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***Research Article** (Open Access)*

CADMIUM INDUCED BIOACCUMULATION AND OXIDATIVE STRESS IN TELEOSTEAN FISH *OREOCHROMIS MOSSAMBICUS*: THE POSSIBLE DETOXIFICATION MECHANISMS OF ZINC AND SELENIUM SUPPLEMENTATION

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ABSTRACT

Cadmium (Cd) is one of the most common non-essential elements, relatively accessible heavy metal in our environment causing wide range of toxic effects. The present study examines the detoxification role of zinc (Zn) and selenium (Se) against Cd induced bioaccumulation and oxidative stress in fresh water teleost *Oreochromis mossambicus*. After acclimatization, fish were exposed to sub lethal concentration of Cd ($1/10^{th}$ of LC_{50}/48h, *i.e.*, 5ppm) for 7, 15 and 30 days (d) period. 15d Cd exposed fish were considered as control and divided into three groups. The first group of fish were subjected to Zn (1ppm) supplementation, second group received only Se (0.5ppm) and third group of fish were supplemented with the combination of both Zn and Se at the above said doses and observed again for 7, 15 and 30d time periods. After specific time intervals liver, kidney, gill, brain and muscle tissues were isolated and used for the estimation of bioaccumulation levels as well as assay of oxidative stress enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). Simultaneously lipid peroxidation (LPO) levels were also measured. Bioaccumulation levels were significantly increased with the increased period of Cd exposure. After supplementation with Zn and/or Se, bioaccumulation levels were progressively decreased. A significant elevation in LPO levels with decreased activity levels of CAT, SOD, GPx and GST were observed during Cd intoxication. With Zn and/or Se supplementation, a significant reversal in the above oxidative stress enzymes was observed. The present study reveals that combined supplementation of Zn and Se tends to detoxify the Cd induced alterations in the test tissues than the other modes of supplementation.

Keywords: *Cadmium, Bioaccumulation, Oxidative Stress, Zinc and selenium, Fish*

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.,* 2010; Obaiah and Usha Rani, 2012, 2013, 2014, 2015, 2016). Bio enhancement of Cd transfer along a food chain was studied by Seebaugh *et al.,* 2005 and fish were reported to be used as biological indicators to assess water pollution (Rashed, 2001). In aquatic systems, as fish occupy the upper trophic level, there are greater chances of transferring Cd to higher organisms particularly to man.

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). 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, Se, Cu 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.,* 2004). 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; Ozdemir and Inanc, 2005, Obaiah and Usha Rani, 2013).

Se is an essential micronutrient with numerous functions in biological systems. It is widely distributed throughout the environment and is found in all living cells as a constituent of GPx and GSH. It is involved in the metabolism of GSH which can reduce toxicity of Cd (Ranzani-Paiva *et al.,* 2011). It was also known that Se has a certain protective role from the toxic effects of Cd and other heavy metals (Ognjanovic, 2008). This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (Shilo *et al*., 2008; Chen *et al.,* 2012).

Hence, an attempt is made in the present investigation on the interactions of Zn and/or Se against Cd induced toxicity 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 selenium as sodium selenite (Na_2SeO_3) 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 Se 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, gill, brain and muscle were isolated and were immediately used for bioaccumulation studies and antioxidant enzymes assay.

Bioaccumulation studies

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

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 tissues were 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 *et al*., (1984) at room temperature. The tissues were 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% ethyl alcohol (EtOH) was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer 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 was estimated by the method of Lowry *et al.,* (1951). 1 % (W/V) homogenates of the tissues was 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 and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 11.5) package. All values are expressed as Mean ± SEM of 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

RESULTS

The accumulation of Cd significantly increased in the selected tissues with the Cd exposure when compared to control (Figure 1). Maximum level of Cd bioaccumulation was observed in 30d fish kidney (22.535 ± 0.41 µg/g). Further liver showed high Cd concentrations when compared to other tissues. Low level of Cd accumulation was found in the brain and muscle tissues of fish over a period of 30 days. Among all the selected tissues, the lowest concentration of Cd was observed in the 30d muscle tissue (4.962±0.23 µg/g).

After supplementation with Zn and/or Se, Cd bioaccumulation levels were progressively decreased in all the test tissues. 30d Se supplemented fish Muscle tissue showed maximum percentage of depletion in Cd accumulation (0.455 ± 0.196 µg/g) than the other tissues (Figure 2). However with Zn alone supplementation, all the test tissues showed moderate levels of Cd accumulation for all the time intervals (Figure 3). Moreover, low level of depletion in Cd concentration was found in the test tissues under combined supplementation of Zn and Se compared to other modes of supplementation (Figure 4). From the above results it is clearly understood that the individual supplementation of Se could tremendously reduce the Cd body burden in the test tissues under 30d long sojourn.

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 Se were depicted in Figures 5-24. 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. However with Zn and/or Se

supplementation there was reversal in the Cd induced LPO. Maximum reduction was found in brain (5.077±0.106) under 30d Se supplementation (Figure 7). 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 (Figure 9). 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).

When 15d Cd exposed fish were supplemented with Zn and/or Se at the above said time intervals, the SOD activity levels significantly increased in all the test tissues. 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 (Figure 13). 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 Cd exposer. When the tissues were supplemented with Zn and/or Se, CAT activity levels were significantly elevated in all the experimental tissues. Maximum elevation in CAT activity was found in the gill tissue of 30d Se supplemented fish (0.385 ± 0.005 µ moles of H_2O_2/mg protein/min).

Figure 17 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 intervals in all the experimental tissues. 30d exposed muscle tissue showed maximum depletion in SOD 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 SOD activity during Cd exposure.

When the Cd exposed fish were subjected to Zn and/or Se supplementation, there was significant elevation in GST activity levels and maximum was observed in 15d Se 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 Se for 30d (Figure 18).

The specific activity levels of GPx were determined in the test tissues of fish exposed to sub lethal concentrations of Cd (Figure 21). 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.

These decreased GPx activity levels were significantly elevated with the Zn and/or Se supplementation in all the test tissues of *O. mossambicus* (Figures 22-24). Maximum increase in GPx activity was found in gill (0.609±0.016 µ moles of NADPH oxidized/mg protein/ min) subjected to Se supplementation for 30d.

DISCUSSION

The results of the present investigation revealed that Cd induces significant alterations in the levels of bioaccumulation, 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 Se and / or Zn. The results revealed that Cd concentrations were significantly increased in all the test tissues at all exposure periods. Maximum accumulation of Cd was observed in kidney and liver of *O. mossambicus* (22.353±0.41µg/g and 15.797µg/g). The increased accumulation of Cd in the liver and kidney over time could be due to the involvement of these organs in the detoxification and removal of toxic substances circulating in the stream. Moreover, since these organs are the major organs of metabolic activities including detoxification of xenobiotics (Klaassen *et al*., 2009). Cd might also be transported into these organs from other tissues like the gills and muscle, for the purpose of subsequent elimination. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis. Within these cells the complex is taken up by lysosomes and degraded by proteases to release Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of the heavy

metal in the kidney during the exposure periods. These findings corroborates those of Asagba *et al*., (2008) studies on fresh water cat fish (*Clarias gariepinus*) and accumulation in fish can be proportionally higher through dietary exposure than through water borne exposure (Szebedinnsky *et al*., 2001; Baldisserotto *et al*., 2005).

Gill also accumulates a higher proportion of Cd (11.580±0.314 µg/g). Several reasons have been proposed to justify the gills as the primary site for Cd uptake, such as proximity to toxicants due to its external position, its highly branched structural and vascular nature with the resultant highly increased surface area through which large volumes of water pass through the gill surface amongst other tissues (Jayakumar and Paul, 2006). In the brain, Cd inhibits enzymes such as Mg^{2+}-ATPase and Na^{+}-K^{+}-ATPase causing metabolic effects and disrupting neurotransmitter uptake (Beauvais*et al*., 2001). In several situations acetylcholine is not broken and accumulates within synapses causing physiologic impairment and alterations in fish swimming behaviour (Glusczak *et al*., 2006). The reason for the consistent low level accumulation of Cd in the brain (3.271±0.40 µg/g) is offered with certainty. However, a possible reason is that the blood brain barrier restricts the entry of Cd into the brain (Crowe and Morgan, 1997).

The muscle of fish accumulated lowest concentration of Cd (2.654±0.30 µg/g), even after 30 days of exposure. This may not be unconnected with the fact that the muscle is not concerned with detoxification and metals like Cd and Pb spread uniformly over the muscle tissue and this may be the reason for low level of Cd accumulation in the muscle (Vinodhini and Narayanan, 2008). It is clear from the present study that the toxicity of metal is affected by Se which in turn reduces the toxic effect of a metal through competitive inhibition at the gill surface. The non toxic Se competes with the toxic metals for the same binding sites (Krishnammal and Navaraj, 2012). If Se occupies the sites, the lamellae are protected from deterioration. Increased Se levels in the medium resulted in a slower transfer of Cd from the gills to the blood and the rate of Cd accumulation was lowered in liver, kidney and other tissues. Similar findings were also reported in rainbow trout by Hollis *et al*., (2000), and in *Cirrhina mrigala* by Ghosh and Adhikari (2006).

