

Influence of Selenium on Lead Caused Variations of Phosphofructokinase in different Brain Regions of Fresh Water Teleosts

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Abstract: *In the present investigation the author made an attempt to investigate the sub-lethal effect of Lead in presence of selenium as chelating agents on differential distribution of phosphofructokinase in various brain regions (cerebrum, diencephalon, cerebellum and medulla oblongata) in L.rohita, C.batrachus and C.punctatus on a comparative approach under chronic studies.*

Keywords: Selenium, Lead, Fishes species, Enzyme Phosphofructokinase and Detoxification.

1. Introduction

Many industrial and agricultural processes have contributed to the contamination of fresh water by release and accumulation of heavy metals in the environment. Heavy metal redox cycling and interaction with organic pollutants contribute to oxidative stress from aquatic pollution. Oxidative stress resulting from reactive oxygen species (ROS) has been identified as a causative agent in a number of pathologies in fish. to counteract the impact of ROS generated by organic pollutants such as heavy metals, endogenous defensive mechanisms exist in fish, including various antioxidant defense enzymes such as superoxide dismutases and glutathione S-transferase. However, vitamins and glutathione S-transferase. However, vitamins and minerals (anti-oxidants) from nutrition play an important role in countering oxidative stress.

Since industrial revolution era, heavy metal waste has been increasing rapidly. Various toxic metal species are produced by industrial activities and fossil fuel consumption. These are accumulated through the food chain, leading to both ecological and health troubles.

Lead is one of the most dangerous, important, and potential toxic metal in marine ecosystem. The toxicity level may affect growth, and enzyme activity and even respiration of organism. The storage of metals by detoxifying mechanism makes them available for assimilation by the biota and bio-magnification along the aquatic food chains (Muhaemin, 2005).

As toxic agents, proper treatment and disposal of used and expired germicides and pesticides are becoming an ever increasing challenge to scientists. Deactivation, such as neutralization of detoxification of germicides and pesticides and the safe disposal of these agents have become crucial for protection of soil, ground and surface water, to ensure a safe environment for human and animal life, for natural microbial ecosystems and for meeting the environmental protection codes (Igwe et al 2009). Deactivation of these agents is scientifically challenging for several reasons:

Chelating agents have been used clinically as antidotes for both acute and chronic metal poisoning (9). Chelators not only enhance excretion but also decrease the clinical signs of toxicity by preventing metals from binding to cellular target molecules. This can be achieved when the chemical affinity of complexing agent for the metal is higher than the affinity for the bioligands. Effective chelation therapy depends on the lipophilic character of the chelating agent and its effectiveness in successful removal of the metal from intracellular spaces where metal is firmly bound. Meso-2,3-dimercaptosuccinic acid (DMSA) is a water soluble, safe and effective chelator and also recommended for clinical use to reduce metal burdens (10).

2. Material and methods

Alive, healthy disease free and active *Labeo rohita* (Ham), *Clarias batrachus* (Linn) and *Channa punctatus* (Bloch.) 120-130 gm and 18-20 cm. (standard length) were obtained from few selected local ponds to avoid ecological variation acclimatized in the laboratory condition for a period of seven days and were subjected for various exposures and investigations.

3. Determination of lethal and sub lethal concentration

Lethal and sub-lethal concentrations of lead was determined on *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* by the Probit analysis method (Finney 1971). Higher concentration of lead were used and slowly reduced the amount of concentration to know the Lc 50/100 value for 45 days exposure.

The *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* (120 -130 gm) of 18-20 cm (standard length) were taken separately and kept in twenty groups and each group contain twenty fish species. No food was given to the above fish species during this period (15,30 and 45 days). The first group of *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* were exposed to sub lethal and lethal concentration of lead preparation of tissue extract and enzyme assays were describe else where (Colowick & Kaplon, 1975 and Shaffi and Habbibula, 1977). Statistical analysis - The experiments

were repeated at least seven times separately to subject the data for analysis of variance (ANOVA).

4. Result

The phosphofructokinase was compartmentalised and subjected the effect of sub lethal lead and also subjected the sub lethal effect of lead in presence of selenium on long term basis. The sub lethal exposure of lead led to optimum fall in phosphofructokinase in diencephalon at 15 days followed by cerebrum at 30 days, medulla oblongata at 45 days and cerebellum at 45 days in *L.rohita*, than in *C.batrachus* (diencephalon at 15 days, cerebrum at 30 days, medulla oblongata at 30 days and cerebellum at 45 days) and in *C.punctatus* (diencephalon at 15 days, cerebrum at 45 days, medulla oblongata at 15 days and cerebellum at 15 days).

