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Impact of Chronic Lead Poisoning on the Hematological and Biochemical Profiles of a Fish, *Barbus conchonius* (Ham)

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Lead is a non-beneficial, non-essential element capable of causing hypertension, atherosclerosis, renal lesions, and neoplasia in laboratory animals (Morgan 1972). The primary source of lead exposure is via food, and the daily intake is estimated to be 200–300 µg for adults. In addition, absorption of lead may also occur from air (20 µg) and drinking water (< 20 µg) (Tsuchiya 1979). The contamination of natural waters by lead is mostly caused by a variety of anthropogenic activities related to increased mining operations and industrial uses of this metal. Adverse effects of lead poisoning in the fishes have been reported with references to both hematological and biochemical variables (Jackim 1973; Hodson 1976; Johansson-Sjobeck & Larsson 1979; Haux & Larsson 1982). Among the clinical symptoms described in fishes are anemia, basophilic stippling of erythrocytes, muscular atrophy, and degeneration of caudal fin and lordoscoliosis (Dawson 1935; Haider 1968). The aim of present investigation was to study the effects of chronically administered sublethal levels of inorganic lead on the hematological and biochemical profiles of a widely distributed freshwater fish, Barbus conchonius. The variables such as erythrocyte numbers, hemoglobin, hematocrit, mean corpuscular volume, blood glucose, glycogen in liver, skeletal muscles, and myocardium, and cholesterol in blood, liver, ovary, and testes were evaluated.

MATERIALS AND METHODS

Adults of Barbus conchonius (Order Cypriniformes) were hand-netted from the local lake (altitude 1937 m) and transported to laboratory for acclimatization under natural photo- and thermal-fluctuations. For chronic lead exposure, test fish were divided into four groups of 24 individuals each. Group I and II were submitted to a nominal 47.4 µg Pb^{2+}/L as nitrate salt (20% of the 96-h LC50) for 30 and 60 days, respectively. Control groups, III and IV, were maintained in the diluent water alone. The water used for experiments possessed the following average characteristics; pH 7.3, hardness 6 mg/L as $CaCO_3$, dissolved oxygen 8.1 mg/L, and temperature 16°C. Fish in all the groups were fed ad libitum throughout the exposure period except for two days prior to sacrifice. All control and experiment-

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al tanks were constantly aerated, and the water replaced biweekly. After the stipulated 30 and 60 days exposures, fish from control and experimental tanks were removed and sacrificed by decapitation without using anaesthesia. Blood samples were drawn into heparin coated tuberculin syringes. Routine hematological procedures were followed for erythrocyte count, Hb and Hct estimations. Photometric methods were used to evaluate blood glucose (Nelson 1944), glycogen (Seifter et al. 1950), and blood and tissue cholesterol (Zlatkis et al. 1953; Rosenthal et al. 1960). To compare the control and experimental mean values, data were subjected to t-test.

RESULTS AND DISCUSSION

The results of this study show considerable influence of chronic lead exposure on the peripheral blood and metabolite levels of Barbus conchonius. The erythrocyte counts showed an 18.8 and 23.6% decrease compared to control values after 30 and 60 days exposure, respectively. The reduction in Hb (14.9 and 11.8%) was accompanied by a lowering of Hct values (31.9 and 12.7%) at the two exposure periods. The MCV showed a reduction after 30 (16.0%) and 60 days (9.72%). The observed decrease in all the three red cell indices reflects a severe anemic state causally related to prolonged lead exposure. Among the various chronic effects of lead on the hematopoietic system, anemia is a common sign in people occupationally exposed to this metal (Tsuchiya 1979). An anemic response to experimental lead poisoning has also been demonstrated in fishes. The rainbow trout, Salmo gairdneri exposed to 300 μg Pb^{2+}/L for 30 days, suffered from anemia and decreased erythrocyte counts and mean corpuscular hemoglobin concentration (Johansson-Sjobeck & Larsson 1979). The Hct remained unchanged in this species related to an increase in the mean corpuscular volume. In the fish under report, on the other hand, anemia was characterised by a reduction in erythrocyte counts, Hb and Hct together with decreased mean corpuscular volume. Therefore, unlike the rainbow trout, anemia in B. conchonius appears to be microcytic. In the mammals, anemia due to lead is reported to be microcytic or normocytic but not macrocytic (Tsuchiya 1979).

Lead is known to inhibit hemoglobin biosynthesis and shorten the survival of red blood cells. An enzyme, delta-aminolevulinic acid dehydratase (delta ALA-D), is strongly inhibited by lead,thereby, blocking the formation of porphobilinogen from aminolevulinic acid (Dresel & Falk 1956). In fishes, the sensitivity of delta ALA-D to lead poisoning has been demonstrated in the liver and kidney of winter flounder, Pseudopleuronectes americanus and mummichog, Fundulus heteroclitus (Jackim 1973), in the erythrocytes of rainbow trout, Salmo gairdneri, brook trout, Salvelinus fontinalis, goldfish, Carassius auratus, pumpkinseed, Lepomis gibbosus (Hodson 1976), and in the erythrocytes, spleen, and renal tissue of rainbow trout, S. gairdneri (Johansson-Sjobeck & Larsson 1979). Other effects of plumbism on the peripheral blood include increased numbers of spindle cells, monocytes, and eosinophils in the catfish, Ameiurus nebulosus (Dawson 1935), and increased number of stippled erythrocytes and reticulocytes in fishes (Haider 1968)

Table 1. Hematological and biochemical status of Barbus conchonius exposed to 47.4 µg Pb^{2+}/L in soft water.