From the data obtained in the present investigation, Zn also plays a major role in reducing the Cd body burden as Se supplementation. Supplementation of Zn either alone or in combination with Se reduced the Cd body burden in the tissues (Peraza *et al.,* 1998; Flora *et al.,* 2008). Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms (Prasad *et al.,* 2004). It regulates MT synthesis. Zn inhibited oxidative stress induced by Cd (Li *et al.,* 2000). Zn prevented damage to the tissues from Cd exposure. This suggests Cd interference with Zn related metabolic functions. The competitive mechanism of interaction is a plausible mechanism of Zn in relation to Cd toxicity. Interactions between Cd and Zn occurs as early as in an intestine during absorption, but more intensive interactions take place during accumulation in the tissues. It has been shown that Cd may inhibit Zn activities at many stages interfering with its absorption, distribution to different tissues, transport into cells and/or transport into several intracellular structures (Oishi *et al.,* 2000; Lonnerdall, 2000; Satarug *et al.,* 2001). The most compelling reason for the protective effects of Zn against Cd toxicity is that Zn induces the synthesis of the metal binding protein, MT in the tissues (Bonda *et al.,* 2004; Amara *et al*., 2008). Interaction of Zn with Cd results in an increase in the excretion of Cd. This has been proposed as a mechanism by which Zn protects against Cd toxicity (Dilek and Cengiz, 2008) because Zn and Cd competes for a common transport mechanism in the organisms. Thus, Zn supplementation has showed beneficial effects on Cd toxicity (Gunnarsson *et al.,* 2004; Bashandy *et al.,* 2006). This may be the reason for the reduced Cd accumulation in the test tissues supplemented with Zn in the present study.

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.,* (2010). 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 Se 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 Se protects against Cd induced toxicity in rainbow trout. However in our study supplementation of Se and/or Zn reduced LPO significantly in all the test tissues. A lower level of LPO means a lower degree of membrane damage. So Se 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 Se 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; Obaiah and Usha Rani, 2012, 2014, 2015), rat (Patra *et al*., 2001; Obaiah and Usha Rani, 2014, 2015) subjected to Se 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. 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 (2009), 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 rapidly dismutase 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 Se, 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 Se supplementation in our study might have a significant role in protecting the cells from Cd induced injury and toxicity.

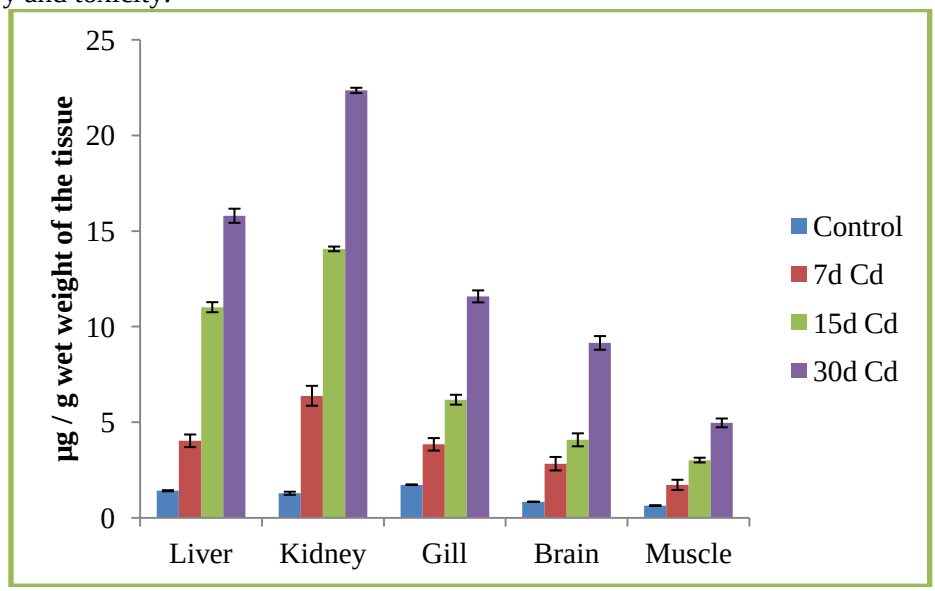

Figure 1: Cd Bio-accumulation levels (µg/g wet weight of the tissue) in selected tissues of Cd exposed *O. mossambicus* (*Tilapia*)

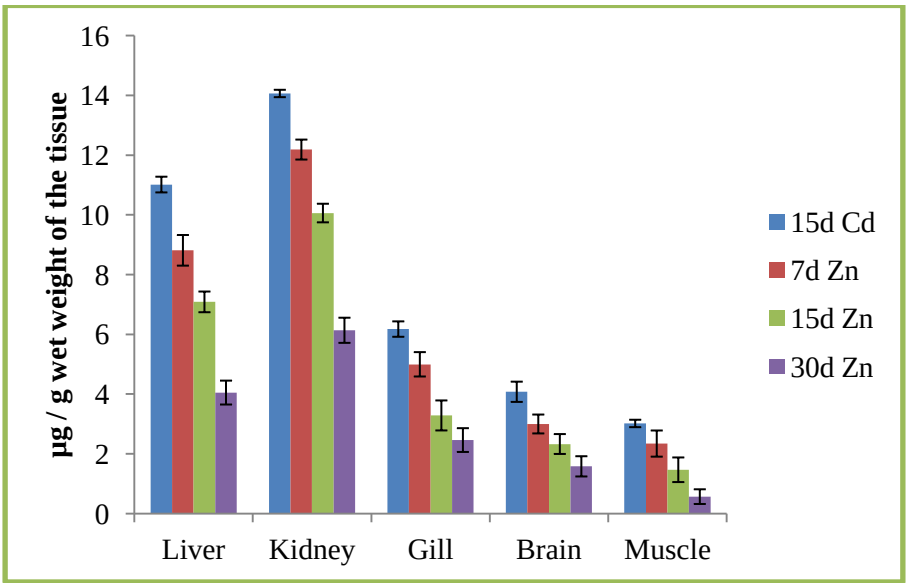

Figure 2: Cd Bio-accumulation levels (µg/g wet weight of the tissue) in selected tissues of *O. mossambicus* (*Tilapia*) after Zn supplementation

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.,* (2010) 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 cysteine, 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 Se supplementation. Increased dietary intake of Zn and Se was known to decrease the gastrointestinal absorption of Cd (Franklin *et al.*, 2005). Interaction between Cd, Zn and Se occur at several sites in the body including cellular mechanisms. There is substantial evidence that Cd, Zn and Se compete for binding sites in tissues such as gill or intestinal cells and further protects against Cd toxicity (Baldisserotto *et al*., 2004).

Cd depleted GPx activity levels in the test tissues of sub lethal concentration of 7, 15 and 30d exposure. 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 Se supplementation during all the experimental periods. Se serves as second messenger for the control of important activities in many cells. Some studies also reported that Se 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).

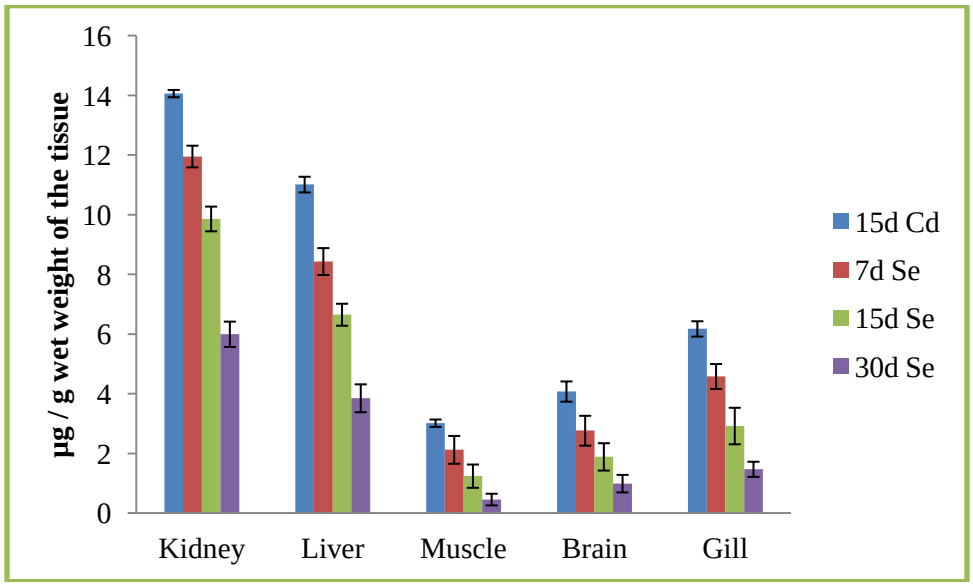

Figure 3. Cd Bio-accumulation levels (µg/g wet weight of the tissue) in selected tissues of *O. mossambicus* (*Tilapia*) after Se supplementation.

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 Se to the Cd exposed fish thereby indirectly suggesting certain therapeutic measures to Cd induced toxicity in vertebrates. It could be therefore concluded that Zn and Se supplementation might play a vital role in reducing the Cd tissue burden of fresh water fish thereby mitigating the risk of potential hazards to human health.

