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Physiological Dysfunction of the Haemopoietic System in a Fresh Water Teleost, *Labeo rohita*, Following Chronic Chlordane Exposure. Part I—Alterations in Certain Haematological Parameters

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Chemical pesticides are used globally on an increasing scale in copious quantities to increase agricultural output. Public health use of these pesticides accounts for about 10 per cent of annual production, the remainder being used in agriculture, forestry, horticulture and the prevention and storage of food products. Their contribution to increased agricultural production and prevention of untold misery in many tropical and semi-tropical countries can not be denied, but at the same time they have also caused unprecedented ecological imbalance, when parts of these eventually end up in rivers, lakes and the sea through leaching and start accumulating in the atmosphere, lithosphere, biosphere and hydrosphere and causing thereby deterioration especially of the aquatic environment to a great extent.

A vast amount of scientific information is available on the pesticide toxicity on fishes but limited information is available on the effect of these pesticides on the physiology of haemopoietic system, thought to be the most sensitive indicator towards environmental pollutants. Therefore, the present study was undertaken to evaluate the effect of chlordane (an organochlorine pesticide) on certain haematological parameters of a fresh water fish, Labeo rohita at different concentrations for a period of 30 and 60 days.

MATERIALS AND METHODS

The fish L. rohita, commonly known as 'Rohu', belonging to the order Cypriniformes and family Cyprinidae were brought to the laboratory from district fish hatchery and kept in big cemented aquaria of about 2000-L capacity for a period of 15 days. The size of the fish varied from 260-290 mm and weight from 450 - 500 g. The fishes of both the sexes were used without any discrimination. The chlordane 20 E.C. made by M/s Rallis India Ltd., Bombay,was used.

After the normal process of acclimatization and washing with 0.1% $KMnO_4$ solution, fishes were trans-

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ferred into the experimental tanks. The sublethal concentrations as 70, 35, 23 and 17 µg/L (1/3, 1/6, 1/9, 1/12th of the TL(50) respectively) as obtained by VERMA et al. (1977) were taken for long term exposure. Controls without the pesticides but with the same amount of organic solvent (N-N, Dimethyl-formamide) were also set up. To avoid the effect of starvation on any of the haematological parameters, fishes were provided with an artificial diet with continuous renewal of water at two days interval. The fishes were anesthetized with MS. 222 (100 mg/L) and blood was collected by severing the caudal peduncle. Blood was collected into vials containing disodium salt of EDTA as anticoagulant.

Aliquots of whole blood were used for electro-metric determination of blood pH through micro-glass electrode and specific gravity of blood was determined by copper sulphate method after PHILLIPS et al. (1950).

RBC and WBC were counted by Neubaur double haemocytometer using Hayem's and Tuerk's solutions as diluting fluid respectively. Hb was measured by Sahli's haemometer, packed cell volume (PCV) or haematocrit value by Wintrobe's method (3,000 rpm for 1 h), erythro-cyte sedimentation rate (ESR) by Westergen tube method, CT by Lee and White method, while mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concen-tration (MCHC) and mean cell volume (MCV) were calculated by the formulae as given in 'Practical Haematology' by DACIE (1963). The values so obtained are given in Table 1 and 2.

RESULTS AND DISCUSSION

Haematological tests have been an important diagnostic tool in medicine for many years, and recent speculations has indicated that they may be equally valuable as indicators of disease or stress in fish. Haematological changes in fish have been related to temperature and season, diet, pesticide stress and metal stress. KHAN AND QAYYUM (1969) observed mean RBC count of 3.24, 2.59, 2.57 and 2.73 millions/cmm in Ophiocephalus striatus, O. punctatus, Clarias batrachus and Saccobranchus fossilis, respectively. Authors, here noted a mean RBC count of 2.00 millions/cmm and Hb 7.5 g/dl in comparision to a RBC count of 3.45 millions/cmm and Hb 14.4 g/dl in S. fossilis of our previous findings (VERMA et al. 1979), suggesting that air breathing fishes possess much amount of RBC and Hb as compared to non-air breathing fishes. This also indicate that fish haematology fluctuates much on ecological conditions.

TABLE 1

Alterations in haematological parameters on exposure to chlordane in L. rohita after 30 days.

