Vol 3 Issue 4 Oct 2013

Impact Factor : 1.2018 (GISI)

ISSN No :2231-5063

Monthly Multidisciplinary Research Journal

GoldenResearch Thoughts

IMPACT FACTOR : 0.2105

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Golden Research Thoughts
Volume-3, Issue-4, Oct-2013
ISSN 2231-5063

Available online at www.aygrt.isrj.net

Calcium Impact On Cadmium Induced Alterations In Selected Oxidative Stress Enzymes In The Fresh Water Teleost, *Oreochromis Mossambicus (Tilapia)*

Obaiah Jamakala And A. Usha Rani
Department of Zoology, Sri Venkateswara University, Tirupati A.P. India

Abstract: Cadmium (Cd) is an important xenobiotic, non biodegradable cumulative pollutant in aquatic ecosystems. The present study was carried out to know the impact of calcium (Ca) on Cd induced oxidative stress enzyme alterations in the selected tissues of fresh water fish, Oreochromis mossambicus *(Tilapia)*. After acclimatization, fish were exposed to sublethal concentration of Cd ($1/10^{th}$ of LC_{50}/48h, i.e., 5ppm) for 7, 15 and 30 days (d) period. Then 15d Cd exposed fish were subjected to Ca supplementation (1ppm) for 7, 15 and 30d time intervals. After specific exposure periods tissues like gill, kidney and brain were isolated and used for Cd for the assay of glutathione-S- transferase (GST), glutathione peroxidase (GPx) and estimation of lipid peroxidation (LPO) levels in both control as well as experimental fish. Significant elevation in LPO levels were observed during Cd exposure and maximum elevation of LPO was found in 30d Cd exposed gill tissue (16.074±0.089 µ moles of MDA formed / gm wet wt. of the tissue). Further the activity levels of GST and GPx were significantly inhibited under Cd stress. However with Ca supplementation there was significant reduction in LPO levels and elevation in the activity levels of the GST and GPx. Maximum activity levels of GST and GPx were observed in 30d Ca supplemented fish kidney. Our findings elucidate the beneficial role of Ca as vital supplement in reducing the Cd burden in the fresh water fish, Oreochromis mossambicus *(Tilapia)*.

Key words: Cadmium, Oxidative stress, Calcium supplementation, Tilapia.

INTRODUCTION :

In aquatic ecosystem, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Among heavy metals, Cd is considered as a major aquatic heavy metal pollutant in many parts of the world (Wu *et al*., 2002). Cd is non biodegradable and once discharged into water bodies it can either be absorbed on sediment particles or accumulated in aquatic organisms. Fish may absorb Cd from surrounding water and food, which accumulated in various tissues in significant amounts (Mohamad, 2008). This metal buildup in food chain and are responsible for chronic illness and death in aquatic organisms, which probably move up to top of the food chain (Kosai et al., 2011). Therefore, fish might prove a better material for detecting metals contaminating the fresh water ecosystems.

Cd can enter fresh waters due to human activities such as mining and smelting of sulfide ores, fuel combustion and application of phosphate fertilizers or sewage sludge. It is taken up by algae, insects and passed through the food chain to fish, which can accumulate Cd in their tissues as gills, liver and kidney (Chowdhury et al., 2004; ASTDR, 2008). Consuming fish or other animals that have accumulated Cd may pose a threat to human health. Sub lethal and chronic concentrations of metals exerts their toxicity on fish by generating free radicals such as hydroxyl radical (OH), superoxide radical (O_2^-) and some non-radical reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2). These ROS can trigger oxidative damage to proteins, nucleic acids and lipids (Siwela et al., 2009).

To attenuate the negative effects of ROS, fish posses an antioxidant defense system like other vertebrates that utilizes enzymatic and non enzymatic mechanisms. The most important antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-tranceferase (GST). More oxidative state or any imbalance between production and degradation of ROS in animal tissues may cause lipid peroxidation, plasma membrane alterations and inactivation of enzymes (Vinodhini and Narayanan, 2009).