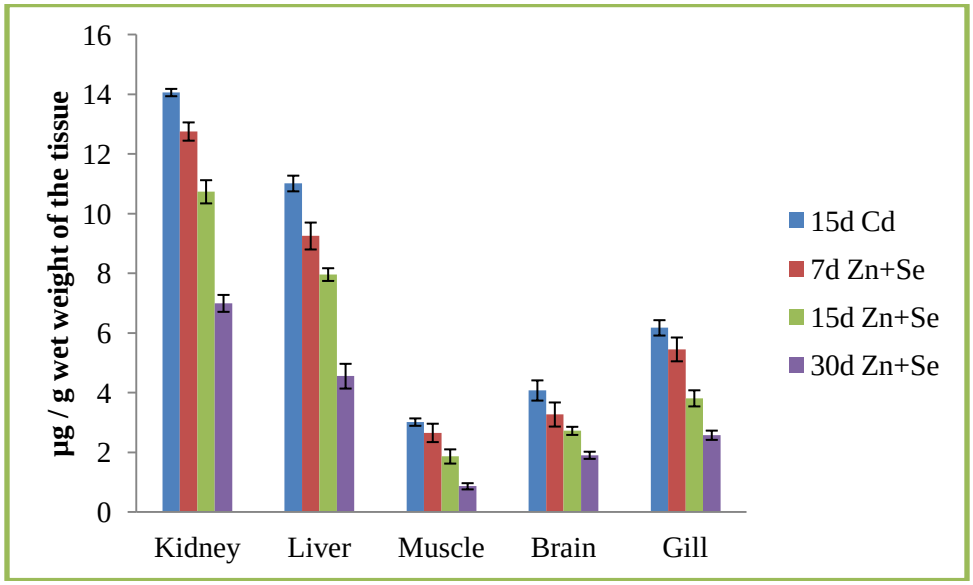

Figure 4. Cd Bio-accumulation levels (µg/g wet weight of the tissue) in selected tissues of *O. mossambicus* (*Tilapia*) after Zn + Se supplementation.

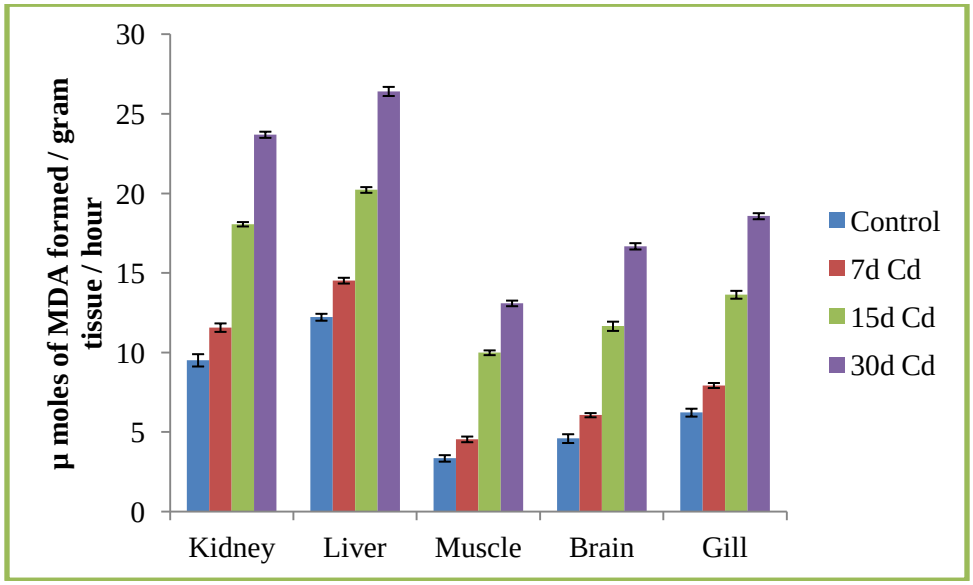

Figure 5. Changes in LPO levels (µ moles of MDA formed/ gram tissue/ hour) in different tissues of Cd exposed *O. mossambicus*.

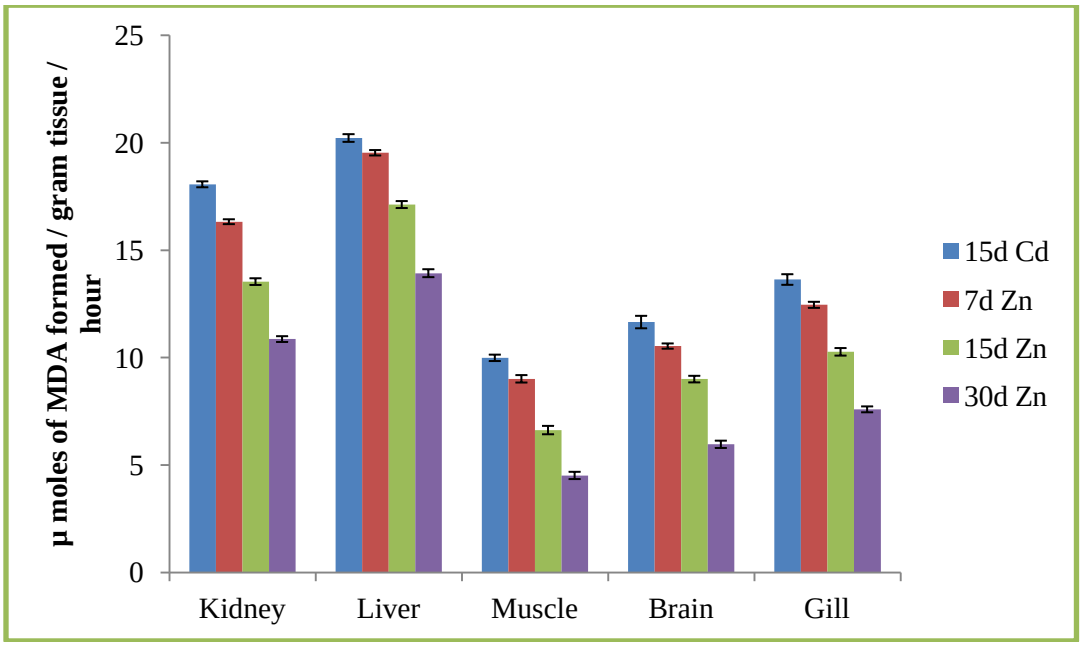

Figure 6. Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in different tissues of Cd exposed *O. mossambicus* under Zn supplementation.

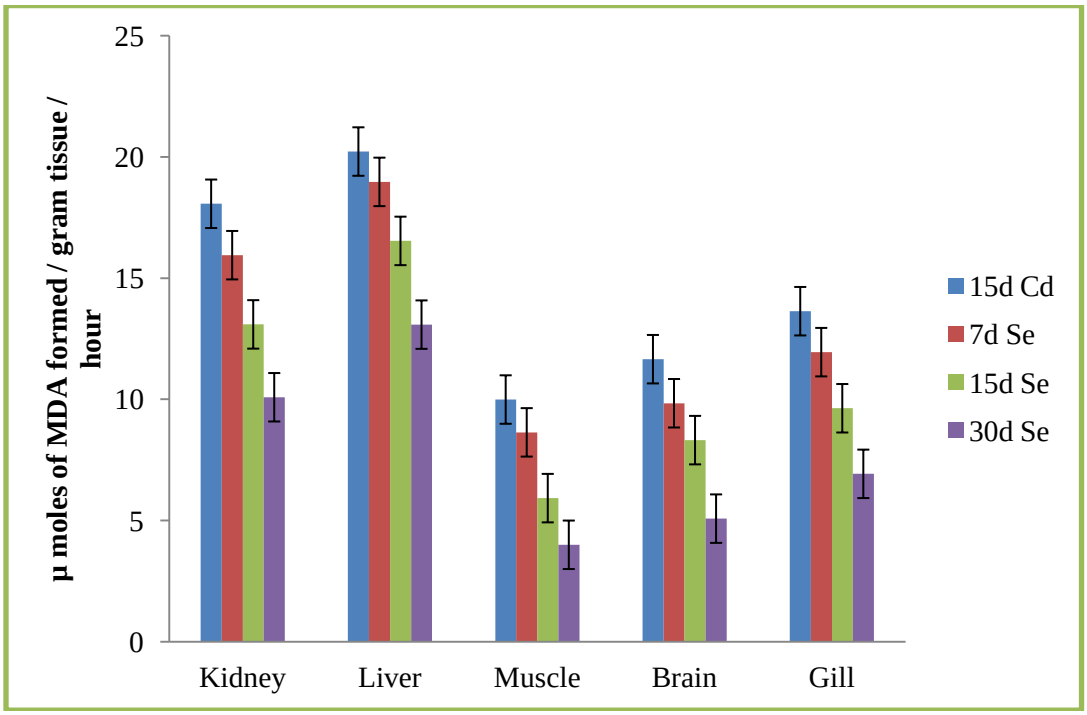

Figure 7. Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in different tissues of Cd exposed *O. mossambicus* under Se supplementation.

