GLUCOSE POST EXPOSURE RECOVERY FROM LEAD INTOXICATED FRESH WATER FISH ANABAS TESTUDINEUS

Shaikh Afsar

Department of Zoology, Vivek Vardhini College, Jambagh, Hyd (A.P)

E-mail of Corresponding author: sk.afsar3@gmail.com

Abstract
Glucose are important amongst the several molecules available in the cells, Carbohydrates play an important role in the cellular process. Under extreme stress conditions, carbohydrate metabolite such as glucose have been known to act as the energy supplier in metabolic pathways and biochemical reactions. In the present investigation fish treated with an equitoxic dose of 10 ppm of lead nitrate and lead acetate intoxicated fish after a period of 15 days of exposure a batch from lead nitrate exposed fish and a batch from lead acetate exposed fish were transferred to lead-free water. Fishes were scarified on 1, 4, 8, 12 and 15 days for the analysis of recovery pattern in tissues viz. liver, muscle, kidney, gill and brain. It is found that lead intoxicated fishes were recovered after 15 days depends upon physical condition of the fish.

Keywords: Carbohydrate; lead; anabas

1. Introduction
Now a day's industrialization is increasing rapidly in our country. The modern industries are making use of various heavy metals such as iron, steel, copper, nickel, platinum and lead. Among the different types of pollutions, chemical pollution appears to be the major type which threatens the living systems very extensively. Among the different habitats aquatic environment is the major target of pollution. Most of the heavy metals are natural constituents of the aquatic environment. Some of them are biologically essential, but some metals like cadmium, lead and mercury are highly hazardous to aquatic biota and normally occur in low concentration. It is clearly known the common forms of lead poisoning result from the mining, processing and commercial dissemination of lead. The primary source of lead exposure to animals are contaminated soils, lead paints that remain on older structures, water from plumbing systems that contain lead, and lead based products, especially batteries, used crankcase oil, and linoleum. The lead containing gasoline fumes from automobile exhausts constitute the chief and wide spread source of lead contamination in urban environments. A Major source of lead to waterfowl and other wildlife is spent lead shot, bullets, cartridge, and lead sinkers used in sport fishing.

2. Materials and Methods
2.1 Material: Anabas testudineus which is selected as test species in the typical representative of Anabantoid fishes in South India. It is fresh water, euryhaline and eurythermal teleost. These fishes are well known for their air breathing ability, and they can survive out of water in moist air for six days. Slender fish with large scales, spines on gill cover, Scales on the head rigidly attached to the skull bone, strongly ctenoid, Grey brown to silver colour, with a dark spot on the base of caudal fin. Omnivorous feeds on macrophyte vegetation, different invertebrates, small fish. No parental care. No sexual dimorphism, the fish will spawn in the evening between plants, and the egg hatch in 24-36 hours. This fish is extremely adaptable, and can be kept in any water, soft, hard, alkaline and acidic, even in brackish water. They are nick-named as ‘Climbing perch’ since they ascent banks and even lower branches of trees. These fish are cultured in ponds in and around Kolleru belt and they have a very good commercial value, because of their nutritive value and taste. The fish Anabas scandens has been selected as the...
test animal because of its euryhaline and eurythermal nature, and unique position in food chain. They are quite sturdy and ideally suited for experimentation in laboratory for longer periods.