Micronutrients in the diet influences heavy metal toxicity (Peraza, 1998). Cd interacts with the essential micronutrients like Ca, zinc (Zn) and iron (Fe). It interacts with these elements and influences Cd bioaccumulation and the enzyme activities of metabolic pathways. It has been well demonstrated that Ca in the water and diet protects fish against the uptake of Cd (Wood et al., 2006). Cd is taken up at

Obaiah Jamakala And A. Usha Rani ,"Calcium Impact On Cadmium Induced Alterations In Selected Oxidative Stress Enzymes In The Fresh Water Teleost, *Oreochromis Mossambicus (Tilapia)*." Golden Research Thoughts Vol-3, Issue-4 (Oct 2013): Online & Print

the gills by Ca transporters and elevated waterborne Ca competes with Cd for these transporters (Niyogi and Wood, 2004). Elevated Ca down regulates the Ca transporters on the gills (Galvez and Wood, 2007) and reduces the subsequent Cd uptake.

In this study, an attempt has been made to determine the impact of Ca supplementation on Cd induced alterations in the selected tissue oxidative stress enzymes of fresh water fish Oreochromis mossambicus (Tilapia). The activities of GST, GPx and the changes in levels of LPO have been determined and the possible generation of heavy metal Cd induced oxidative stress is discussed.

MATERIALS AND METHODS

Chemicals:

Cadmium as cadmium chloride ($CdCl_2$) 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 ± 20C. 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 two groups. First group served as control and other group as experimental. The experimental group was exposed to sub lethal concentration of $CdCl_2$ i.e., 5 ppm ($1/10^{th}$ of LC50 / 48 hrs) daily for 7, 15 and 30 days (d) time periods. Then 15d Cd exposed animals were subjected to Ca supplementation (i.e., 1 ppm) for again 7, 15 and 30d long sojourn.

After specific time period's fish were sacrificed and tissues like kidney, brain and gill were isolated and used for the assay of oxidative stress enzymes.

Lipid peroxidation (LPO):

The lipid proxides were determined by the TBA method of Ohkawa et al., (1979). The tissues were homogenized (20 % W/V) in 1.5 % KCl. To 1 ml of tissue homogenate, 2.5 ml of 20 % trichloro acetic acid (TCA) was added and the contents were centrifuged at 3500 g for 10 min. Residue was dissolved in 2.5 ml of thiobarbituric acid (TBA) was added and the samples were kept in a hot water bath for 30 min. The samples were cooled and malondialdehyde (MDA) was extracted with 4 ml of n- butanol and read at 530 nm in a spectrophotometer against the reagent blank. Trimethoxy pentane (TMP) was used as external standard. The values were expressed in µ moles of malondialdehyde formed / gm.

Glutathione- S – transferase (GST): EC: 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 Xg for 15 min at 40C and the supernatant was again centrifuged at 16,000 Xg for 1 hour at 40C. 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 / minute.

Glutathione Peroxidase (GPx): EC: 1.11.1.9

Glutathione Peroxidase (GPx) was determined by a modified version 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 rpm for 10 min at 00C 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 X103 M cm-1 used to determine the activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

DATA ANALYSIS

The data was subjected to statistical analysis such as mean, standard deviation (SD) and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 16) software. All values are expressed as Mean ± SD of 6 individual samples. Significant differences were indicated at $p < 0.05$ level.

RESULTS

The amount of oxidative damage was recorded by measuring the LPO by the TBARS in the selected tissues of both control and experimental fishes (Table.1; Fig.1). A significant increase in LPO was observed in all the selected tissues exposed to Cd for the above said time intervals. Gill tissue documented highest LPO (16.074± 0.089 µ moles of MDA formed/gm wet wt. of the tissue) followed by kidney and brain respectively in 30d Cd exposure. However with Ca supplementation there was reversal in the Cd induced LPO. The LPO was reduced with Ca supplementation in all the selected tissues during all the time intervals of supplementation. Maximum reduction was found in brain (5.077±0.106 µ moles of MDA formed/gm wet wt. of the tissue) under 30d Ca supplementation (Fig.2).

The specific activity levels of GST were

determined in the selected tissues of Cd exposed fish and also in controls (Table.2; Fig.3). Cd significantly reduced the activity levels of GST in the kidney (0.285 ± 0.015 µ moles of thioether formed / mg protein / min) showed higher decrement than the other tissues of 30d exposed fish. Low level of GST activity was found in gill tissue (0.233±0.018 µ moles of thioether formed / mg protein / min). When the tissues were supplemented with Ca, GST activity levels were significantly elevated in all the experimental tissues (Fig.4). Maximum elevation in GST activity was found in the kidney tissue of 30d Ca supplemented fish. (0.584 ± 0.013 µ moles of thioether formed / mg protein / min).