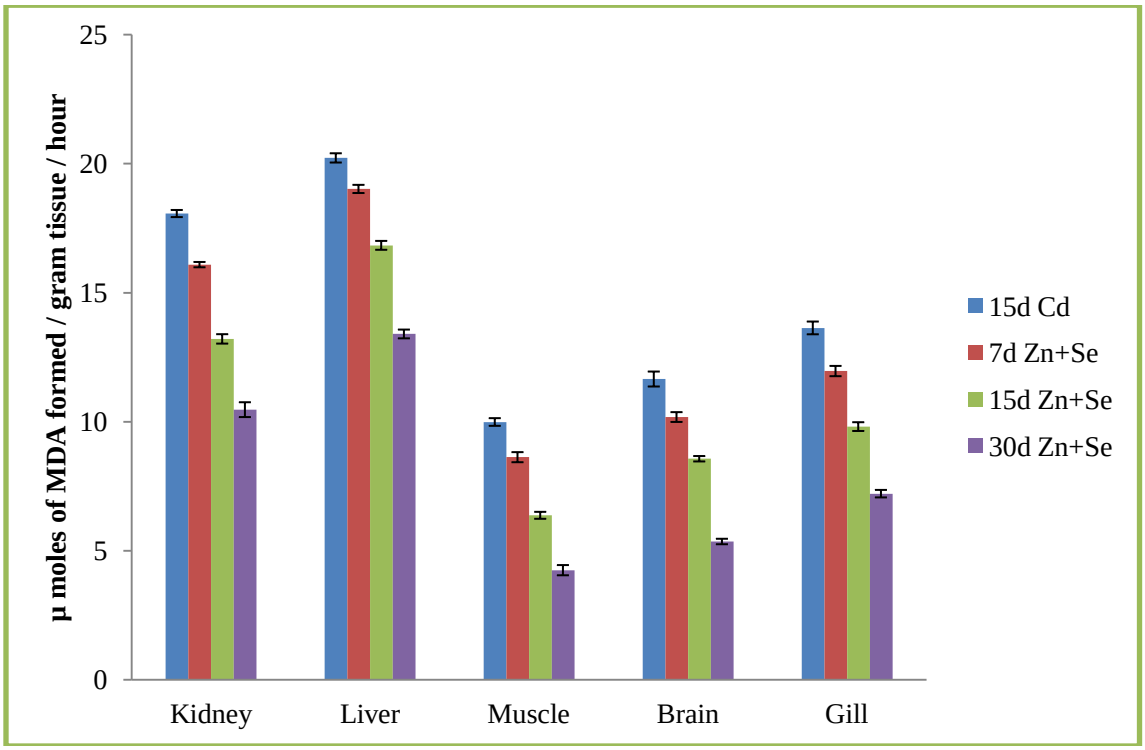

Figure 8. Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in different tissues of Cd exposed *O. mossambicus* under Zn+Se supplementation.

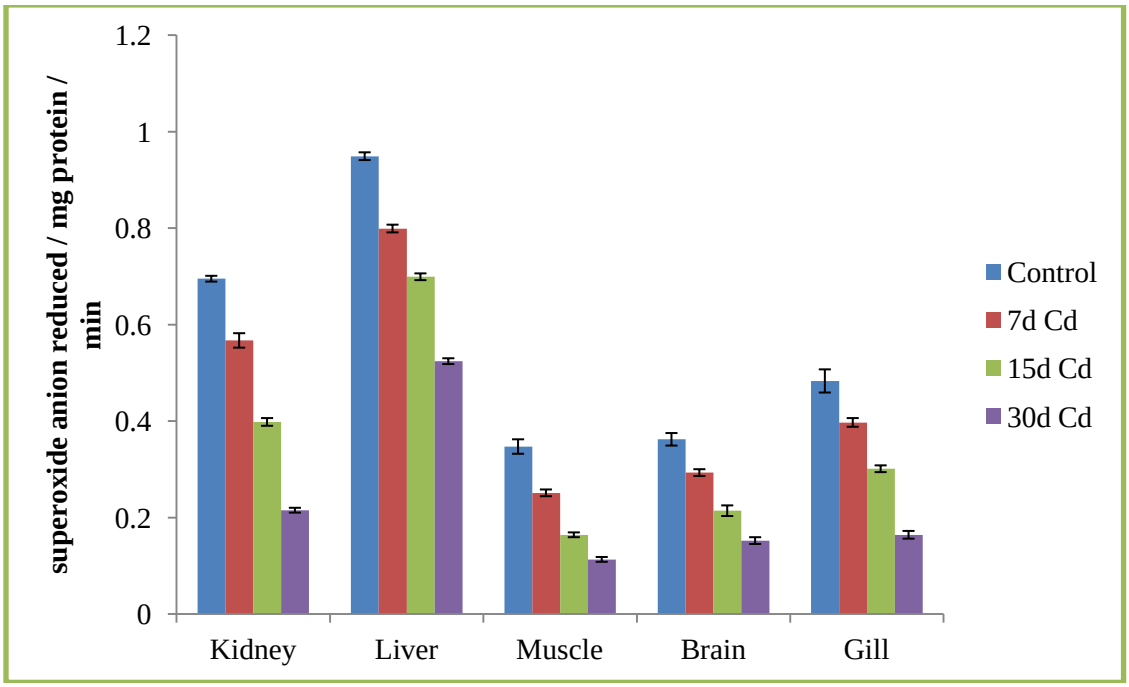

Figure 9. Changes in SOD (superoxide anion reduced / mg protein / min) activity in different tissues of Cd exposed *O. mossambicus.*

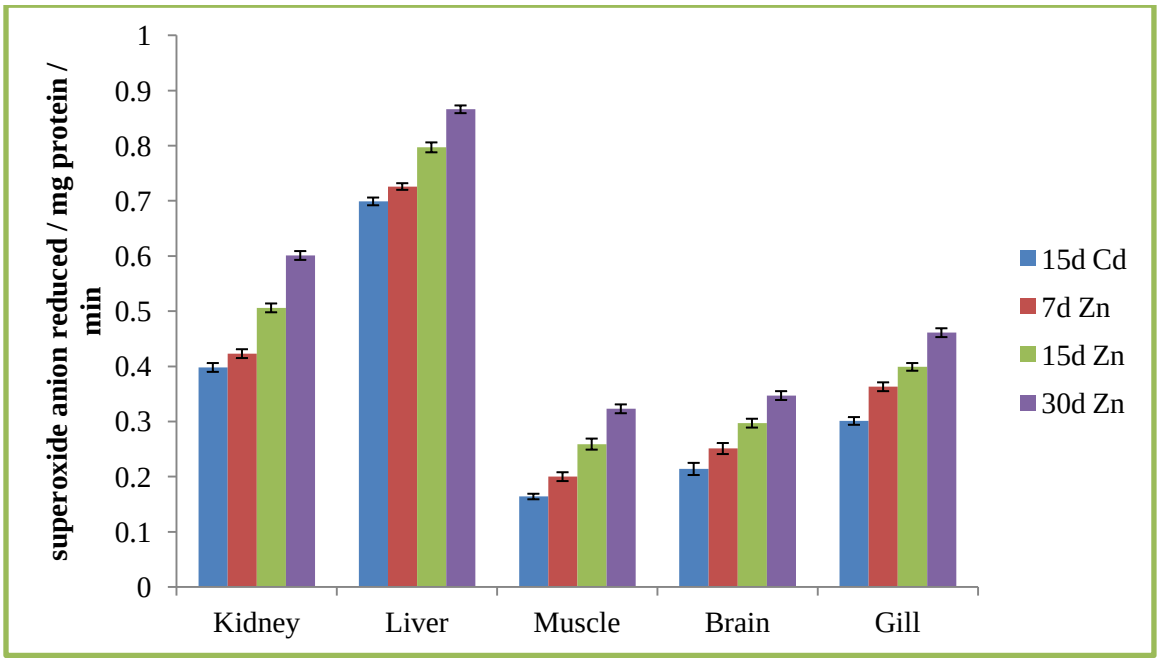

Figure 10. Changes in SOD (superoxide anion reduced / mg protein / min) activity in different tissues of Cd exposed *O. mossambicus* under Zn supplementation.

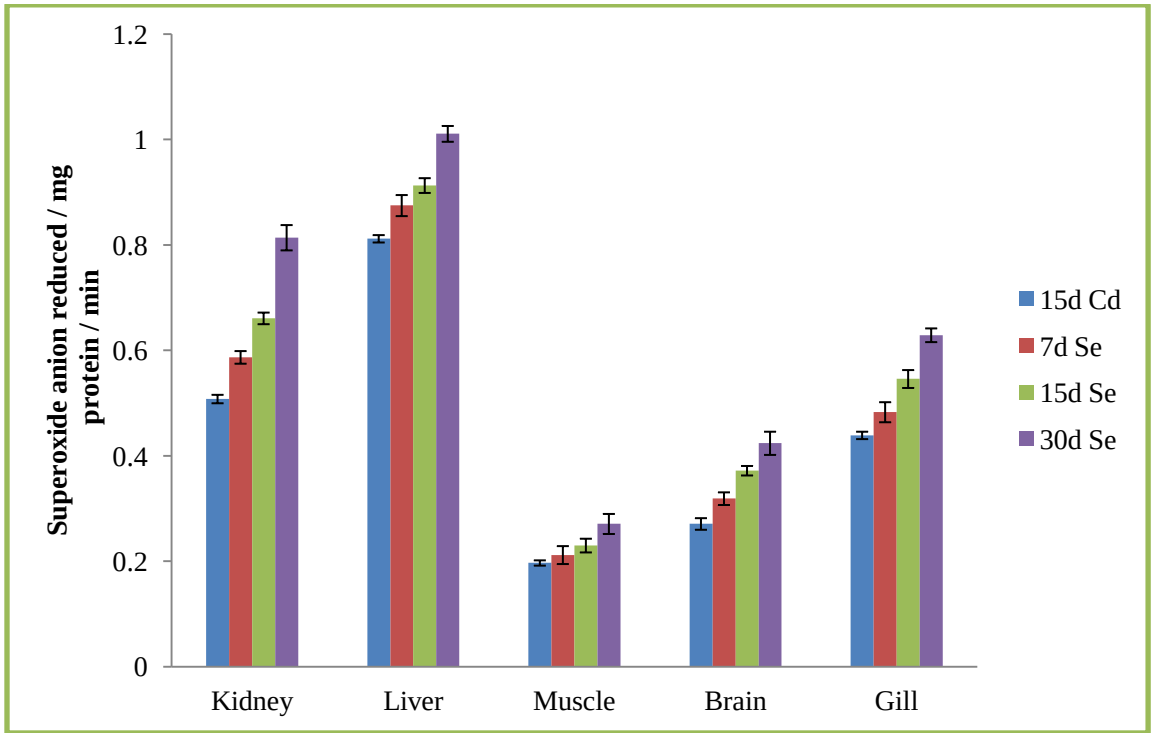

Figure 11. Changes in SOD (superoxide anion reduced / mg protein / min) activity in different tissues of Cd exposed *O. mossambicus* under Se supplementation.