The sub-lethal impact of lead in presence of selenium led to maximum fall in phosphofructokinase in diencephalon at 30 days, followed by cerebrum at 15 days, medulla oblongata at 45 days and cerebellum at 45 days in *L.rohita*, in comparison to *C.batrachus* (diencephalon at 30 days, cerebrum at 15 days, medulla oblongata at 45 days and cerebellum at 30 days) and in *C.punctatus* (diencephalon at 30 days, cerebrum at 15 days, medulla oblongata at 45 days and cerebellum at 45 days) (Tab and Graph 1).

5. Discussion

From the above research investigations it is well established beyond doubt that metal/pesticide/ toxicant/pollutant exposure caused oxidative stress followed by free radical formation and reactive oxygen species irrespective of experimental animal as a mammal /fish/ any other lower animal species. Similar situation must have taken place even in the present investigation too when *L.rohita*, *C.batrachus* and *C.punctatus* was exposed to sub-lethal and lethal concentrations of lead alone and in presence of selenium on various brain regions (cerebrum, diencephalon, cerebellum and medulla oblongata) through the functions of phosphofructokinase on a comparative basis.

For enzyme (mentioned above) the highest fall was recorded in diencephalon followed by cerebrum, medulla oblongata and cerebellum irrespective of acute and chronic investigations. The more fall of the enzymes in diencephalon indicates more oxidative stress than cerebrum, medulla oblongata and cerebellum.

Due to oxidative stress the metabolic rate and the total metabolism is in disparity and diencephalon being the metabolic centre, being autonomous nervous system centre and reproductive centre got disturbed and the fall in phosphofructokinase due to stress and recovery in presence of selenium would indicate that chelating agents detoxifying role in the enzyme level should be interpreted at this juncture.

The cerebrum being the largest part not only in area but also its coordinating with other sensory regions of the body and regulating various vital processes like learning memory, thought, feelings, decisions, making and few other voluntary activities too got affected due to sub-lethal and lethal concentrations of lead and recovery when studied the effect of sub-lethal and lethal lead through phosphofructokinase emphasizes that chelating agents like selenium are helping to reduce the body burden of toxicant and provide protection to organism through the metabolism.

The third highest enzyme fall was recorded in medulla oblongata which is a vital involuntary centre (centre for reflex, respiration, circulation, excretion, vomiting and glycolysis) and the fall was ascribed to the sub-lethal and lethal lead and the recovery was related to the role of selenium indicates that chelating agents are capable of trapping the active centers of lead and the recovery of the above said enzymes may be realized on the above school of thought.

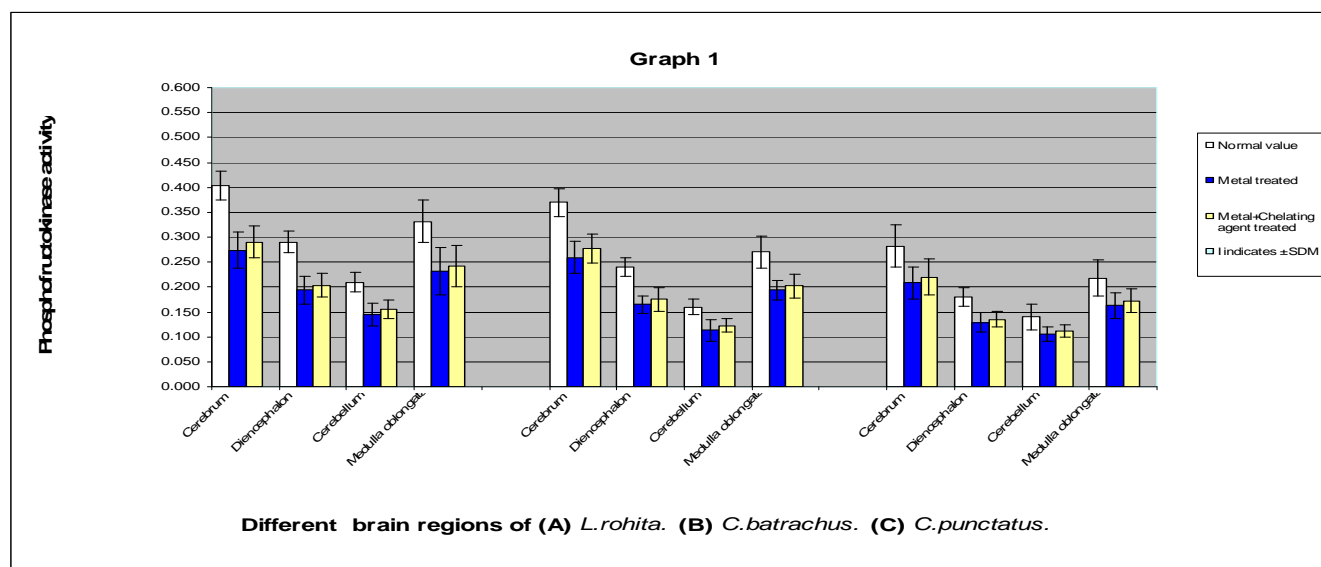
The last highest fall in phosphofructokinase was recorded in cerebellum and (cerebellum is a voluntary centre, balance, posture, soul, protein synthesis and reflex) this is due to less receptor sites for metal like lead and the recovery in enzymes is due to the chelation therapy of selenium.