Table.3 shows decreased activity levels of GPx in all the selected tissues of fish during 7, 15 and 30d Cd exposure. Maximum depletion of GPx activity was observed in 30d Cd exposed gill (0.301 ± 0.056 µ moles of NADPH oxidized / mg protein / min) (Fig. 5). When 15d Cd exposed fish was supplemented with Ca at the above said time intervals, the GPx activity levels significantly increased in all the test tissues (Fig.6). Maximum increase in GPx activity was observed in gill (0.609 ± 0.016 µ moles of NADPH oxidized / mg protein / min) of 30d Ca supplemented fish. Lowest increment was found in brain tissue (0.414 ± 0.016 µ moles of NADPH oxidized / mg protein / min).

DISCUSSION

Fresh water teleost is highly susceptible to water borne Cd toxicity. The process of Cd poisoning in fishes is not clear; however, various mechanisms were reported for the process of Cd poisoning such as disrupted respiratory process, kidney damage and whole body burden (Cirillo et al., 2012). Cd can cause significant metabolic alterations such as the enzymatic activities (Siraj Basha and Usha Rani, 2003), membrane transport mechanisms and injuries of biological systems at different levels (Pratap and Wandelaar Bonga, 1990). General mechanisms of oxidative toxicity in both mammalian and piscine systems are similar, with comparable lesions and responses serving as markers of oxidative stress (Pratama Yoga, 2002). Impaired antioxidant defenses by cells can be the result of the inhibitory effects of Cd on various enzymes which inturn causes the cells to be more susceptible to oxidative attacks. Toxic forms of activated oxygen react with cellular components resulting in protein oxidation, oxidative DNA damage as well as LPO, further leads to the inactivation of enzymes, disruption of membranes, mutations and ultimately cell death (Elia et al., 2003).

In the present study the generation of free radicals was measured indirectly by measuring the content of malondialdehyde (MDA) and activity levels of GST and GPx. Our studies revealed that LPO was enhanced during Cd exposure (Fig.1), which may be due to interaction of Cd with membrane phospholipids and thus causing membrane disorganization and further fragility. Similar findings were observed in common carp (Cyprinus carpio) by Jia et al., (2010). MDA elevation induced by Cd was also reported in different fish tissues (Dallinger *et al*., 1997).

The activity levels of GST were significantly reduced in all the test tissues of Cd exposed fish for 7, 15 and 30d time periods (Fig.3), the decreased activity could be due to its inactivation by over production of ROS induced by Cd. Among the selected tissues brain is susceptible to oxidative damage through free radicals as it contains high amount of unsaturated lipids and utilizes about 20% of total oxygen demand of the body. Pretto et al., (2010) also reported a significant decrease in GST activity in Cd exposed catfish, Rhamdia quelen. According to Han et al., (2006) addition of Cd suppresses the GST activity in liver and kidney tissues of growing pigs.

Cd depleted GPx activity levels in the test tissues alterations of sub lethal concentration of 7, 15, and 30d exposure. GPx catalyzes the conversion of H202 to water and reduces tissue injury from LPO directly. In the present study there was a significant decrease in GPx activity in the kidney, gill 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. 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.

Ca serves as second messenger for the control of important activities in many cells. Supplemented Ca activates Ca dependent enzymes which inturn may mobilize the transport of Cd from the metal sensitive organelles to other sub cellular components (Ng *et al*., 2009). Interaction between Cd and Ca occur at several sites in the body including cellular mechanisms. In the present study Ca supplementation significantly decreased the enhancement of LPO caused by Cd. Lower level of LPO means a lower degree of membrane damage. GST and GPx activity levels were significantly increased after Ca supplementation. So Ca might have alleviated the Cd induced membrane damage and aids in protecting the cell.