Variable	Exposure period (days)	Control	Pb-exposed	% change
Erythrocytes (x 10^6/mm³)	30	3.07 ± 0.13[a]	2.49 ± 0.12*	-18.8
	60	3.25 ± 0.19	2.48 ± 0.06**	-23.6
Hemoglobin (g/dl)	30	12.05 ± 0.24	10.25 ± 0.14	-14.9
	60	11.77 ± 0.12	10.37 ± 0.31	-11.8
Hematocrit (%)	30	55.77 ± 3.42	37.95 ± 3.53	-31.9
	60	54.45 ± 4.93	37.50 ± 2.03	-31.1
Mean corpuscular volume (µm³)	30	181.6[b]	152.4	-16.0
	60	167.5	151.2	-9.72
Blood glucose (mg%)	30	92.9 ± 5.56	143.4 ± 24.5**	+54.5
	60	91.5 ± 3.00	129.7 ± 6.93	+41.8
Liver glycogen (mg/g)	30	13.2 ± 0.61	10.2 ± 2.46**	-22.5
	60	11.0 ± 1.00	4.5 ± 0.61*	-59.0
Skeletal muscle glycogen (mg/g)	30	1.59 ± 0.20	1.00 ± 0.06	-37.1
	60	1.01 ± 0.07	0.79 ± 0.11	-21.7
Myocardium glycogen (mg/g)	30	16.0 ± 1.35	16.5 ± 2.02*	+3.1
	60	15.6 ± 1.19	11.6 ± 0.65**	-25.6
Blood cholesterol (mg%)	30	379.8 ± 17.7	264.5 ± 29.3**	-30.3
	60	404.8 ± 25.5	194.7 ± 21.7*	-51.8
Liver cholesterol (mg%)	30	271.0 ± 31.1	165.5 ± 12.9**	-38.9
	60	292.0 ± 15.9	120.7 ± 13.4	-58.6
Ovary cholesterol (mg%)	30	93.5 ± 5.37	79.0 ± 11.06	-15.5
	60	65.5 ± 4.62	50.5 ± 5.85**	-22.9
Testes cholesterol (mg%)	30	432.0 ± 8.1	172.6 ± 7.8**	-60.0
	60	370.3 ± 28.1	118.0 ± 4.3	-68.1

[a]Mean ± SE of 8 samples; [b]Calculated from the mean values; * $p < 0.05$; ** $p < 0.01$

and higher vertebrates (Botts 1977). The basophilic stippling has been associated with ribosomal abnormality that leads to a decreased hemoglobin synthesis (Albahary 1972).

Lead induced biochemical alterations included a hyperglycemic response at both 30 and 60 days when the respective blood glucose values were 54.4 and 41.8% higher than the control values. By contrast, a significant reduction occurred in the blood glucose level of rainbow trout, S. gairdneri exposed to 10, 75, and 300 µg Pb/L for 30 days. However, when allowed to recover in lead-free brackish water for 49 days, the rainbow trout became slightly hyperglycemic (Haux & Larsson 1982). The glycogen reserves in B.conchonius were depleted by 22.5 and 59% in the liver after 30 and 60 days, respectively. A decrease in skeletal muscle glycogen occurred at both the observation times but the glycogen content of myocardium fell only after 60 days exposure. Physiologically, the observed mobilization of energy reserves appears to be part of the secondary stress responses which are apparently mediated via hormones of pituitary-adrenal axis (Mazeaud & Mazeaud 1981). In addition, the lead induced structural impairments (branchial and/or renal lesions) also result in physiological dysfunctions. Thus, in the mammals, an increase in urine concentration of glucose, amino acids, and phosphate is causally related to lead-induced kidney damage (WHO 1977).

Information on the influence of environmental pollutants on the blood and tissue levels of cholesterol is rather limited (Wassermann et al.1970; Dutta & Haghighi 1986). A reduction in both circulating and tissue levels of cholesterol was observed in the present study. The decrease in cholesterol levels after 30 and 60 days of exposure was; blood 30.3 and 51.8%, liver 38.9 and 58.6%, ovary 15.5 and 22.9%, and testes 60 and 68.1%, respectively. Since cholesterol is an important constituent of the cell membranes and a precursor for steroid hormones, the toxicant-induced changes in this parameter may be related to either a disruption of plasma membranes and/or altered steroidogenesis. As stated earlier, lead exposure elicits a stress response in B. conchonius, and therefore, depressed cholesterol levels may be related to its enhanced utilization in corticosteroidogenesis and/or a decreased de novo synthesis. Involvement of thyroid hormones has also been suggested in cholesterol metabolism, and an enhanced breakdown in hyperthyroidism is known to result in hypocholesterolemia (Wassermann et al. 1970). In the bluegills, Lepomis macrochirus, exposure to methyl mercuric chloride caused a decrease in serum cholesterol (Dutta & Haghighi 1986). In this fish, a mercury-stimulated enhanced protein synthesis in liver with resultant increased levels of serum high density lipoproteins was suggested to be the cause of hypocholesterolemia. The exact mechanism through which cholesterol metabolism is altered by lead in B. conchonius requires further investigation.

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