Tab - 1 Influence of selenium on lead induced Phosphofructokinase variations in various brain regions in three freshwater teleosts-chronic studies

Name of Species	Regions of the brain	Lead exposure					Lead exposure with selenium				
		Control	15 days	30 days	45 days	% of F/R	Control	15 days	30 days	45 days	% of F/R
<i>Labeo rohita</i> (Ham.)	Cerebrum	0.404 ±0.029	0.376 ±0.032	0.282 ^{cd} ±0.022	0.274 ^{cd} ±0.036	32	0.404 ±0.029	0.346 ±0.038	0.302 ^e ±0.018	0.290 ^e ±0.032	28
	Diencephalon	0.290 ±0.022	0.231 ±0.024	0.212 ^e ±0.018	0.194 ^d ±0.028	33	0.290 ±0.022	0.276 ±0.029	0.228 ^e ±0.022	0.203 ^e ±0.024	30
	Cerebellum	0.210 ±0.019	0.192 ±0.024	0.178 ±0.019	0.145 ^e ±0.023	31	0.210 ±0.019	0.196 ±0.018	0.182 ±0.016	0.155 ±0.019	26
	Medulla oblongata	0.332 ±0.042	0.302 ±0.028	0.284 ±0.034	0.232 ^e ±0.047	30	0.332 ±0.042	0.302 ±0.026	0.286 ±0.036	0.242 ^e ±0.041	27
<i>Clarias batrachus</i> (Linn.)	Cerebrum	0.370 ±0.028	0.356 ±0.022	0.288 ^{cd} ±0.012	0.259 ^{cd} ±0.032	30	0.370 ±0.028	0.308 ±0.014	0.292 ^e ±0.022	0.277 ^e ±0.029	25
	Diencephalon	0.240 ±0.019	0.194 ±0.024	0.188 ±0.021	0.165 ^e ±0.018	31	0.240 ±0.019	0.224 ±0.029	0.192 ±0.019	0.175 ±0.024	27
	Cerebellum	0.160 ±0.016	0.149 ±0.014	0.138 ±0.018	0.113 ±0.021	29	0.160 ±0.016	0.150 ±0.019	0.135 ±0.016	0.123 ±0.014	23
	Medulla oblongata	0.270 ±0.032	0.246 ±0.030	0.214 ±0.034	0.194 ^d ±0.020	28	0.270 ±0.032	0.256 ±0.028	0.242 ±0.038	0.202 ^e ±0.024	25
<i>Channa punctatus</i> (Bloch)	Cerebrum	0.282 ±0.042	0.256 ±0.032	0.232 ±0.022	0.208 ^e ±0.032	26	0.282 ±0.042	0.240 ±0.038	0.232 ±0.024	0.220 ^e ±0.036	22
	Diencephalon	0.180 ±0.019	0.159 ±0.022	0.145 ±0.016	0.129 ±0.019	28	0.180 ±0.019	0.164 ±0.016	0.142 ±0.012	0.135 ±0.016	25
	Cerebellum	0.140 ±0.026	0.124 ±0.010	0.116 ±0.012	0.106 ±0.014	24	0.140 ±0.026	0.132 ±0.012	0.126 ±0.010	0.112 ±0.012	20
	Medulla oblongata	0.218 ±0.036	0.198 ±0.032	0.182 ±0.021	0.163 ^e ±0.026	25	0.218 ±0.036	0.202 ±0.024	0.190 ±0.018	0.172 ±0.024	21

Values are mean ± SDM of 7 Replicates. The data was subjected to test of ANOVA and Superscripts a-e indicates that p> 0.01, 0.02, 0.03, 0.04 & 0.05.

