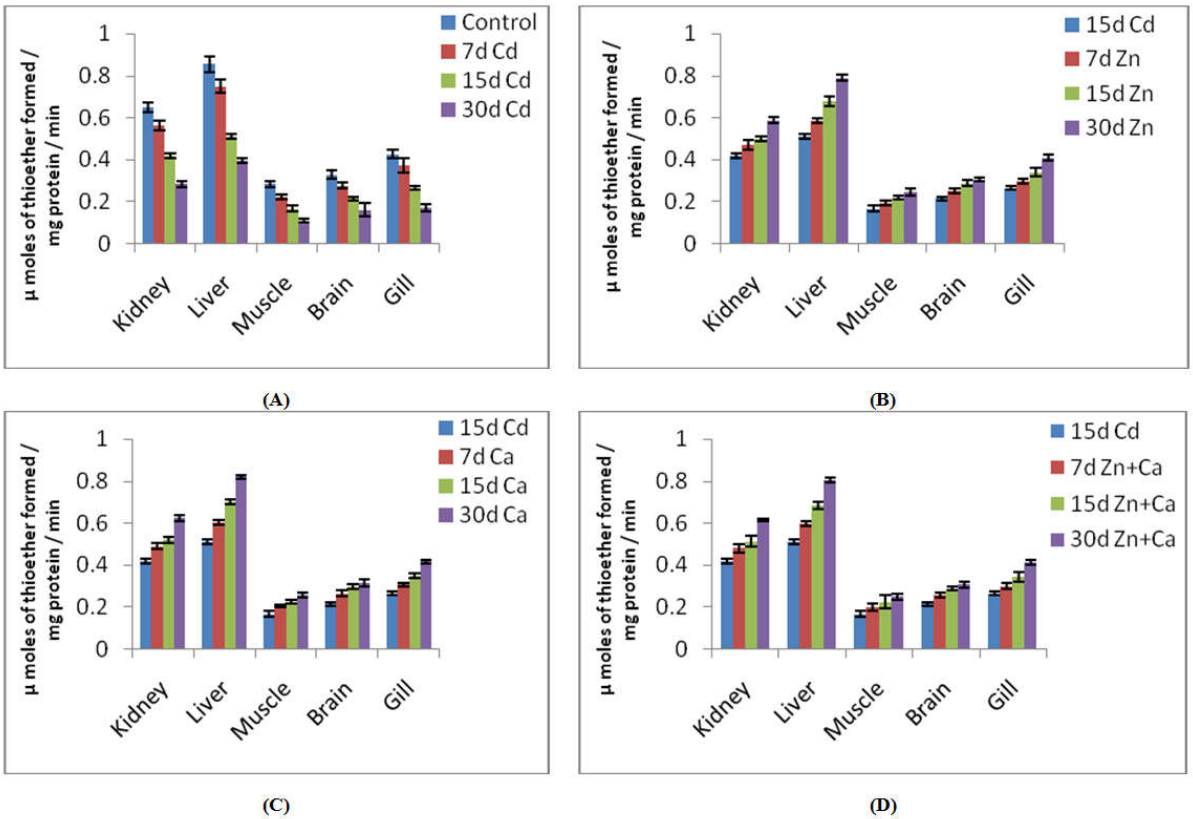

Fig 4 (A). Changes in GST activity in different tissues of Cd exposed *O. mossambicus*. (B). Changes in GST activity in different tissues of Cd exposed *O. mossambicus* under Zn supplementation. (C). Changes in GST activity in different tissues of Cd exposed *O. mossambicus* under Ca supplementation. (D). Changes in GST activity in different tissues of Cd exposed *O. mossambicus* under Zn+Ca supplementation. 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. 15d Cd treated rats considered as control for supplementation groups (B, C, D).