2.2 Methods: Biochemical assays were made in different tissues from both experimental (exposed to toxicant) and Normal (toxicant free) fishes. Fish approximately of same size and weight were selected and grouped into 6 batches. 2 batch of fish served as controls, 2 batches of fish were exposed to lead nitrate and the remaining two batches were exposed to lead acetate for a period of 15 days. After a period of 15 days of exposure a batch from lead nitrate exposed fish and a batch from lead acetate exposed fish were transferred to lead-free water and scarified at the same intervals to observe the recovery responses. In all the experiments, a minimum of six individual observations were made. The values of different parameters were expressed as mean with their standard error. Significance of the values obtained were tested using student ‘t’ test. The glucose content in the tissues were estimated by the method \(^{14}\). The 10% (W/V) homogenate was prepared carefully in 10% TCA. 1 ml of the TCA supernatant was used for the estimation of carbohydrates. For the estimation of glycogen, 5 ml of absolute alcohol was added to 1 ml of TCA extract and incubated for three hours at 37\(^{\circ}\) C. After the precipitation was complete, the tubes centrifuged at 3000 rpm for 15 minutes. The supernatant was rejected. The glucose pellet was dissolved by addition of 1 ml of double distilled water. Reagent blank was prepared by pipetting 1 ml of double distilled water in a separate tube. At this point 5 ml of freshly prepared anthrone reagent added to each tube with vigorous, constant blowing and the tubes were capped and cooled to room temperature. Then they were immersed in a boiling water bath to a depth, a little above the level of the liquid in the tubes and boiled for 15 minutes and then cooled to room temperature. The color developed was read at 620 nm against the blank. The values were expressed as mg glucose per gram wet weight of the tissue.

3. Result
The glucose content was reduced progressively throughout the exposure span indicating its utilization during lead toxicity. The depletion of glucose was time-dependent and tissue-specific. After 1st of exposure, maximum amount of reduction was found in liver (-29.48%, \(P < 0.001\) for lead nitrate and -39.53%, \(P < 0.001\) for lead acetate) followed by kidney (-33.95% for lead nitrate and -37.65% for lead acetate both significant at \(P < 0.05\)), gill (-34.38% for lead nitrate, -35.82% for lead acetate, both arte significant at \(P < 0.001\)), muscle (-26.56 % for lead nitrate and -29.19 % for lead acetate significant at \(P < 0.001\) ) and brain (9.86% for lead nitrate and -14.55% for lead acetate, the values are statistically insignificant).

After 4\(^{th}\) day of exposure the depletion was statistically significant in all the tissues at \((P < 0.001; P < 0.05)\). Maximum amount of reduction was recorded in the liver (-50.12% for lead nitrate and -51.61% for lead acetate) followed by kidney (-49.28% lead nitrate and -51.01% for lead acetate) gill (-44.55% lead nitrate, -45.51% for lead acetate) and brain (-16.09% lead nitrate, -21.46% for lead acetate).

For 8\(^{th}\) day of exposure all the tissues except gill exhibited a reduction statistically significant at \(P < 0.001\). Maximum amount of depletion was recorded in kidney (-56.45% for lead nitrate, -52.96% for lead acetate) followed by liver (-50.08% for lead nitrate, 48.74% for lead acetate), gill (-49.47% for lead nitrate and -49.73% for lead acetate), muscle (-34.25% for lead nitrate, -35.80% for lead acetate) and brain (-25.48% for lead nitrate, -28.85% for lead acetate).

On 12\(^{th}\) day of exposure the depletory response was significant at \((P < 0.01 \text{ and } P < 0.001)\). Maximum depletion was found in liver (-59.79% for lead nitrate, -62.08% lead acetate) followed by gill (-59.80% for lead nitrate, -60.80% for lead acetate), muscle (-52.67 for lead nitrate, -51.56% for lead acetate), kidney (-48.22% for lead nitrate,-52.66 for lead acetate) and brain (-30.52% for lead nitrate, -29.72% for lead acetate).

On 15\(^{th}\) day of exposure glucose content was found depleted maximum in all the tissues when
compared to preceding exposure periods. Maximum depletion was found in kidney (-59.89% lead nitrate, significant at P < 0.01, and -61.93% for lead acetate and significant at P < 0.001) followed by liver (-59.74% for lead nitrate and -60.47% for lead acetate, significant at P < 0.001), gill (-58.73 % for lead nitrate,- 60.32% for lead acetate significant at P < 0.01), muscle (-51.53% for lead nitrate; -52.81% for lead acetate), and brain (-37.41% for lead nitrate and -40.48% for lead acetate).

During recovery period, depletion in glucose content was gradually reduced in all the tissues maximum recovery was achieved on 15th day of recovery period. The percent reduction over controls were statistically insignificant at the end of the 15th day for liver, muscle, kidney and gill while in the brain % difference between control and experimental were statistically insignificant on 12th day of recovery period (Table and Fig.1).

