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Bioassay of Cadmium and its Effect on Differential Distribution of Dehydrogenases in Different Brain Regions in *Labeo rohita* (HAM)

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Summary

The sublethal effect of cadmium on the specific activities of lactic, malic and succinic dehydrogenases in different brain regions in *Labeo rohita* (HAM) was assessed with reference to acute, chronic and recovery conditions. Cadmium enhanced succinic, malic and lactic dehydrogenases to a marked extent in the cerebrum from 0 to 12 h exposure. However, a subsequent fall of the above enzymes in some regions was recorded from 12 to 24 h. In chronic studies, the greatest decrease in succinic dehydrogenase was noted in the cerebrum (0 to 15 days) and the least reduction in the cerebellum (30 to 45 days) in comparison with malic and lactic dehydrogenase. In recovery studies an optimum rise in lactic, malic and succinic dehydrogenase was found in the cerebrum (30-45 days). In general, cadmium accumulation was highest in the cerebrum (12 h and 15 days) and least in the cerebellum (24 h and 45 days). This was markedly above the safety level in acute and chronic situations.

Key words

Labeo rohita • Brain • LDH • MDH • SDH • Freshwater teleost

Introduction

Heavy metals exert a broad spectrum of effects on aquatic organisms, especially on the fish species. These effects range from behavioral and ecological to physiological changes which in turn reflect on economically, nutritionally and culturally important fish species. Heavy metals are soft but highly toxic as they compete for binding with essential metals (Allen 1995, Chattopadhyay *et al.* 1995, Kumar *et al.* 1994, Sastry and Shukla 1994) and consequently they interfere with sulphydryl groups that play an important role for normal function of enzymes and structural proteins. Moreover, these sulphydryl groups assist in the metabolic pathways in various organs for better growth and optimum yield from body water (Shaffi 1999).

Reports on cadmium-induced stress are scarce and inconsistent with reference to energy-producing metabolic cycles in various viscera but not specific for

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any organ including the nervous system. Dehydrogenases play a pivotal role in dehydrogenation to release energy for the normal function of biological systems under various situations (Giesy and Weiner 1977).

In the present investigation the sublethal effect of cadmium was studied on the differential distribution of lactic, malic and succinic dehydrogenase and also on cadmium accumulation in the cerebrum, diencephalon, cerebellum and medulla oblongata in *Labeo rohita* (HAM) under acute, chronic and recovery conditions.

Methods

Disease free and healthy *Labeo rohita* (HAM) fish (total length 17-19 cm, 80-90 g weight) were obtained from a several selected local ponds and conditioned to the laboratory for 10 days. They received commercial meal before exposure.

Cadmium source and LD_{50} values

Analytical grade cadmium chloride (BDH) was obtained from a local chemist and dissolved in water to obtain the required dilution. LD_{50} value was determined after exposure to seven serial concentrations of cadmium and such concentration was repeated seven times with appropriate controls. The recorded death rate was subjected to probit analysis (Finney 1971) and the LD_{50} value was 8.5 mg of cadmium *per* liter.

Details of exposure

Fifty *Labeo rohita* were kept in two separate aquaria (4 × 1.5 × 2 feet containing 40 l water) marked as "A" (with sublethal cadmium as the treated group) and the other marked as "B" (without cadmium) for a period of 24 hours in the control group.

A similar number of *Labeo rohita* was kept in two different aquaria as the treated and control groups for a period of 45 days under chronic conditions. For the recovery studies, two groups of *Labeo rohita* were kept in two different aquaria as treated and untreated groups for 45 days. Seven *Labeo rohita* from the control and treated groups were dissected separately after 12 and 24 h for acute studies, 15, 30 and 45 days for chronic studies and 15, 30 and 45 days for recovery from the above mentioned groups. The fishes were fed a commercial pellet food *ad libitum* during the experimental period. The aquaria were examined every 4 h during the acute, chronic and recovery stages to remove the faeces, food remnants and dead fish. On each occasion, food was added to each aquaria till the fish stopped eating. They were not fed during the first 24 h and the last 24 h of the experiment to avoid and possible metabolic differences in food intake. Treated and untreated water (dechlorinated) were renewed every 24 h after feeding.