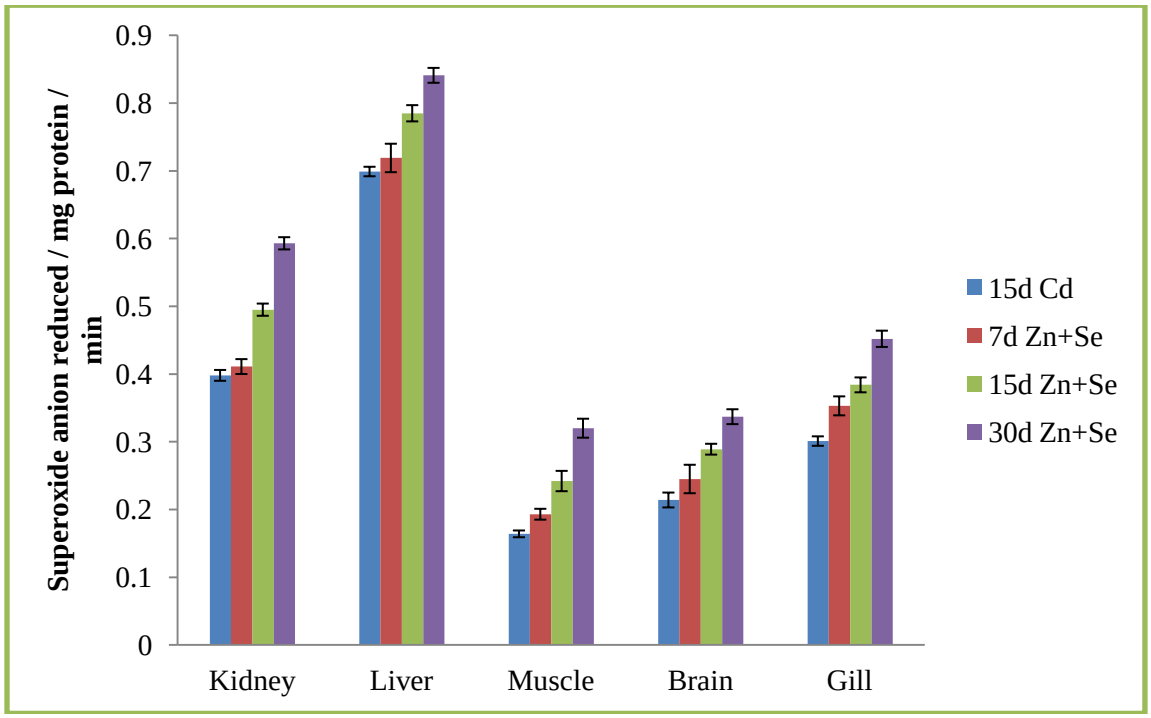

Figure 12. Changes in SOD (superoxide anion reduced / mg protein / min) activity in different tissues of Cd exposed *O. mossambicus* under Zn+Se supplementation.

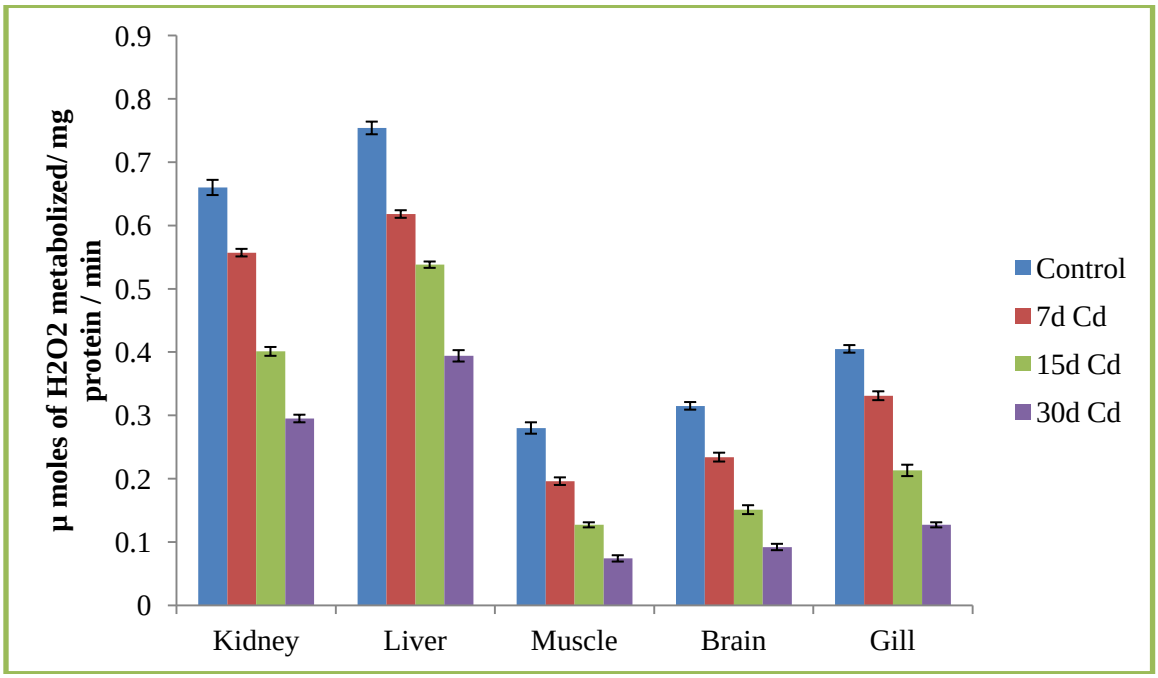

Figure 13. Changes in CAT activity (µ moles of H_2O_2 metabolized/ mg protein / min) in different tissues of Cd exposed *O. mossambicus.*

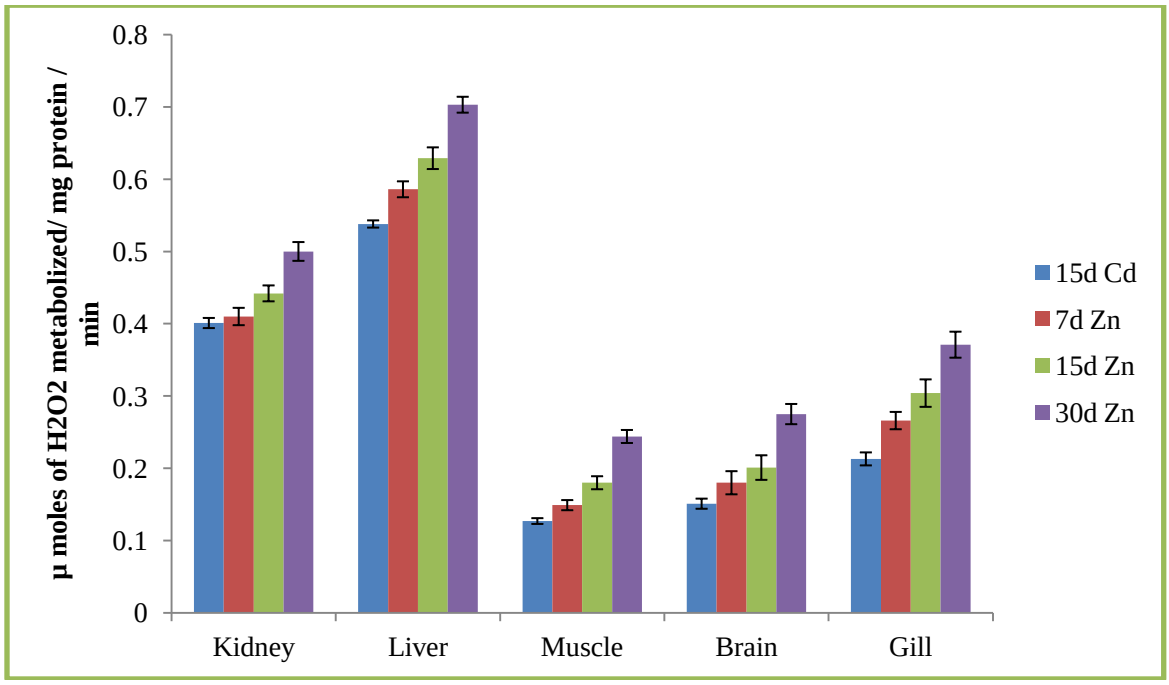

Figure 14. Changes in CAT activity (µ moles of H_2O_2 metabolized/ mg protein / min) in different tissues of Cd exposed *O .mossambicus* under Zn supplementation.