4. Discussion
In the present investigation the tissue glucose level was found depleted throughout the exposure period. The depletion was progressive in all the tissues during entire exposure period, indicating persistent and cumulative inhibitory effects of lead5. The magnitude of depletion was more in fish treated with lead acetate. The inorganic lead i.e. lead nitrate is not greatly soluble in cell membranes and owing to its reduced mobility, accumulates in lesser amounts when compared to organometallic form, and therefore produces less toxic effects. The investigations of 1,6 also reveal that organic lead is more toxic than inorganic form. Tissue-specific depletion of glucose as observed in the present study may be due to its rapid utilization to meet the energy demands under toxic manifestations. The tissues-specific responses may be due to differential accumulation and movement of lead in tissues which depends on various factors like age, temperature, perfusion, vascularity and residual blood volume59. Some observations revealed that liver and kidney retain more amount of lead incomparison to heart muscle and brain.3,10,15. Hence it can be speculated that excess retention of this metal in these organs was responsible for pronounced decrease in the glucose and glycogen content.

Another possible reason for the observed depletion of glucose content be due to imbalanced release of hormones as observed in some fish models under heavy metal intoxication11.

A significant decrease in tissues glucose level was observed during the period of 15 days in mercuric chloride intoxication 7. The heavy metals are reported to accumulate in the pancreatic tissue and known to cause an impairment in the regulatory release of insulin and glucagons21. Impairment of insulin release during heavy metal toxicosis probably check the absorption of glucose into the tissues causing its depletion in tissues and raising the blood glucose levels18. Loss of glucose content was recorded in the tissues of fish Prochilodus lineatus after lead intoxication 12 and cadmium intoxication 16 and in Channa punctatus exposed to mercuric chloride13 and Cadmium chloride8. The loss in the glucose in the tissues may be due to lead induced malabsorption of glucose through the intestines17.

References
1. Bondy, C. Stephen (1984). Neurotoxicity of organic and inorganic lead compounds. Abstract ITRC-IBRO Symposium, Neurotoxic substances and their impact on Human Health. ITRC Lucknow, India.
2. De Francisco N, J.D.Ruiz Troya, and E.I. aguera (2003). Lead and lead toxicity in domestic and free living birds. Avian pathol, 32:3-13.
3. Gbem TT, Balogun J, K Lawal, FA., Annune PA, (2001). Trace metal accumulation in Clarias gariepinus (Teugels) exposed to sublethal levels of tannery effluent. The Sci. of the total Environ. 271(1-3)1-9.
4. Hammond P.B. (1969) Lead poisoning. “An old problem with a new dimension. In : Blood FR (ed) Essays in Toxicology. Academic Press New york. (1): 115-155
5. Harrison, P.R., W.R.Matson and J.W.Winchester. (1971).Atoms. Environ.pp 5613
6. Hodson, P.V. (1979) Factors affecting the sublethal toxicity of lead to fish. Proc. of heavy metals Conference. London U.K. pp.135-138.
7. Kannan K , Rajasekaran G and Raveen R (2010). Heavy metal mercuric chloride induced biochemical changes in the freshwater catfish Mystus vitattus J. Ecotoxicol. Environ. Monit. 20 (1) 97-99.
8. Krishna Murthy, G. (1989). In vivo effects of cadmium chloride on some aspects of physiology and histology of fish Channa punctatus (bloch) doctoral dissertation, Osmania University, Hyderabad, India
9. Mali, R.P, (2002). Studies on some aspects of physiology of freshwater female crab Brytelphusa guerini with special reference to inorganic pollutants. P.hD Thesis submitted to Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra.
10. Mazher Sulthana and Boja Rajan, A. (2007). Histopathological lesions induced by copper sulphate, lead nitrate and zinc sulphate in the liver, intestine and kidney of Tilapia mossambica J.Aqua.Biol., Vol. 22(1) 189 - 192.
11. Mathan Ramesh, Manoharan Saravanan and Chokkalingam Kavitha (2009). Hormonal responses of the fish, Cyprinus carpio, to environmental lead exposure. African Journal of Biotechnology Vol. 8 (17) 4154-4158
12. Martinez, C. B. R, Nagae, M. Y, Zaia, C. T. B. V, Zaia, D. A. M. (2004) Acute morphological and physiological effects of lead in the neotropical fish Prochilodus lineatus Braz. J Biol. 64 No.4
13. Malla Reddy, M. (1979). Physiological responses of fish to pollution. Doctora dissertation, Osmania University, Hyderabad, India
14. Nicholas, V., Carroll, Robert, W., Longley and Joseph, H. Roe (1956). Determination of glycogen in liver and muscle by use of Anthrone reagent J. Biochem., 22:583-593.