Brain compartmentation

Untreated and treated *Labeo rohita* were dissected at different intervals after the beginning of the experiment and their brain was dissected into the cerebrum, diencephalon, cerebellum and medulla oblongata. The details were described elsewhere (Kun and Abood 1949, Shaffi and Habibulla 1977, Shaffi 1995)

Enzyme and protein assays

The protein content was estimated according to Lowry *et al.* (1951). The assays on succinic, lactic and malic dehydrogenase were described in detail elsewhere (Srikanthan and Krishnamurthy 1955, Shaffi 1993).

Cadmium estimation

The brains of control and treated *Labeo rohita* were dissected out and subdivided into the cerebrum, diencephalon, cerebellum and medulla oblongata. The separated brain regions were weighed, lyophilized to constant weight and digested. The digested regions were analyzed for cadmium by atomic absorption spectrophotometry (Perkin-Elmer 2380) as described by Giesy and Weiner (1977) and A.P.H.A (1980). The experiment was repeated with seven separate samples for acute, chronic and recovery studies and the data were evaluated by ANOVA.

Results

Cadmium in sublethal concentrations elevated succinic, malic and lactic dehydrogenase activity to a marked extent in the cerebrum (between 0 to 12 h exposure) followed by medulla oblongata, diencephalon and cerebellum in *Labeo rohita.* A fall in the activity of these dehydrogenases (more pronounced in succinic followed by malic and lactic dehyrogenase) was found between 12 to 24 h exposure especially in the cerebrum, less so in other brain regions. The maximum decrease concerned the succinic dehydrogenase in the cerebrum (from 0 to 15 days) and medulla oblongata (15 to 30 days) when compared to malic dehydrogenase and lactic dehydrogenase (Table 1).

Table 1. Cadmium sublethal effect on differential distribution of dehydrogenases in diffeerent brain regions in *Labeo rohita* (HAM) under acute (A), chronic (B) and recovery (C) conditions.

Brain Regions	A CONT	12 h	24 h	B CONT	15 days	30 days	45 days	C CONT	15 days	30 days	45 days
Cerebrum											
LDH	0.121 ± 0.018	0.156 ± 0.015	0.060[ab] ± 0.015	0.119 ± 0.022	0.086 ± 0.017	0.079[a] ± 0.014	0.066[ab] ± 0.012	0.060 ± 0.010	0.069 ± 0.013	0.076 ± 0.018	0.112[a] ± 0.015
MDH	0.189 ± 0.030	0.212[a] ± 0.034	0.056[ab] ± 0.012	0.149 ± 0.026	0.089[a] ± 0.015	0.076[a] ± 0.019	0.075[a] ± 0.013	0.075 ± 0.018	0.084 ± 0.020	0.098 ± 0.022	0.141[a] ± 0.016
SDH	0.189 ± 0.026	0.272 ± 0.050	0.047[ab] ± 0.010	0.186 ± 0.034	0.122[a] ± 0.028	0.098[a] ± 0.016	0.074[ab] ± 0.018	0.073 ± 0.013	0.092 ± 0.024	0.115[a] ± 0.019	0.0178[ac] ± 0.026
Diencephalon											
LDH	0.086 ± 0.024	0.097 ± 0.030	0.059 ± 0.013	0.083 ± 0.020	0.079 ± 0.016	0.076 ± 0.014	0.069 ± 0.013	0.068 ± 0.015	0.074 ± 0.018	0.078 ± 0.014	0.080 ± 0.080
MDH	0.111 ± 0.019	0.131 ± 0.028	0.065[ab] ± 0.016	0.108 ± 0.026	0.099 ± 0.021	0.088 ± 0.018	0.072[a] ± 0.014	0.071 ± 0.019	0.088 ± 0.018	0.096 ± 0.021	0.103[a] ± 0.014
SDH	0.127 ± 0.020	0.161 ± 0.030	0.069[a] ± 0.023	0.124 ± 0.030	0.116 ± 0.018	0.102 ± 0.015	0.078[a] ± 0.012	0.077 ± 0.014	0.101 ± 0.016	0.116 ± 0.022	0.121[a] ± 0.024
Cerebellum											
LDH	1.049 ± 0.011	0.053 ± 0.010	0.042 ± 0.009	0.055 ± 0.012	0.051 ± 0.012	0.048 ± 0.010	0.040 ± 0.008	0.039 ± 0.007	0.046 ± 0.012	0.049 ± 0.009	0.052 ± 0.011
MDH	0.063 ± 0.014	0.076 ± 0.018	0.047 ± 0.012	0.064 ± 0.015	0.059 ± 0.013	0.052 ± 0.009	0.042 ± 0.011	0.043 ± 0.012	0.054 ± 0.010	0.060 ± 0.015	0.064 ± 0.012
SDH	0.083 ± 0. 022	0.097 ± 0.016	0.052 ± 0.010	0.083 ± 0.026	0.076 ± 0.017	0.068 ± 0.014	0.056 ± 0.014	0.057 ± 0.010	0.073 ± 0.022	0.080 ± 0.018	0.089 ± 0.014
Medulla oblongata											
LDH	0.067 ± 0.013	0.077 ± 0.017	0.039[a] ± 0.008	0.063 ± 0.014	0.058 ± 0.012	0.046 ± 0.008	0.042 ± 0.011	0.040 ± 0.013	0.043 ± 0.010	0.057 ± 0.012	0.059 ± 0.014
MDH	0.085 ± 0.019	0.105 ± 0.022	0.045[ab] ± 0.010	0.081 ± 0.018	0.075 ± 0.015	0.059 ± 0.013	0.050 ± 0.010	0.049 ± 0.014	0.052 ± 0.012	0.069 ± 0.020	0.078[a] ± 0.017
SDH	0.107 ± 0.024	0.145 ± 0.030	0.043[a] ± 0.009	0.102 ± 0.020	0.084 ± 0.019	0.064[a] ± 0.016	0.056 ± 0.012	0.054 ± 0.010	0.059 ± 0.013	0.082 ± 0.024	0.097[a] 0.030