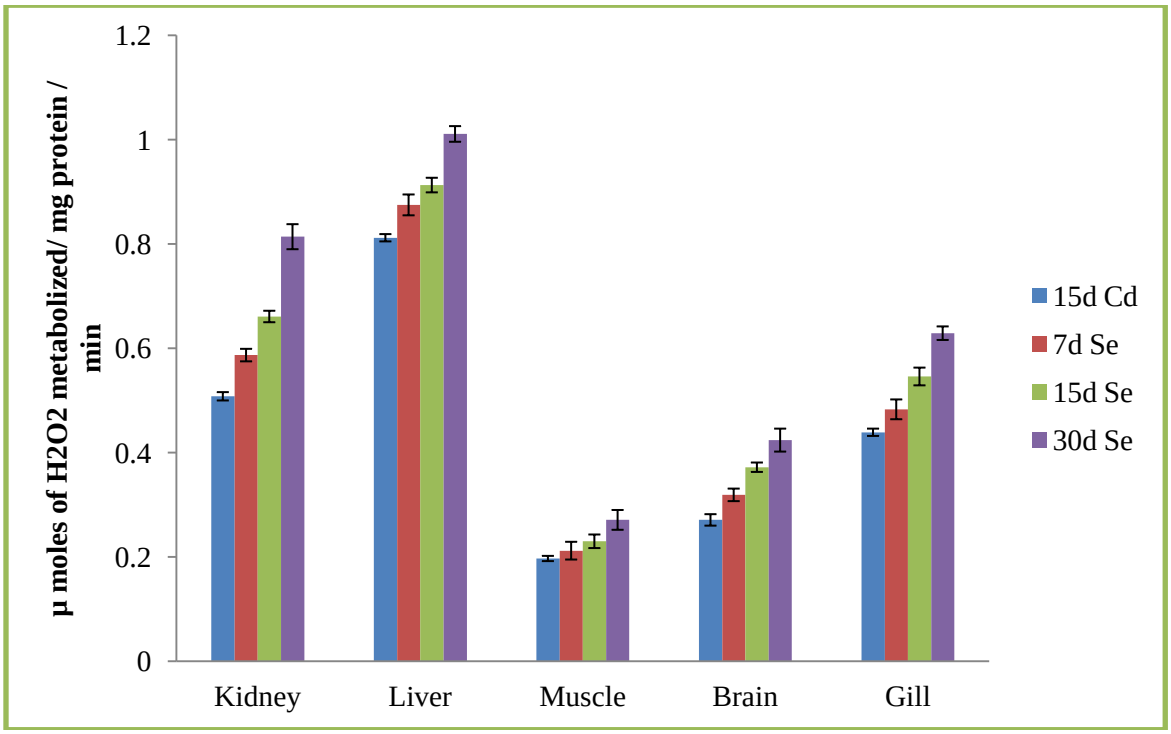

Figure 15. Changes in CAT activity (µ moles of H_2O_2 metabolized/ mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Se supplementation.

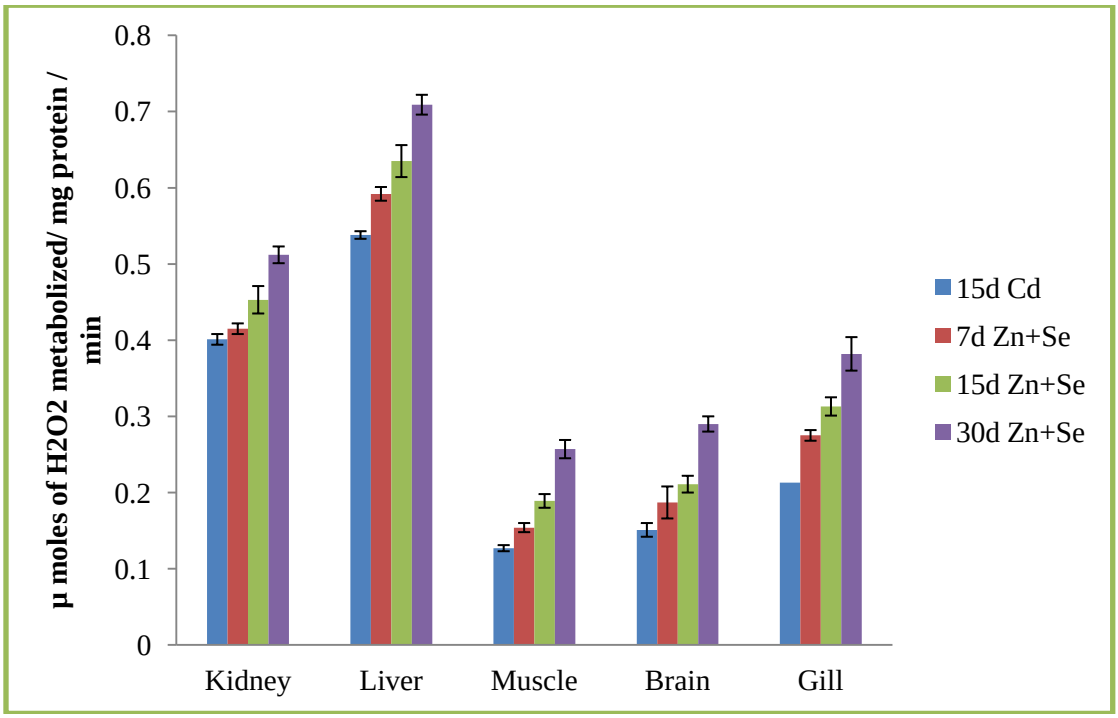

Figure 16. Changes in CAT activity (µ moles of H_2O_2 metabolized/ mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Zn+Se supplementation.

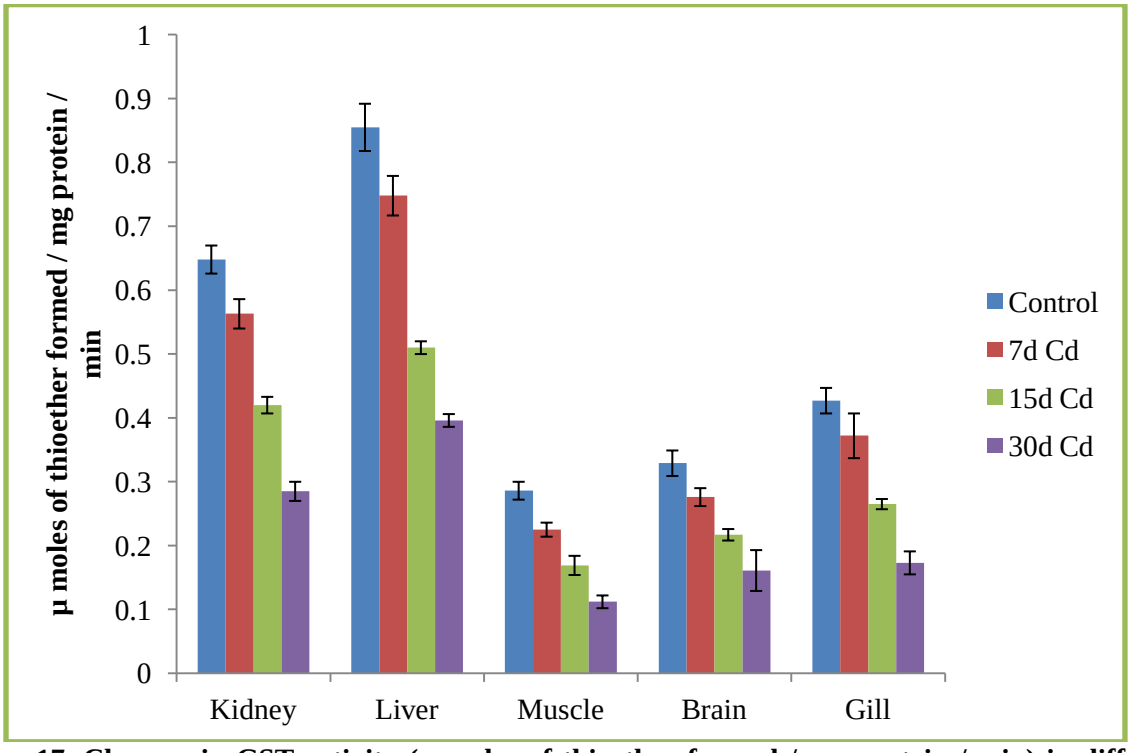

Figure 17. Changes in GST activity (µ moles of thioether formed / mg protein / min) in different tissues of Cd exposed *O .mossambicus.*