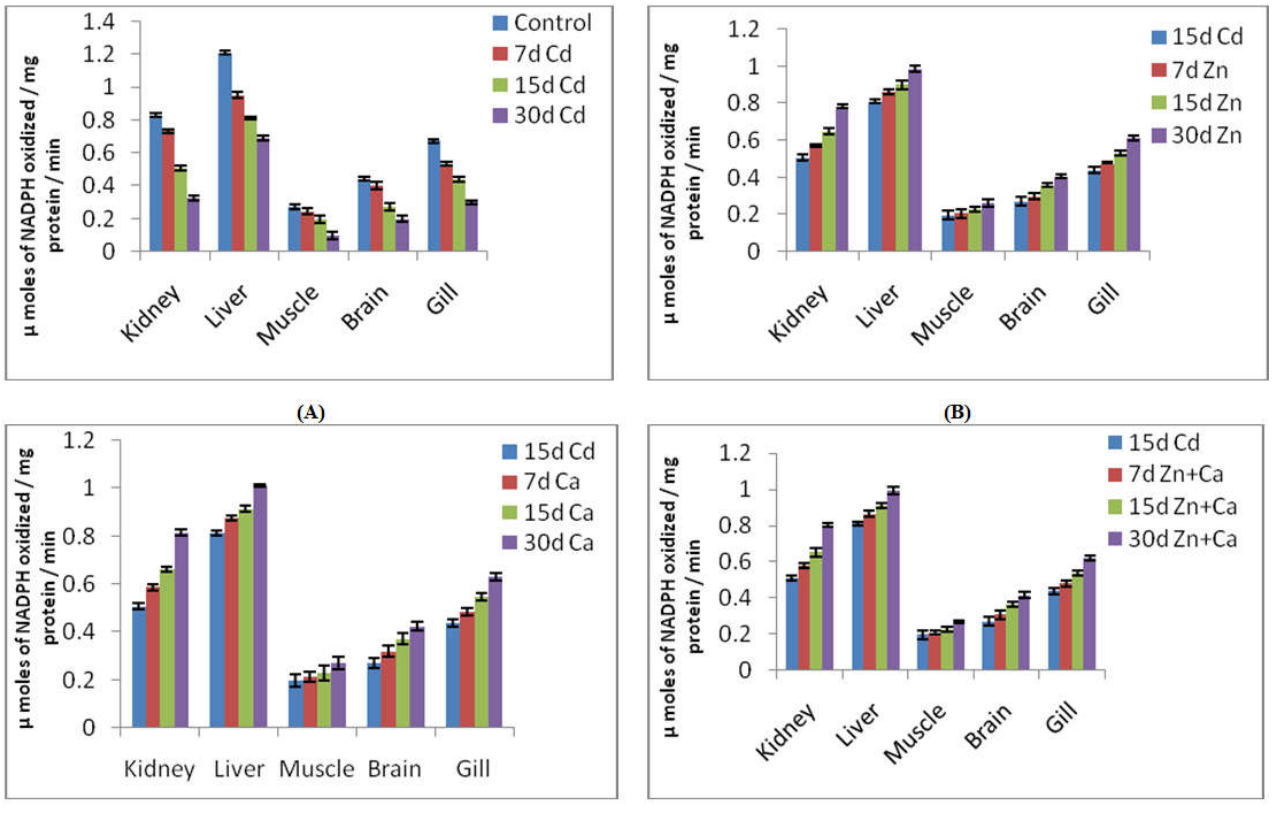

Fig.5 (A). Changes in GPx activity in different tissues of Cd exposed *O. mossambicus*. (B). Changes in GPx activity in different tissues of Cd exposed *O. mossambicus* under Zn supplementation. (C). Changes in GPx activity in different tissues of Cd exposed *O. mossambicus* under Ca supplementation. (D). Changes in GPx activity in different tissues of Cd exposed *O. mossambicus* under Zn+Ca supplementation. 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. 15d Cd treated rats considered as control for supplementation groups (B, C, D).

Maximum increase in SOD activity was observed in kidney (0.601 ± 0.008 superoxide anion reduced / mg protein / min) of 30d Zn supplemented fish. The specific activity levels of CAT were determined in the selected tissues of Cd exposed fish and also in controls (Fig.3). CAT activity levels were significantly reduced in gill (0.127 ± 0.004 µ moles of H_2O_2 / mg protein / min) followed by brain, muscle, kidney and liver tissues under Cd exposer. The decreased activity levels of CAT were as follows:

Gill > Brain > Muscle > Kidney > Liver

When the fishes were supplemented with Zn and / or Ca, CAT activity levels were significantly elevated in all the experimental tissues. Maximum elevation in CAT activity was found in the gill tissue of 30d Ca supplemented fish (0.385 ± 0.005 µ moles of H_2O_2 / mg protein / min).

Fig.4 explains the GST activity levels in selected tissues of fish under Cd intoxication. Exposure to Cd markedly depletes the activity of GST from 7d to 30d time periods in all the selected tissues. 30d Cd exposed muscle tissue showed maximum depletion in GST activity (0.112±0.010 µ moles of thio- ether formed / mg protein / min). Further all the tissues except gill showed more or less similar reduction in GST activity during Cd exposure. Depletion in the activity of GST was in the following order:

Muscle > Kidney > Liver > Brain < Gill

When the Cd exposed fish were subjected to Zn and / or Ca supplementation, there was significant elevation in GST activity levels and maximum was observed in 15d Ca supplemented fish. Further maximum increment was observed in muscle (0.257±0.01 µ moles of thioether formed / mg protein / min) of fish supplemented with Ca for 30d.