From the available literature it is clearly evident that there are other possible interactions of essential trace elements as nutrients with the secondary mechanisms of toxicity of non essential metals (Peraza, 1998). Therefore Ca supplementation in our study was effective in ameliorating the Cd body burden of the Oreochromis mossambicus. Cd in the form of divalent cation (Cd^{2+}) enters the cells via channels in the apical membrane and the transepithelial calcium (Ca^{2+}) is comparatively inhibited by Cd. At the gill surface Cd^{2+} competes with Ca^{2+} for high affinity Cd binding sites (Niyogi and Wood, 2004) and the cumulative effect of this process can cause disruption of Ca homeostasis in fish under Cd exposure. It is clear from the present study that the toxicity of Cd is affected by Ca which inturn reduces the toxic effects of Cd through competitive inhibition at the gill surface. The non toxic Ca competes with the toxic metal Cd for the same binding sites. If Ca occupies these sites the lamellae are protected from deterioration. Increased Ca levels in the medium resulted in a slower transfer of Cd from the gills to blood and the rate of Cd accumulation was lowered in kidney, gill and other tissues. Similar findings were reported in rainbow trout by Zohouri et al., (2001) and Baldisserotto, (2004). Ng et al., (2009) also stated that elevated Ca levels generally protects against toxicity of Cd in juvenile rainbow trout.

As there have not been many studies designed

specifically to address the effects of micronutrient status on toxicity from exposure to non essential heavy metals like Cd particularly in the aquatic animals like fish, perhaps this study might be of significance in reducing the risk assessment for heavy metals by simple dietary supplementation of micronutrient Ca. It could be therefore concluded that Ca supplementation might play a vital role in reducing the Cd tissue burden of fresh water fish Oreochromis mossambicus, thereby reducing the risk of potential hazards to human health.

ACKNOWLEDGEMENTS

The authors are highly thankful to CSIR, New Delhi for the financial support rendered with the award of Major Research Project (No. 37(1450)/10/EMR-II, dated 09-12-2010) to Prof. A. Usha Rani, Dept. of Zoology, Sri Venkateswara University, Tirupati.

REFERENCES

I.Agency for toxic substances and disease registry (ASTDR) (2008). ASTDR toxicological profiles for cadmium. Draft for public comment: public comment period ends on February 26, 2009: accessed November 19, 2010., 2008 http://www.astdr.cdc.gov/tox profiles/tp.asp?id= 48&tid=15.

II.Baldisserotto, B., Kamunde, C., Matsuo, A. and Wood,C.M. (2004). A protective effect of dietary calcium against acute waterbone cadmium uptake in rainbow trout. Aquat. Toxicol 67: 57-73.

III.Chowdhury, M.J. and Wood, C.M. (2007). Renal function in the fresh water rainbow trout after dietary cadmium acclimation and water borne cadmium challenge. Comp. Biochem. Physiol.C145: 321-332.

IV.Cirillo, T., Cocchieri, R.A., Fasano, E., Lucisano, A., Tafuri, S., Ferrante, M.C., Carpene, E., Andreani, G. and Isani, G. (2012). Cadmium accumulation and antioxidant responses in Sparus aurata exposed to waterborne cadmium. Arch. Environ. Contam. Toxicol 62: 118-126.

V.Dallinger, R., Egg, M., Köck, G. and Hafer, R. (1997). The role of metallothionein in cadmium accumulation of Arctic char (Salvelinus alpinus) from high alpine lakes. Aquat.Toxicol 38: 47-66.

VI.Elia, A.C., Galarini, R., Taticchi, M.I., Dorr, A.J. and Mantilacci, L. (2003). Antioxidant responses and bioaccumulation in Icttalurus meals under mercury exposure. Ecotoxicol. Environ. Safe 55: 162-167.

VII.Flohe, L. and Gunzler, W.A. (1984). Assays of glutathione peroxidase. Methods Enzymol 105: 114-121.

VIII.Galvez, F. and Wood, C.M. (2007). Interactions of waterborne and dietary cadmium on the expression of calcium transporters in the gills of rainbow trout: Influence of dietary calcium supplementation. Aquat. Toxicol 84: 208-214.

IX.Habig, W.H., Pabast, M.J. and Jackoby, W.B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem 249: 7130-7139.

X.Han, X.Y., Xu, Z.R., Wang, Y.Z. and Huang, Q.C. (2006). Effect of cadmium on lipid peroxidation and activities of antioxidant enzymes in growing pigs. Biological Trace Element Research 110: 251-263.

XI.Huang, D.J., Zhang, Y.M., Song, G., Long, J., Liu, J.H. and Ji, W.H. (2007). Contaminants induced oxidative damage on the carp Cyprinus carpio collected from the Upper Yellow River, China. Environ. Monit. Assess 128: 483-488.

