MALATE DEHYDROGENASE ACTIVITY POST EXPOSURE RECOVERY FROM LEAD INTOXICATED FRESHWATER FISH ANABAS TESTUDINEUS

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Abstract
Malate dehydrogenase activity are important amongst the several enzymes available in the cells, Carbohydrates play an important role in the cellular process. Under extreme stress conditions, carbohydrate enzyme such as Malate dehydrogenase 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 scarificed 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 days 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 testudineus 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 Malate dehydrogenase activity in the tissues were estimated by the method of 6. 10% (W/V) homogenate of liver, kidney, gills, muscle and brain were prepared in 0.25 M ice cold sucrose solution and centrifuged at 2,500 rpm for 15 minutes. The clear supernatant was used for the assay of the enzyme activity. The incubation mixture contained in 2 ml volume: 100 µ moles of phosphate buffer (pH 7.4), 2 µ moles of INT, 50 µ moles of sodium malate in phosphate buffer (pH 7.4), 0.1 µ mole of NAD and 0.5 ml of homogenate. The reaction was initiated by adding enzyme extract. After incubating the reaction mixture at 37° C for 30 minutes, the reaction was stopped by the addition of 5 ml of glacial acetic acid. Zero time controls were maintained for each tissue separately by adding 5 ml of glacial acetic acid to the reaction mixture, prior to the addition of homogenate supernatant. Colour was extracted by adding 5 ml of toluene and kept overnight in the refrigerator. The extracted formazan was measured at 495 nm in a spectrophotometer. The enzyme activity was expressed as µ moles of formazan formed/mg protein/hour.

3. Results and Discussion
3.1 Results: During 1st and 4th day of exposure periods malate dehydrogenase activity was increased. Later on a steady fall in activity was noticed. On 1st day maximum activity was elicited in muscle tissue(+7.94% for lead nitrate and +12.69% for lead acetate; P < 0.01) followed by gill (6.25% for lead nitrate and +10.79% for lead acetate; P < 0.05), kidney (9.73 for lead nitrate and +10.62% for lead acetate; P < 0.05), liver (8.49% for lead nitrate and +9.81% P < 0.01) and brain (+5.56% for lead nitrate and +6.48% for lead acetate; P < 0.05). On 4th day maximum enhancement was noticed in muscle (+19.23% lead nitrate P < 0.01, +22.30% lead acetate P < 0.001) followed by liver (+17.55% lead nitrate, +19.22% lead acetate; P < 0.05), kidney (+17.39% lead nitrate, +18.26% lead acetate P < 0.05), brain (+10.00% lead nitrate, +9.15% acetate, P < 0.001). The gill tissue recorded a drop in MDH activity (-7.88% lead nitrate, -7.27% lead acetate, P < 0.05).
On 8th day of exposure inhibition in MDH activity was noticed in all the tissues. Maximum inhibition was seen in kidney (-16.36% lead nitrate, -18.18% lead acetate, P < 0.05) followed by liver (-15.10% lead nitrate, 18.49% lead acetate, P < 0.001), gill (-15.63% for lead nitrate, -16.25% lead acetate, P < 0.01), Muscle (-12.69% for lead nitrate P < 0.05; -14.93% lead acetate; P < 0.001) and brain (-4.71% lead nitrate P < 0.01 -5.49% acetate P < 0.001).
On 12th day of exposure depletion in MDH activity was noticed. The percent depletion ranged between -10.04% to -26.05% for lead nitrate and -12.91% to -25.20% for lead acetate. Kidney exhibited maximum inhibition (-26.05% for lead nitrate, P < 0.01 -23.53% for lead acetate P < 0.05) and minimum inhibition was noticed in brain (-10.04% for lead nitrate; -12.91% for lead nitrate; -12.91% for lead acetate; P < 0.001). The percent inhibition in MDH activity was statistically significant at P < 0.001, P < 0.01, P < 0.05.
On 15\textsuperscript{th} day of exposure maximum inhibition in MDH activity was notice in all the tissues. Out of all selected tissues kidney exhibited maximum drop in activity (-33.88% lead nitrate P < 0.05; -35.54% lead acetate P < 0.001) followed by liver (-32.39% lead nitrate P < 0.01 -34.08% lead acetate P < 0.001), muscle (-29.29% lead nitrate P < 0.01; -31.43% lead acetate P < 0.001), gill (-27.22% lead nitrate, -29.44% lead acetate; P < 0.05) and brain (-16.26% lead nitrate, -16.87% lead acetate; P < 0.001).

During recover periods inhibitory response was continued in all the tissues. However the inhibition was gradually reduced over remaining recovery periods. The responses of tissues varied from each other during recovery periods. Liver, muscle, kidney reached near normal levels of MDH activity on 15\textsuperscript{th} day, whereas brain and gill reached the control levels on 8\textsuperscript{th} day 12\textsuperscript{th} days respectively, by exhibiting statistically insignificant variation over control levels. On 15\textsuperscript{th} day of recovery the liver exhibited variation of -2.11% for lead nitrate and -1.58% for lead acetate where as muscle exhibited -1.56% and -3.13% variation for lead nitrate and lead acetate. Kidney reported to show -4.59% and -5.50% variation over control for these two salts of lead. The variation recorded in gill on 15\textsuperscript{th} day of recovery was +1.20% and -0.60% for lead nitrate and lead acetate. Brain exhibited minimum variation over control out of all tissues. (Fig.1).

3.2 Discussions: Malate dehydrogenase an another krebs cycle enzyme that catalyses the interconversion of malate and oxaloacetate in the present study almost exhibited similar response with that of SDH. However the initial elevation of MDH was found upto 4\textsuperscript{th} day of exposure whereas in SDH, the elevation was recorded on 1\textsuperscript{st} day of exposure. The initial rise in MDH activity indecates the higher metabolic rate during the early stages of lead toxicosis. The decrease in MDH activity in the present study could be attributed to low succinate oxidation as evidenced in the present study and due to binding of toxicant with enzyme protein\textsuperscript{7} and toxic effects produced by the lead on the tissues\textsuperscript{1}. The decrease in MDH activity was found to be a shift in the carbohydrate metabolism from aerobic to anaerobic type due to toxicity of copper sulphate toxicity\textsuperscript{9}. Time dependent and tissue specific changes in the MDH activity recorded in mammalian models during lead toxicity\textsuperscript{4} and fishes exposed to various metals\textsuperscript{10} lends support for the observed changes in MDH activity in the present study. The decreased SDH and MDH activity indicates the decreased operation of krebs cycle probably by limiting the flow of substrates into the cycle or impairment of mitochondrial organization\textsuperscript{11}, dissociated coupled phosphorylation and depressed rate of metabolism\textsuperscript{8}. A fall in the respiratory enzymes as observed in the present study indicates the impairment of vital energy yielding and phosphorylation process.

Figure – 1 Malate dehydrogenase activity in the tissues of \textit{Anabas testudineus} during exposure and recovery period after Lead nitrate and Lead acetate intoxication

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