Values (mmoles of formazon / mg protein / hour) are means ± S.D. of 7 separate replicates. Treated values significantly different from untreated controls: superscripts a, b, c, indicate P<0.001, P<0.01 and P<0.05, respectively.

Table 2. Cadmium accumulation in different brain regions in *Labeo rohita* (HAM) under acute (A), chronic (B) and recovery (C) conditions.

	A			B				C			
Regions of the brain	CONT	12 h	24 h	CONT	15 days	30 days	45 days	CONT	15 days	30 days	45 days
Cerebrum	0.018 ± 0.004	10.08[a] ± 2.02	14.40[ab] ± 2.56	0.020 ± 0.007	28.60[a] ± 3.26	35.80[ab] ± 3.94	48.80[abc] ± 3.40	43.60 ± 4.26	36.20[a] ± 6.12	18.00[ab] 2.40	0.360[abc] 0.054
Diencephalon	0.010 ± 0.003	2.06[a] ± 0.39	6.04[ab] ± 0.72	0.013 ± 0.004	4.20[a] ± 1.16	10.60[a] ± 1.88	13.50[abc] ± 2.96	12.34 ± 1.72	10.20[a] ± 2.18	5.55[ab] 1.24	0.019[abc] 0.006
Cerebellum	0.007 ± 0.002	1.52[a] ± 0.28	3.52[ab] ± 0.43	0.010 ± 0.003	2.18[a] ± 0.32	6.44[a] ± 1.26	8.26[abc] ± 1.30	7.24 ± 0.98	4.16[a] ± 0.74	2.26[abc] 0.82	0.012[abc] 0.004
Medulla oblongata	0.013 ± 0.005	6.20[a] ± 0.84	7.80[a] ± 0.98	0.015 ± 0.005	15.40[a] ± 1.96	21.20[ab] ± 2.64	26.62[abc] ± 4.12	24.14 ± 3.06	20.33[a] ± 2.84	7.15[ab] 1.39	0.028[abc] 0.008

Mean values (mg/g dry weight) are means ± S.D of 7 separate replicates. Treated values significantly different from untreated controls: superscripts a, b, c indicate that $P<0.001$, $P<0.01$, and $P<0.05$.