XII.Jia, X., Zhang, H. and Liu, H. (2010). Low levels of cadmium exposure induce DNA damage and oxidative stress in the liver of oujiang coloured common carp Cyprinus carpio. Var. Color. Fish Physiol. Biochem. DOI: 10. 1007/ s 10695-010-94165.

XIII.Kosai, P., Jiraungkoorskul, W., Synsatayakul, A. and Jiraungkoorskul, K. (2011). Efficacy of calcium reducing lead toxicity in hematology of Oreochromis niloticus. Science Alert DOI: 10.3932/jfas.2011.346-355.

XIV.Mohamed, F.A.S. (2008). Bioaccumulation of selected metals and histopathological alterations in tissues of Oreochromis niloticus and Lates niloticus from Lake Nasser, Egypt. Global Veterinia 2(4): 205-218.

XV.Ng, T.Y.T., Klink, J.S. and Wood, C.M. (2009). Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? Aquat. Toxicol 91: 75-86.

XVI.Niyogi, S. and Wood, C.M. (2004). Kinetic analyses of waterborne Ca and Cd transport and their interactions in the gills of rainbow trout (Oncorhynchus mykiss) and yellow perch (Perca flavescens), two species differing greatly in acute waterborne Cd sensitivity. J.Comp.Physiol. B Biochem. Syst. Environ. Physiol 174: 243-253.

XVII. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358.

XVIII.Peraza, M.A., Ayala – Fierro, F., Barber, D.S., Casarez, E. and Rael, L.T. (1998). Effects of micronutrients on metal toxicity. Environ. Health. Perspect 106: 203-216.

XIX.Pratama Yoga, G. (2002). Effect of cadmium exposure on lipid peroxidation in tilapia. Limnotek 9 (1): 23-32.

XX.Pratap, H.B. and Wendelaar Bonga, S.E. (1990). Effects of waterborne cadmium on plasma cortisol and glucose in the cichlid fish, Oreochromis mossambicus. Comp. Biochem. Physiol. C 95: 313-317.

XXI.Pretto, A., Loro, V.L., Baldisserotto, B., Pavanato, M.A., Moares, B.S., Menezes, C., Cattaneo, R., Clasen, B., Finamor, I.A. and Dressler, V. (2010). Effects of water cadmium concentrations on bioaccumulation and various oxidative stress parameters in Rhamdia quelen. Arch. Environ. Contam. Toxicol DOI: 0.1007/s00244-010-9586-2.

XXII.Siraj Basha, P. and Usha Rani, A. (2003). Cadmium induced antioxidant defense mechanism in fresh water teleost Oreochromis mossambicus (Tilapia). Ecotox. Environ. Safe 56: 218-221.

XXIII.Siwela, A.H., Nyathi, C.B. and Naik, Y.S. (2009). Metal accumulation and antioxidant enzyme activity in C. gariepinus, cat fish and O. mossambicus, tilapia, collected from Lower Mguza and Wright Dams, Zimbambwe. Bull. Environ. Contam. Toxicol 83: 648-651.

XXIV.Vinodhini, R. and Narayanan, M. (2009). Biochemical changes of antioxidant enzymes in common carp (Cyprinus carpio L.) after heavy metal exposure. Turk. J. Anim. Sci 33(4): 273-278.

XXV.Wood, C.M., Franklin, N.M. and Niyogi, S. (2006). The protective role of dietary calcium against cadmium

uptake and toxicity in freshwater fish: An important role for the stomach. Environ. Chem 3: 389-394.

XXVI.Wu, S.M., Chou, Y.Y. and Deng, A.N. (2002). Effects of exogenous cortisol and progesterone on metallothionein expression and tolerance to waterborne cadmium in tilapia (Oreochromis mossambicus). Zoological Studies 41(1): 111-118.

XXVII.Zohouri, M.A., Pyle, G.G. and Wood, C.M. (2001). Dietary Ca inhibits waterborne Cd uptake in Cd- exposed rainbow trout, Oncorhynchus mykiss. Comp. Biochem.Physiol130C (2/3): 347-356.

Table –1: Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in different tissues of Cd exposed *O. mossambicus* before and after Ca supplementation.