The specific activity levels of GPx were determined in the test tissues of fish exposed to sub lethal concentrations of Cd (Fig.5). GPx activity levels were markedly decreased in all the test tissues at all the time intervals. Among the tissues, muscle showed more decrement (0.098 ± 0.030 µ moles of NADPH oxidized / mg protein / min) under 30d Cd exposure. The decreased GPx activity levels in the tissues were as follows:

Muscle > Gill > Kidney > Brian > Liver

These decreased GPx activity levels were significantly elevated with the Zn and / or Ca supplementation in all the test tissues of *O. mossambicus*. Maximum increase in GPx activity was found in gill (0.609±0.016 µ moles of NADPH oxidized / mg protein / min) subjected to Ca supplementation for 30d.

DISCUSSION

The results of the present investigation revealed that Cd induces significant alterations in the levels of LPO and certain oxidative stress enzymes status in liver, kidney, gill, brain and muscle of teleostean fish *Oreochromis mossambicus* at all the time intervals. These activities were progressively reversed after using trace element supplements like Ca and / or Zn. Cd may induce oxidative damage in different tissues by enhancing per oxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles.

The present study findings revealed that LPO was enhanced during Cd exposure, which may be due to interaction of Cd with membrane phospholipids and thus causing membrane disorganization and further fragility. The enhanced LPO in this study could also be due to inhibition on activity levels of antioxidants, which were more concern with defense against free radical induction due to Cd intoxication. Similar findings were observed in the liver of common carp (*Cyprinus carpio*) by Jia *et al.,* (2011). MDA elevation induced by Cd was also reported for different fish tissues (Dallinger *et al.,* 1997).

Similar results were observed by several workers in different animals exposed to heavy metals. Wang and Wang (2009) reported that Cd significantly increased the LPO level in the contaminated copepods after 12d of Cd exposure, so the treated animals had encountered oxidative injury. Company *et al.,* (2004) also demonstrated that Cd exposure (101.2 mg/ l) notably increase the LPO levels in the vent mussel. Talas *et al.,* (2008) reported that there was significant increase in the LPO levels in liver tissue of rainbow trout exposed to Cd / Cr at the dose of 2ppm. In one study, Cd exposed fish showed increased LPO in gills of the calm *Ruditapes decussates* (Florence *et al.,* 2002).

Zn and / or Ca supplementation significantly counteracted the enhancement of LPO caused by Cd. This finding is in harmony with the findings of Ng *et al*., 2009 who reported that elevated Ca protects against Cd induced toxicity in rainbow trout. However in our study supplementation of Ca and / or Zn reduced LPO significantly in all the test tissues. A lower level of LPO means a lower degree of membrane damage. So Ca and Zn might have alleviated the Cd induced membrane damage and aids protection to the cell.

Decrease in SOD activity could be due to its inhibition by the excess production of ROS as evidenced by LPO in the present study. Decreased SOD levels indicate the product of O_2 radicals increased by the lowered ability of the tissues that can scavenge free radicals. Therefore, the enhanced LPO in the liver, kidney and other tissues might result from the reduction of their SOD activity. These findings are in accordance with Hisar *et al.,* (2009) on rainbow trout. Casalino *et al.,* (2002) proposed that Cd binds to the imidazole group of the His – 74 in SOD, which is vital for the breakdown of H_2O_2, thus causing its toxic effects and Cd probably interacting with metal moieties of SOD (Cu, Zn or Mn) and thus reducing its activity. Alternatively, Cd may alter the protein conformation by interacting with the enzyme, there by altering its functional activity (Nagaraj *et al.,* 2000). The alterations in SOD activity may depend on several factors such as Cd dose, Cd exposure time, type of Cd administration and the state of the animal (Yalin *et al.,* 2006). In this study, a significant reduction in SOD activity with the exposure period of the test tissues were observed in Cd subjected animals. Similar observations have been reported by Talas *et al.,* (2008). Oost *et al.,* (2003) observed that a significant decrease in SOD activity in the liver tissue of rainbow trout exposed to 2 ppm heavy metal (Cd, Cr). When the test fish were supplemented with Ca and / or Zn there was a significant increase in SOD activity in the experimental tissues. Similar findings were reported in Cd exposed rainbow trout (Baldisserotto *et al*., 2004), rat (Patra *et al*., 2001; Amara *et al.,* 2008) subjected to Ca and Zn supplementation.