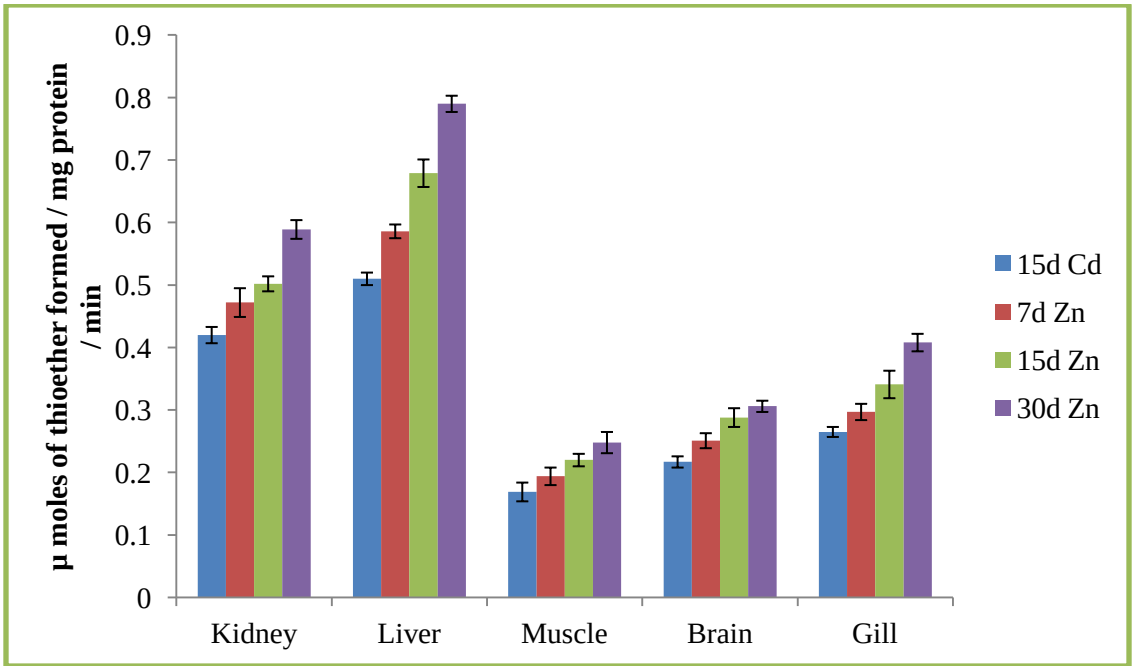

Figure 18. Changes in GST activity (µ moles of thioether formed / mg protein / min) in different tissues of Cd exposed *O .mossambicus* under Zn supplementation

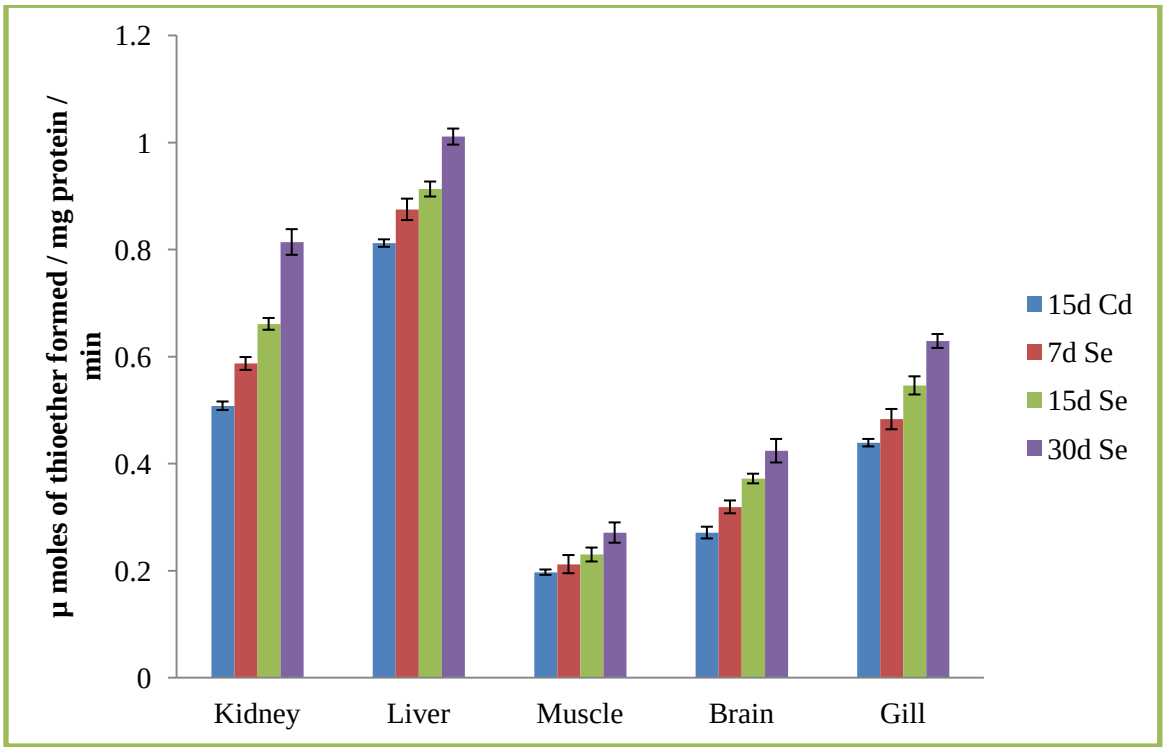

Figure 19. Changes in GST activity (µ moles of thioether formed / mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Se supplementation

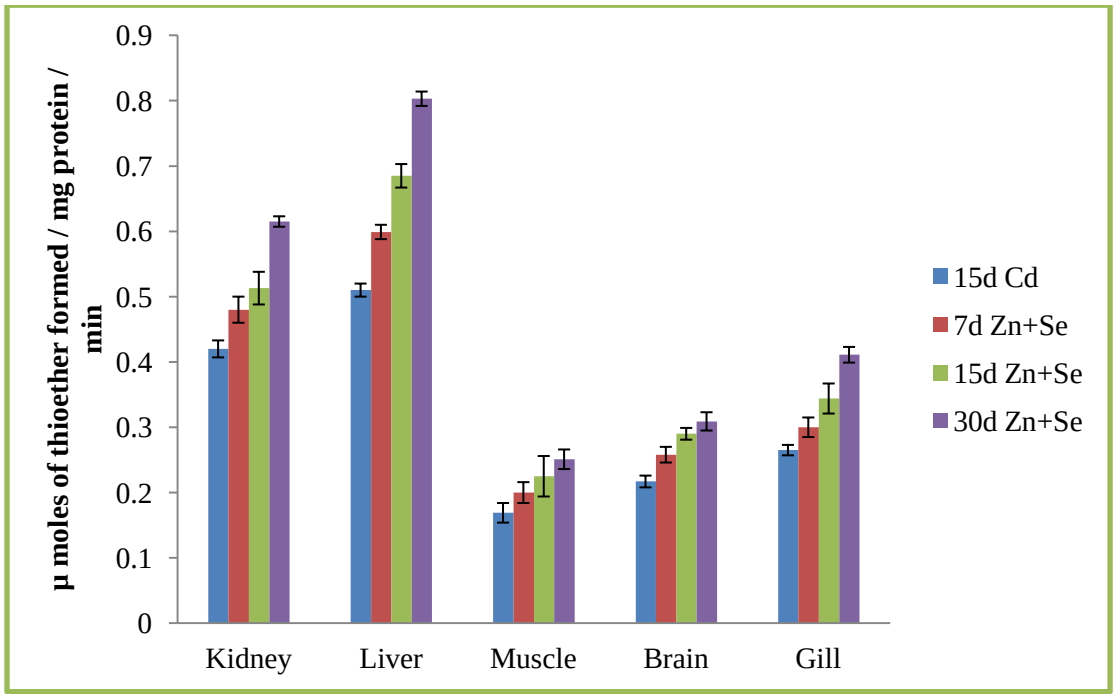

Figure 20. Changes in GST activity (µ moles of thioether formed / mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Zn+Se supplementation.

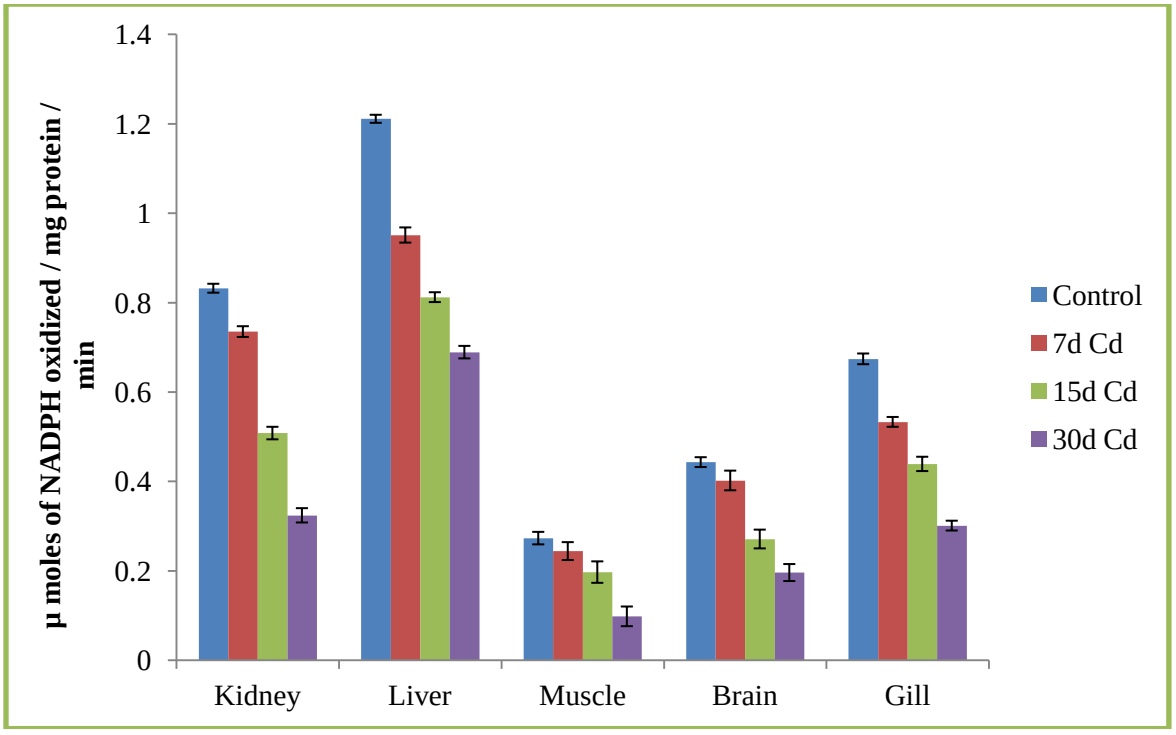

Figure 21. Changes in GPx activity (µ moles of NADPH oxidized / mg protein / min) in different tissues of Cd exposed *O. mossambicus.*