*F-Fall /R-Rise



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References

- [1] A.P.H.A. 1995: Standard methods for examination of water & waste water. 25th Edi. Washington, D.C 10.
- [2] Abid,M.S., Siddiqui,S.S. and Rizvi,S.J. 2004 : Effect of selenium on lead induced changes in the rat brain nucleic acid and protein. *Poll.Res.*23:619-622.
- [3] Archana,S., Injal and Raut,P.D. 2010 : Bioaccumulation of lead (Pb) and remediation by calcium chelation therapy in the gills and mantle of fresh water bivalve, *Lamellidens marginalis*. *J. Ecophysiol. Occup. Hlth.* 10:21-26.
- [4] Ashour, Abdel-Rahiem,A., Yassin,M.M., Abu Aasi,N.M. and Ali,R.M. 2007 : Blood,serum glucose and renal parameters in lead-loaded albino rats and treatment with some chelating agents and natural oils. *Turk J.Biol.* 31 :25-34.
- [5] Banerjee,P., Bhattacharya,S.S., Bhattacharjee,N., Pathak,S., Belon,P. and Khuda-Buksh,A.R. 2009 : Ascorbic acid combats arsenic-induced oxidative stress in mice liver. *Ecotox. Environ. Safe.* 72: 639-649.
- [6] Bergan,T., Klaveness,J. and Aasen,A.J. 2001 : Chelating agents: *Intern. J. Exp. and Clin. Chemo.* 47(1) : 10-14.
- [7] Daniel,S., Limson,J.L., Dairam,A., Watkins,J.M. and Daya,S. 2004 : Through metal binding, curcumin protects against lead and cadmium -induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain.*J. Inorg. Biochem.* 98(2):266-275.
- [8] Das,S. and Kaviraj, A. 2000 : Cadmium accumulation in different tissues of common carp, *Cyprinus carpio* treated with activated charcoal, EDTA and single superphosphate. *Geobios.* 27:69-72.
- [9] El-Missiry,M.A. 2000 : Prophylactic effect of melatonin on lead-induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rats. *J. Biochem. Mol. Toxicol.* 14:57.
- [10] El-Shebly,A.A. 2009 : Protection of Nile tilapia (*Oreochromis niloticus*) from lead pollution and enhancement of its growth by α -tocopherol vitamin E. *Res. J. Fisheries and Hydrobiol.* 4 (1) : 17-21.
- [11] Fernandez, L.G. and Olalla, H.Y. 2000: Toxicity and bio-accumulation of lead & cadmium in marine protozoan communities: *Ecotoxicol. and Env. Safty.*47:266-276.
- [12] Flora, S.J.S., Flora,G., Saxena,G. and Mishra,M. 2007 : Arsenic and lead induced free radical generation and their reversibility following chelation. *Cell Mol. Biol.* 53(1):26-47.
- [13] Flora,S.J.S., Pandey,M. and Mehta,A. 2003 : Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. *Chem. Biol. Interact.* 145(3):267-280.
- [14] Flora,S.J.S., Saxena,G. and Mehta,A. 2007 : Reversal of lead induced neuronal apoptosis by chelation treatment in rats: Role of reactive oxygen species and intracellular Ca^{2+} . *J. Pharm. and Exp. Therap.* 322:108-116.
- [15] Gomez,M., Domingo,J.L., Llobet,J.M. and Corbella,J. 2006 : Effectiveness of some chelating agents on distribution and excretion of vanadium in rats after prolonged oral administration. *J. Applied Toxicol.*11 (3): 195-198.
- [16] Gupta,R. and Flora,S.J.S. 2005 : Therapeutic value of *Hippophae rhamnoides* against sub chronic arsenic toxicity in mice.*J. Med. Food.* 8:353-361.
- [17] Igwe,J.C., Ekwuruke,A., Gbaruko,B.C. and Abia,A.A. 2009 : Detoxification of copper fungicide using EDTA-modified cellulosic material. *African J. Biotechnol.* 8(3) : 499-506.
- [18] Jayaprakash,K. and ChinnaSwamy,P. 2005 : Effect of spirulina & Liv -52 on cadmium induced toxicity in albino rats. *Indian J. Exp. Bio.* 43:773-781.
- [19] Johri,S., Shukla,S. and Sharma,P. 2002 : Role of chelating agents and antioxidants in beryllium induced toxicity. *Indian J. Exp. Bio.* 40 : 575-582.
- [20] Joshi,D.N., Flora,S.J.S. and Kalia,K. 2006: Characterization of arsenic detoxification mechanism in micro organisms and their response to some thio