15. Olojo, E.A.A., Olurin, K. B., Mbaka, G. and Oluwemimo, A. D. (2005). Histopathology of the gill and liver tissues of the African catfish Clarias gariepinus exposed to lead. African Journal of Biotechnology Vol. 4 (1) 117-122.
16. Sadath Sulthana (1990). “Physiological responses of freshwater fish Anabas scandens (cuvier) during exposure and recovery periods after CdCl2 intoxication. Doctoral dissertation submitted to Osmania University
17. Sastry K.V. and Agrawal, M.K. (1979) Effects of lead nitrate on the activity of a few enzymes in the kidney and ovary of Heteropneustes fossilis, Bull. Environm. Contam. Toxicol 22: 55-59
18. Usha rani and R. Rama Murthi (1989). Histopathological alterations in the liver of teleost Tilapia mossambica in response to cadmium toxicity Ecotoxicol Environ. Safety 17(2) 221-226.
19. Villarrel-trevino, C.M., A. Vilegas-Navorro (1987)” Differentia acc of lead by soft tissues of rabbit” Bull. Environ. Contam. Toxicol 1987. 39: 334-342.
20. Waldner, C.S, Checkley, B, Blakley, C, Pollock, and B, Mitchell (2002). Managing lead exposure and toxicity in cow-calf herds to minimize the potential for food residues J.Vet.Diagn.Invest. 14:481-486.
21. Whittle, E., R.L.Singhal, M. Collins, and P.D. Hrdina (1983). Effect of subacute low level lead exposure on glucose homeostatis. Res. Commun. Chem. Pathol. Pharmol. 40(1), 141-154.

Figure – 1: Glucose content in the tissues of Anabas testudineus during exposure and recovery period after Lead nitrate and Lead acetate intoxication
### TABLE 1

**GLUCOSE CONTENT IN THE TISSUES OF ANABAS TESTUDINEUS DURING EXPOSURE AND RECOVERY PERIODS AFTER LEAD NITRATE AND LEAD ACETATE INTOXICATION**