In the present study the activity levels of CAT were significantly reduced in all the test tissues of Cd exposed fish for 7, 15 and 30d (Fig.3). The decrease in CAT activity could

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. Similar findings were observed in Cd exposed rainbow trout (Hisar *et al.,* 2009). Vaglio and Landriscina (1999) also reported that CAT activity levels were decrease in the fish *Sparus aurata* following *in vivo* exposure to Cd. According to Radhakrishnan (2008), different tissues of fresh water fish, *Heteropneustes fossilis (Bloch)* exposed to Cd showed decrement in CAT activity. Pratama yoga (2002) suggests that Cd causes LPO through inhibition of the CAT activity. CAT is a manganese or heme containing enzyme, functions to rapidy dismutate H_2O_2 to water and oxygen. By inhibiting this enzyme activity, H_2O_2 production within the cell is increased and leads to the production of hydroxyl radical and subsequently results in the cellular damage via the metal catalyzed Haber-Weiss reaction. After supplementation with Zn and / or Ca, CAT activity levels were significantly increased in all the test tissues. Cd is known to decrease the trace element absorption in the body (Flora *et al*., 2008). Hence the CAT activity levels were decreased during all the Cd exposure periods. However with trace elements like Zn and / or Ca supplementation in our study might have a significant role in protecting the cells from Cd induced injury and toxicity.

The activity levels of GST progressively decreased in the present study with the increased exposure periods in all the test tissues. The decrement in GST activity might be explained by the high production of ROS induced by Cd. These findings are in support of Pretto *et al.,* (2011) who reported a significant decrease in GST activity in Cd exposed gills of cat fish, *Rhamdia quelen.* In addition, some investigators have suggested that severe oxidative stress might suppress the activity of antioxidant enzymes due to oxidative damage and loss of the compensatory mechanisms (Zhang *et al.,* 2004; Atli *et al.,* 2006; Liu *et al.,* 2006).

The heavy metals are spontaneously conjugated with GSH and cystein, but interact with GSTPi by binding directly to this protein. This binding could have protective function against heavy metals. It has been demonstrated that addition of Cd suppresses the GST activity in liver and kidney tissues of rainbow trout (Baldisserotto *et al*., 2004). In our study reduced GST activity levels by Cd exposure were enhanced with the Zn and / or Ca supplementation. Increased dietary intake of Zn and Ca was known to decrease the gastrointestinal absorption of Cd (Franklin *et al.,* 2005). Interaction between Cd, Zn and Ca occur at several sites in the body including cellular mechanisms. There is substantial evidence that Cd, Zn and Ca compete for binding sites in tissues such as gill or intestinal cells and further protects against Cd toxicity (Baldisserotto *et al*., 2004; Dilelk and Cengiz, 2008). Antonio *et al*., 2003 observed similar results in Cd/Pb treated rats after supplementation with Ca.

Cd depleted GPx activity levels in the test tissues of sub lethal concentration of 7, 15 and 30d exposure (Fig.5). GPx catalyzes the conversion of H_2O_2 to water and reduces tissue injury from LPO directly. Thus, the decrease in GPx activity would induce free radical generation and thereby injuring the tissues. There was a significant decrease in GPx activity in the liver, kidney and other test tissues during the exposure period. Such depletion in GPx activity has been reported by Huang *et al*., (2007) in fresh water fish *Cyprinus carpio* exposed to organic / metallic contaminants. Company *et al.*, (2004) also demonstrated that Cd exposure (101.2 µg/ l) obviously inhibits GPx activity in vent mussel. Decreased GPx activity indicates the increased levels of H_2O_2 in the tissues. Therefore, the enhanced LPO in the tissues might result from the reduction in GPx activity.

GPx converts H_2O_2 to water and lipid peroxides to unreactive hydroxyl fatty acids. It was reported that organic pollutants such as pesticides (Oruc *et al.,* 2004) and various contaminants including heavy metals (Huang *et al*., 2007) could significantly decrease the GPx activity in kidney and intestine of carp (*Cyprinus carpio*). In our study decreased GPx activity levels were elevated significantly with Zn and / or Ca supplementation during all the experimental periods. Ca serves as second messenger for the control of important activities in many cells. Some studies also reported that Ca supplemented diets reduced the Cd induced alterations in the gills, kidney and liver in fresh water fish *Oncorhynchus mykiss* (Baldisserotto *et al*., 2004). Zn acts as an antioxidant and reducing the Cd body burden through induction of metallothionein in the tissues (Peraza *et al*, 1998; Flora *et al.,* 2008; Hijova, 2004). However the toxicity of Cd is influenced by the trace elements like Ca, Zn, Fe and Se and certain of the physiological mechanisms that control Ca metabolism have been shown experimentally to delay absorption and tissue distribution of Cd in experimental animals (Bhavani *et al.,* 2012; 2014).

In the present study it is clear that LPO, SOD, CAT, GST and GPx showed a trend towards normalcy in their activity levels in all the test tissues after supplementation with Zn and / or Ca to the Cd exposed fish thereby indirectly suggesting certain therapeutic measures to Cd induced toxicity in vertebrates.

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