- chelator. *J. Cell and Tissue Res.* 6:30.
- [21] Kannan,G.M. and Flora,S.J.S. 2004 : Chronic arsenic poisoning in the rats:Treatment with combined administration of succimers and an antioxidants. *Ecotoxicol. Environ. Safety.* 58: 37-43.
- [22] Kosnett,M.J. 2010 : Chelation for heavy metals (arsenic, lead and mercury): Protective or perilous. *Clin.Pharmacol. Ther.* 88 (3) : 412-415.
- [23] Kumar,P., Prasad, Y., Patra,A.K. and Sarup,D. 2007 : Levels of cadmium and lead in tissues of freshwater fish (*Clarias batrachus*) and chicken in western UP (India). *Bull. Environ. Contamin. and Toxicol.* 79:396-400.
- [24] Mishra,D. and Flora,S.J.S. 2008 : Quecetin administration during chelation therapy protects arsenic-induced oxidative stress in mice. *Biol. Trace Elem. Res.* 122(2): 137-147.
- [25] Moh.Muhaemin. 2005 : Chelating ability of crab shell particles and extracted acetamido groups (Chitin and Chitosan) from *Portunus* sp. to lead.(Pb²⁺). *J Coastal Develop.* 9 :1-7.
- [26] Palaniappan,PL. RM., Krishnakumar,N. and Vadivelu,M. 2008 : FT-IR Study of the effect of lead and the influence of chelating agents, DMSA and D-Penicillamine on the biochemical contents of brain tissues of *Catla catla* fingerlings. *Aquat. Sci.* 70:314-322.
- [27] Palaniappan,PL.RM, Sabhanayakam,S., Krishna Kumar,N. and Vadivelu,M.2008 : Morphological changes due to lead exposure and the influence of DMSA on the gill tissues of the fresh water fish, *Catla catla*. *Food and Chem. Toxicol.* 7 : 2440-2444.
- [28] Palaniappan,PL.RM., Krishnakumar,N. and Vadivelu,M. 2009 : Bio-accumulation of lead and the influence of chelating agents in *Catla catla* fingerlings. *Envir.Chem.Lett.*7(1):51-54.
- [29] Saxena,G. and Flora,S.J.S. 2004 : Lead -induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. *J Biochem. and Mol.Toxicol.* 18(4) : 221 -233.
- [30] Shaffi,S.A., Prakash,B.V.B.S., Nichat,A. and Raibole,M. 2007 : Microbes as antidote to detoxify the metal caused toxicity in some fresh water teleosts. *Nat. Sem. on New Horizons in Toxicol. and Sustenance of Life* 64(Abs.).
- [31] Sivaprasad,R., Nagaraj,M. and Varalakshmi,P. 2003 : Combined efficacies of lipoic acid and meso-2,3-dimercaptosuccinic acid on lead-induced erythrocyte membrane lipid peroxidation and antioxidant status in rats. *Hum. Exp. Toxicol.*22 (4): 183-192.
- [32] Sohini and Rana,S.V. 2007 : Protective effect of ascorbic acid against oxidative stress induced by inorganic arsenic in liver and kidney of rat. *Indian J. Exp. Bio.* 45:371-375.
- [33] Soudini,N., Sefi,M., Amara,I.B., Boudwara,T. and Zaghal,N. 2010: Protective effects of selenium on chromium (VI) induced nephrotoxicity in adult rats.*Ecotoxicol and Environ. Safety.*73:671- 678.