S. No	Tissue	Control	Cd exposed			Ca Supplementation		
			7d	15d	30d	7d	15d	30d
1	Kidney	9.515 ± 0.038	11.570 ± 0.026	15.068 ± 0.013	21.692 ± 0.019	13.947 ± 0.010	13.091 ± 0.018	10.085 ± 0.087
2	Brain	4.596 ± 0.027	6.069 ± 0.013	10.656 ± 0.090	13.681 ± 0.019	9.738 ± 0.019	8.613 ± 0.010	5.077 ± 0.106
3	Gill	5.027 ± 0.024	7.933 ± 0.015	11.636 ± 0.024	16.074 ± 0.089	10.646 ± 0.099	9.231 ± 0.170	6.925 ± 0.145

All values are expressed as Mean ± SD of 6 samples.
All values are significant at P<0.05

Table –2: Changes in GST activity (µ moles of thioether formed / mg protein / min) in different tissues of Cd exposed *O.mossambicus* before and after supplementation with Ca.

S. No	Tissue	Control	Cd exposed			Ca Supplementation		
			7d	15d	30d	7d	15d	30d
1	Kidney	0.618 ± 0.022	0.563 ± 0.023	0.420 ± 0.013	0.285 ± 0.015	0.489 ±0.014	0.502 ± 0.014	0.584 ±0.013
2	Brain	0.329 ± 0.020	0.276 ±0.014	0.217 ±0.009	0.161 ±0.032	0.265 ±0.015	0.297 ±0.010	0.306 ±0.116
3	Gill	0.427 ± 0.023	0.372 ±0.035	0.305 ±0.008	0.233 ±0.018	0.369 ± 0.009	0.402 ±0.012	0.408 ±0.008

All values are expressed as Mean ± SD of 6 samples.
All values are significant at P<0.05.

Table –3: Changes in GPx activity (µ moles of NADPH oxidized / mg protein / min) in different tissues of Cd exposed *O.mossambicus* before and after Ca supplementation.

S. No	Tissue	Control	Cd exposed			Ca Supplementation		
			7d	15d	30d	7d	15d	30d
1	Kidney	0.832 ± 0.010	0.731 ± 0.007	0.588 ± 0.014	0.404 ± 0.016	0.647 ± 0.013	0.647 ± 0.009	0.794 ± 0.012
2	Brain	0.443 ± 0.011	0.402 ± 0.022	0.327 ± 0.021	0.231 ± 0.036	0.369 ± 0.025	0.392 ± 0.025	0.414 ± 0.016
3	Gill	0.674 ± 0.012	0.533 ± 0.011	0.439 ± 0.016	0.301 ± 0.056	0.483 ± 0.015	0.546 ± 0.014	0.609 ±0.016

All values are expressed as Mean ± SD of 6 samples.
All values are significant at P<0.001

Fig. 1: Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in selected tissues of Cd exposed O. mossambicus (Tilapia).

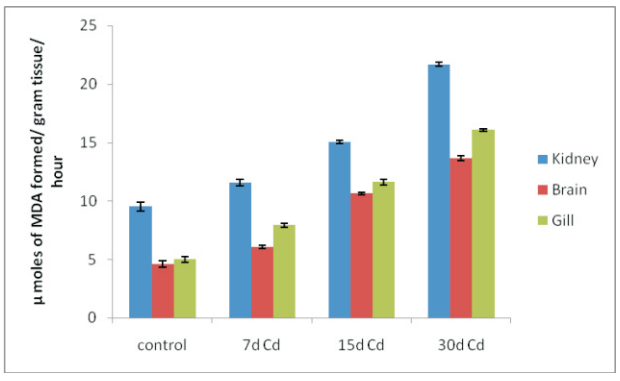


Fig. 2: Changes in LPO levels (µ moles of MDA formed / gram tissue / hour) in selected tissues of Cd exposed O. mossambicus (Tilapia) after Ca supplementation.

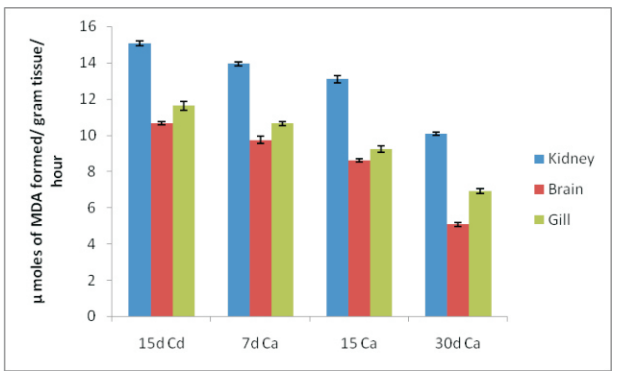


Fig. 3: Changes in GST activity (µ moles of thioether formed / mg protein/ min) in selected tissues of Cd exposed O. mossambicus (Tilapia).