| Tissues | Exposure Period (in days) | Recovery period (in days) |
|---------|---------------------------|---------------------------|
|         | 1  | 4  | 8  | 12 | 15 | 1  | 4  | 8  |
| Liver   |    |    |    |    |    |    |    |    |
| C       | 22.39±0.15 | 24.97±0.42 | 26.24±0.24 | 25.82±0.39 | 24.40±0.69 | 23.75±0.65 | 21.52±0.49 | 23.30±0.72 | 20.92±0.35 | 24.35±0.73 |
| A       | 15.72±0.72 | 12.42±0.05 | 10.10±0.33 | 9.42±0.72 | 9.09±0.72 | 10.79±0.72 | 12.60±0.82 | 13.11±0.49 | 17.75±0.25 | 20.74±0.30 |
| B       | 13.54±0.54 | 12.05±0.57 | 9.75±0.52 | 8.25±0.72 | 8.34±0.72 | 10.84±0.49 | 12.02±0.23 | 16.49±0.49 | 17.15±0.49 | 22.92±0.17NS |
| %N      | -35.23 | -21.61 | -48.74 | -66.08 | -32.47 | -56.46 | -44.14 | -32.19 | -18.02 | -5.87 |
| Muscle  |    |    |    |    |    |    |    |    |
| C       | 6.86±0.01 | 7.28±0.29 | 8.34±0.27 | 8.34±0.95 | 7.92±0.15 | 8.32±0.15 | 6.98±0.04 | 8.58±0.04 | 6.89±0.09 | 7.39±0.79 |
| A       | 6.14±0.08 | 4.72±0.45 | 4.71±0.41 | 4.25±0.84 | 3.88±0.41 | 4.12±0.41 | 4.90±0.22 | 4.46±0.18 | 4.90±0.06 | 7.21±0.55NS |
| B       | 5.82±0.15 | 4.65±0.91 | 4.26±0.56 | 4.35±0.55 | 3.70±0.77 | 3.57±0.24 | 5.06±0.52 | 4.86±0.15 | 5.09±0.04 | 7.14±0.75NS |
| %N      | -29.19 | -35.30 | -49.59 | -51.66 | -53.21 | -49.36 | -39.80 | -26.65 | -14.89 | -3.33 |
| Kidney  |    |    |    |    |    |    |    |    |
| C       | 5.21±0.23 | 5.23±0.23 | 5.34±0.11 | 5.39±0.32 | 5.17±0.23 | 5.25±0.25 | 3.99±0.11 | 3.64±0.44 | 5.51±0.45 | 3.95±0.45 |
| A       | 2.14±0.06 | 1.75±0.14 | 1.62±0.06 | 1.75±0.59 | 1.58±0.48 | 1.45±0.22 | 1.62±0.52 | 1.85±0.45 | 3.05±0.66 | 1.32±0.75NS |
| B       | 2.02±0.07 | 1.69±0.20 | 1.75±0.14 | 1.62±0.12 | 1.50±0.29 | 1.39±0.29 | 1.67±0.12 | 1.82±0.05 | 2.09±0.01 | 3.25±0.33NS |
| %N      | -6.82 | -6.85 | -6.77 | -5.91 | -6.67 | -6.02 | -4.39 | -6.77 | -5.98 | -6.41 |
| Gill    |    |    |    |    |    |    |    |    |
| C       | 3.09±0.34 | 3.15±0.45 | 3.17±0.38 | 3.15±0.33 | 3.08±0.03 | 3.18±0.09 | 3.63±0.03 | 2.38±0.03 | 2.63±0.09 | 2.99±0.09 |
| A       | 2.10±0.07 | 2.15±0.07 | 2.10±0.05 | 2.08±0.04 | 2.06±0.03 | 2.03±0.03 | 2.06±0.03 | 2.10±0.03 | 2.35±0.05 | 2.55±0.02NS |
| B       | 0.56±0.05 | 0.74±0.05 | 0.53±0.05 | 0.39±0.05 | 0.42±0.05 | 0.39±0.05 | 0.26±0.05 | 0.27±0.05 | 0.22±0.05 | 0.27±0.05 |
| %N      | -25.6 | -25.8 | -25.7 | -25.6 | -25.6 | -25.6 | -25.6 | -25.6 | -25.6 | -25.6 |
| Beam    |    |    |    |    |    |    |    |    |
| C       | 2.15±0.15 | 2.05±0.11 | 2.08±0.03 | 2.04±0.03 | 2.02±0.05 | 2.04±0.03 | 2.15±0.09 | 2.15±0.09 | 2.15±0.09 | 2.15±0.09 |
| A       | 1.92±0.03 | 1.72±0.05 | 1.55±0.05 | 1.47±0.05 | 1.38±0.05 | 1.29±0.05 | 1.38±0.05 | 1.47±0.05 | 1.29±0.05 | 1.38±0.05 |
| B       | 1.12±0.03 | 1.04±0.04 | 1.02±0.04 | 1.00±0.04 | 0.98±0.04 | 0.96±0.04 | 1.00±0.04 | 1.02±0.04 | 1.02±0.04 | 1.02±0.04 |
| %N      | -14.55 | -21.46 | -28.85 | -38.72 | -40.48 | -32.00 | -15.51 | -9.88 | -5.26 | -1.43 |

Values are mean ± S.E. of six observations and are expressed as mg glucose / gm wet weight of tissues. C= control, A= lead nitrate, B = Lead acetate, %N= percent variation from control. Values are significant at *P < 0.001, **P < 0.01, ***P < 0.05, NS = not significant.