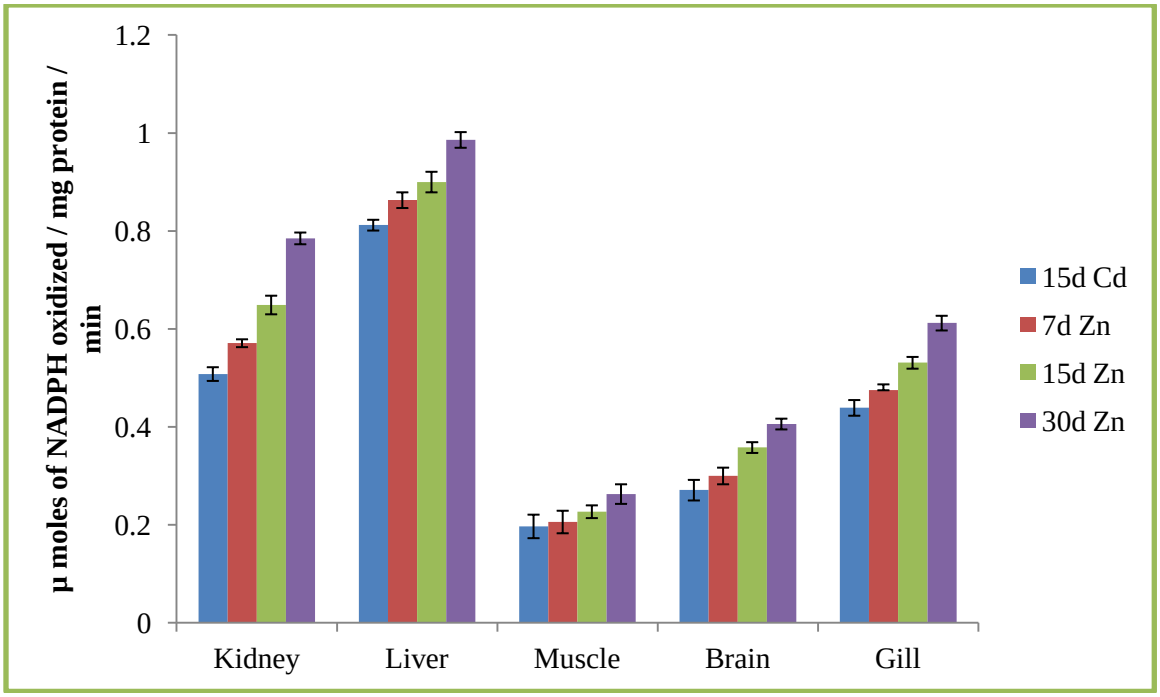

Figure 22. Changes in GPx activity (µ moles of NADPH oxidized / mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Zn supplementation.

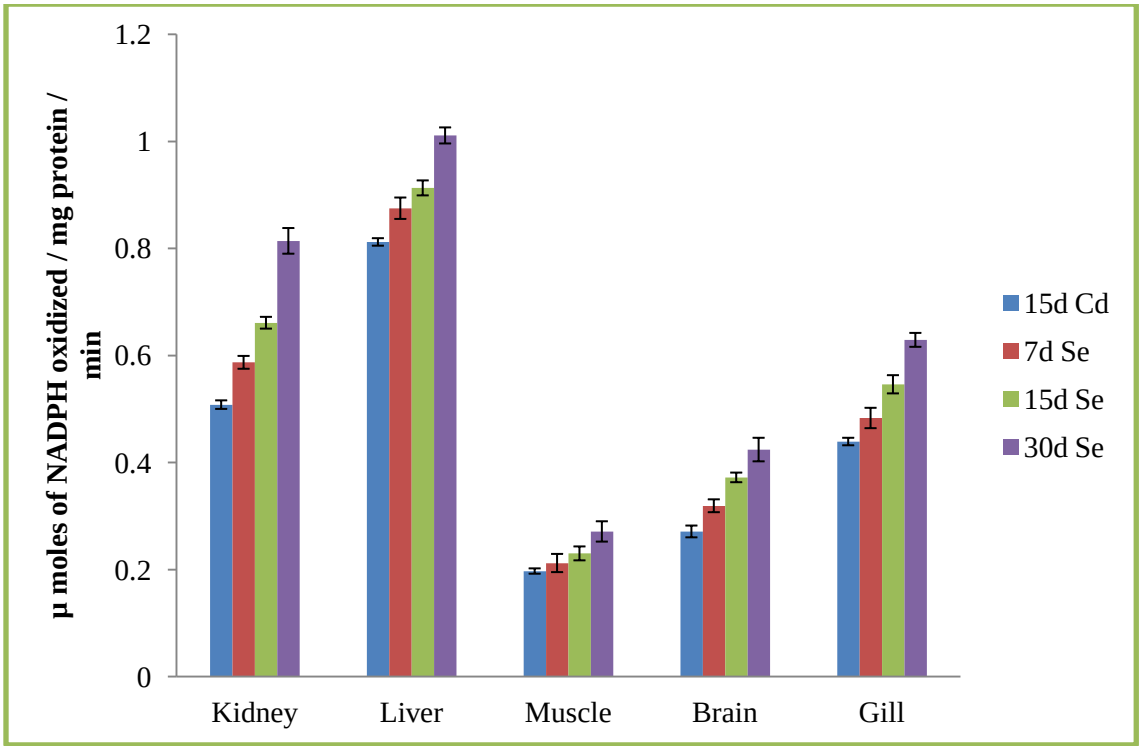

Figure 23. Changes in GPx activity (µ moles of NADPH oxidized / mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Se supplementation.

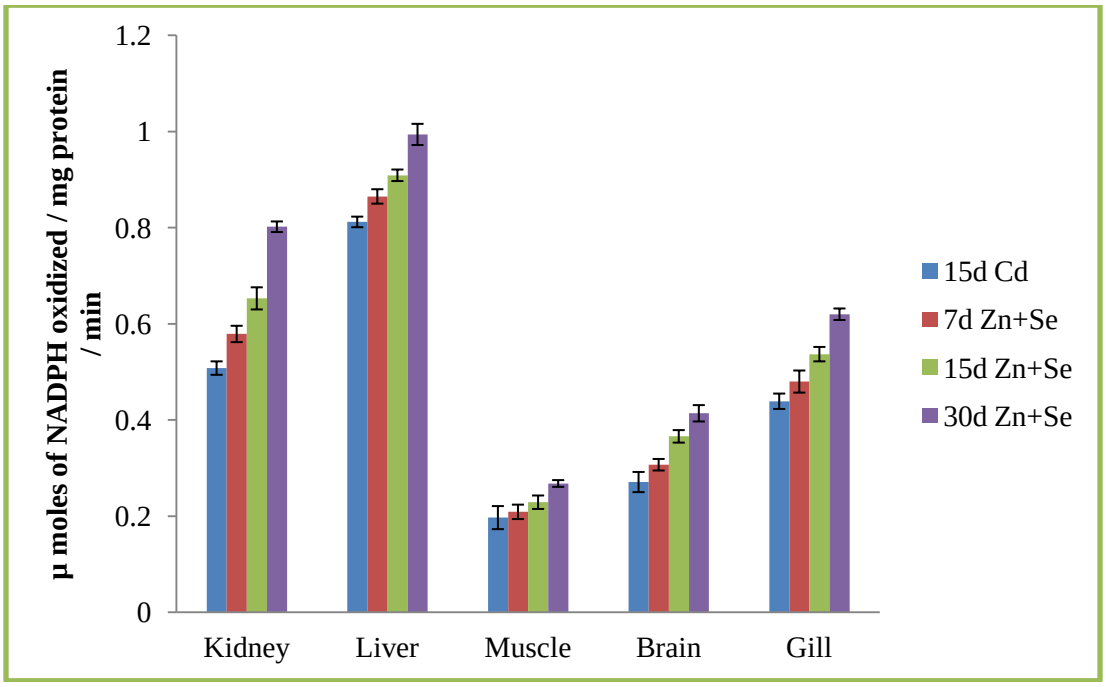

Figure 24. Changes in GPx activity (µ moles of NADPH oxidized / mg protein / min) in different tissues of Cd exposed *O. mossambicus* under Zn+Se supplementation.

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