In the recovery studies, an optimum rise in lactic, malic and succinic dehydrogenase was noted in the cerebellum (0 to 15 days), diencephalon (9 to 15 days), medulla oblongata (15 to 30 days) and cerebrum (30 to 45 days, Table 1). Cadmium accumulation was highest in the cerebrum (12 h and 15 days) followed by medulla oblongata (12 h and 15 days), diencephalon (24 h and 30 days) and the cerebellum (24 h and 45 days). These values were significantly above the safety levels in the both acute and chronic groups (Table 2).

Discussion

The presence of heavy metals in the water and consequent changes in its physicochemical parameters lead to organ damage. Perturbed biochemical changes, anemia, neurological disorders, acute and chronic ailments, mortality of target and non-target organisms, consumption of intoxicated organisms may promote the accumulation of pollutants in vital organs of humans and may compromise their normal processes (Shaffi 1978, 1995, Shastry and Shukla 1994).

Cadmium accumulation was found especially in the cerebrum in comparison with medulla oblongata, diencephalon and the cerebellum in both acute and chronic studies in *Labeo rohita*. This indicates most probably the presence of more optimum binding sites on mitochondria and cytochromes in the cerebrum than in the medulla oblongata, diencephalon and cerebellum. Subsequent complex interaction with sulphydryl groups may play a major role in essential life processes.

After the 12 h exposure, the highest rise in succinic, malic and lactic dehydrogenases was noted in the cerebrum, while a lesser rise was recorded in medulla oblongata, diencephalon and the cerebellum. This reflects the metabolic response of *Labeo rohita* to provide extra energy for survival and to overcome the intoxication.

The greatest reduction in the activity of cerebral dehydrogenases in cadmium intoxication during acute and chronic studies are due to its maximum surface area, participation in all physiological processes and co-ordination with other organ system. The fall in succinic, malic and lactic dehydrogenases at 24 h and 15 to 45 days' exposure during acute and chronic studies indicate that intermediary metabolic processes are unable to cope with the cadmium-induced stress. This would consequently suppress oxidative phosphorylative processes that are concerned with energy utilization for maintaining the hydro-mineral balance (Ewers 1991, Shaffi 1995).

Under stressful conditions, the adrenals do help to elevate many organ activities including higher blood glucose levels and to maintain functional coordination under changed situations. Such mechanisms must have been involved and the fall in the above mentioned dehydrogenases in different brain regions can thus be explained in this way (Hutagalung 1989, Shaffi 1993).

The accumulated cadmium must have inhibited dehydrogenase synthesis and further formation of a complex with the enzyme protein by damaging cell organelles including mitochondria from which TCA cycle enzymes are synthesized. This must operate maximally in the cerebrum as compared with other investigated regions of the brain (Kumar *et al.* 1994, Allen 1995)

It is well known that acute and chronic exposure to cadmium reduces oxygen uptake by the whole body and different tissues significantly which turns the exposed animal towards anaerobic metabolism. Thus the activity of dehydrogenases is bound to decline in different brain regions and the present results are not unexpected (Ewers 1991).

The fall in dehydrogenase activity and the rise in cadmium concentration in different brain regions were found to be directly proportional. During the detoxication studies (treated fish kept in untreated water), the fast recovery in lactic, malic and succinic dehydrogenases were recorded in the cerebellum (0-15 days), diencephalon (0-15 days), medulla oblongata (15-30 days) and the cerebrum (30-45 days) in *Labeo rohita*. At the end of 45 days exposure, the cadmium concentration in different brain regions was just above the control value which indicates that the presence of cadmium in the protein part of enzyme still persists.

Cadmium accumulation in the organism may cause a deficit of zinc that influences the subsequent fall of dehydrogenase activity. This can account for the dehydrogenase variations in different brain regions of this fish and might be responsible for their impaired growth.

Cadmium accumulation in different brain regions under acute and chronic conditions was found to be much higher than the levels recommended by the German Federal Health Agency. The acceptable cadmium levels have also been reported for other fish species (Papoutsoglou and Abel 1988). Cadmium can thus be also harmful to a certain proportion of the human population. The recovery of lactic, malic and succinic dehydrogenases in different brain regions during detoxication processes may be due to revival of biological processes due to *de novo* synthesis.

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