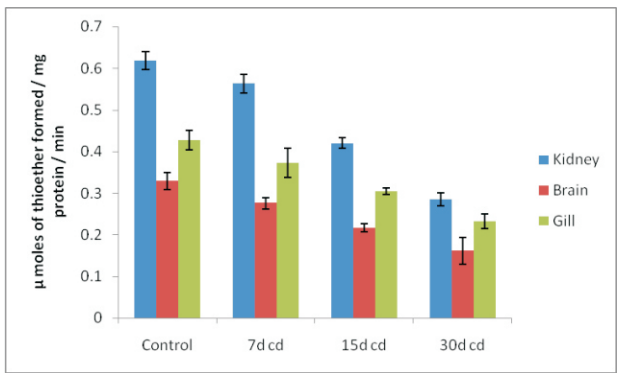


Fig. 4: Changes in GST activity (µ moles of thioether formed / mg protein/ min) in selected tissues of Cd exposed O. mossambicus (Tilapia) after Ca supplementation.

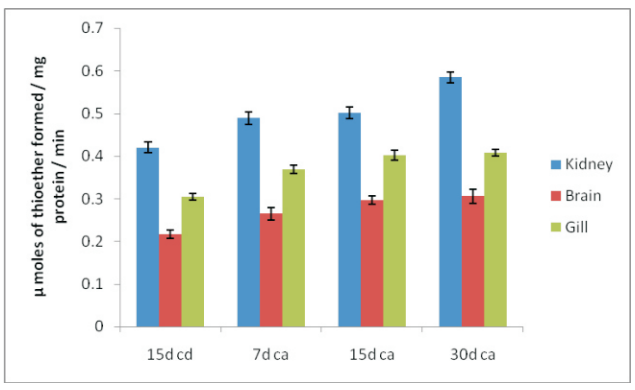


Fig.5: Changes in GPx activity (µ moles of NADPH oxidized /mg protein/min) in selected tissues of Cd exposed O. mossambicus (Tilapia).

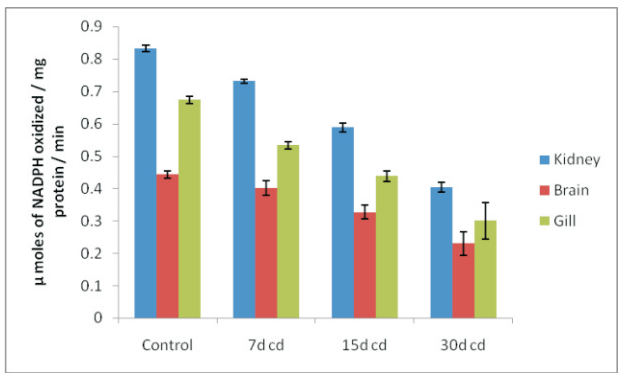
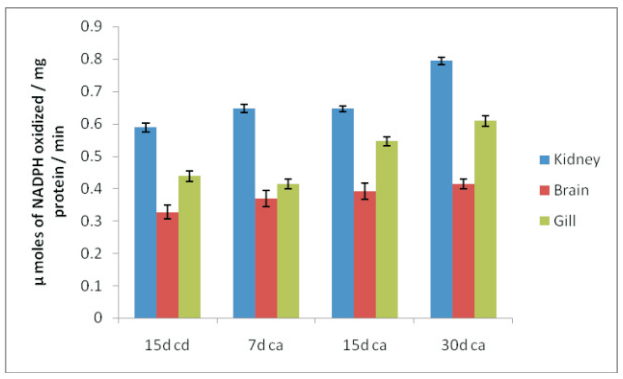


Fig. 6: Changes in GPx activity (µ moles of NADPH oxidized /mg protein/min) in selected tissues of Cd exposed O. mossambicus (Tilapia) after Ca supplementation.



OBAIAH JAMAKALA
Department of Zoology, Sri Venkateswara University, Tirupati A.P. India