Parameters[a]	Control	17 µg/L	23 µg/L	35 µg/L	70 µg/L
CT (Sec)	140.0 ±10.0	138.0 ± 12.0	120.0 ± 8.0	105.0 ± 7.0*	95.0 ± 5.0*
Hb (g. /dl)	7.5 ± 0.5	8.0 ± 0.9	9.4 ± 0.4*	8.5 ± 0.8	9.2 ± 0.6*
RBC ($x10^{12}$/L)	2.2 ± 0.3	2.3 ± 0.4	3.1 ± 0.8	2.9 ± 0.2	3.5 ± 0.5
WBC ($x10^{9}$/L)	3.2 ± 0.2	3.5 ± 0.3	3.6 ± 0.4	3.6 ± 0.5	3.8 ± 0.4
ESR (mm/h)	1.5 ± 0.1	1.5 ± 0.2	1.8 ± 0.1	1.3 ± 0.3	1.5 ± 0.2
PCV (%)	30.5 ± 1.5	30.0 ± 1.8	32.5 ± 2.1	31.0 ± 2.5	33.2 ± 3.0
MCH (pg)	34.9 ± 2.4	34.8 ± 2.0	30.8 ± 2.8	29.3 ± 2.0	25.7 ± 1.9*
MCHC (%)	24.6 ± 1.5	26.7 ± 1.9	28.9 ± 1.8	27.4 ± 2.1	27.1 ± 2.3
MCV (fl)	141.9 ± 4.8	130.0 ± 3.2	106.6 ± 3.0**	106.9 ± 2.8**	94.9 ± 2.5***
HSI (%)	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.3	1.1 ± 0.4	1.0 ± 0.3
GSI (%)	0.35 ± 0.1	0.41 ± 0.2	0.42 ± 0.1	0.39 ± 0.2	0.38 ± 0.1
	1.45 ± 0.5	1.47 ± 0.3	1.50 ± 0.5	1.45 ± 0.5	1.51 ± 0.3
Sp. gravity	1.0 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	1.1 ± 0.4
pH	7.4 ± 0.6	7.4 ± 0.2	7.3 ± 0.5	7.4 ± 0.8	7.3 ± 0.7

a Values are mean ± S.E. (10 observations).
*$P<0.05$; **$P<0.01$; ***$P<0.001$ (Fisher's 't' test).

TABLE 2

Alterations in haematological parameters on exposure to chlordane in L. rohita after 60 days.

Parameters[a]	Control	17 µg/L	23 µg/L	35 µg/L	70 µg/L
CT (Sec)	138.0 ±10.0	140.0 ±12.0	110.0+± 5.0	80.0 ± 8.0*	85.0 ± 4.0**
Hb (g/dl)	8.0 ± 1.0	8.6 ± 0.8	9.2 ± 1.5	10.8 ± 1.1	9.9 ± 0.5
RBC ($x10^{12}$/L)	2.0 ± 0.4	2.9 ± 0.5	2.3 ± 0.3	3.0 ± 0.8	3.8 ± 0.4*
WBC ($x10^{9}$/L)	3.8 ± 0.5	3.4 ± 0.8	3.9 ± 0.6	3.9 ± 0.7	3.9 ± 0.5
ESR (mm/h)	1.8 ± 0.2	1.5 ± 0.1	1.5 ± 0.3	1.8 ± 0.2	2.0 ± 0.6
PCV (%)	31.7 ± 1.5	32.0 ± 0.5	34.7 ± 1.8	33.0 ± 1.0	35.3 ± 0.5
MCH (pg)	40.0 ± 2.7	29.9 ± 2.3*	40.9 ± 3.6	36.2 ± 3.4	26.1 ± 2.1*
MCHC (%)	23.7 ± 2.1	26.9 ± 2.4	26.5 ± 1.8	32.7 ± 2.1*	28.1 ± 2.9
MCV (fl)	158.5 ± 6.1	113.9 ± 8.1*	154.4 ±10.7	110.7 ± 8.6**	92.8 ± 9.4**
HSI (%)	1.1 ± 0.2	1.3 ± 0.3	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.2
GSI (%)	0.38 ± 0.1	0.40 ± 0.2	0.39 ± 0.1	0.35 ± 0.1	0.45 ± 0.2
	1.55 ± 0.5	1.58 ± 0.7	1.53 ± 0.3	1.48 ± 0.4	1.60 ± 0.3
Sp. gravity	1.1 ± 0.1	1.1 ± 0.4	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.5
pH	7.5 ± 1.0	7.4 ± 0.7	7.4 ± 0.9	7.3 ± 0.8	7.5 ± 1.5

a Values are mean ± S.E. (10 observations).
*P<0.05; **P<0.01; (Fisher's 't' test).

Several haematological parameters were found either increased or decreased. A significant decrease ($P < 0.05$ and $P < 0.01$) in CT was observed after 30 and 60 days at 35 and 70 µg/L but no significant increase or decrease was observed at 17 and 23 µg/L of chlordane. A decrease in CT was also observed by VERMA et al. (1979) after different time intervals of chlordane exposure in S. fossilis. MEHROTRA et al. (1974) observed a slight decrease at 0.1%, a slight increase at 0.5% and a significant ($P < 0.05$) increase at 3.0% concentration than control in rats, fed with metanil yellow.

An increase in Hb was observed at all concentrations but a significant increase ($P < 0.05$) was observed only after 30 days at 23 and 70 µg/L of the toxicant. RBC increase at all concentrations at both time intervals but a significant increase ($P < 0.05$) from 2.0 to 3.81 x 10^{12}/L was noted only after 60 days at 70 µg/L. WBC showed no significant increase or decrease at any concentration after both time intervals. PCV values showed increase at all concentrations but significant alterations were not observed at any concentration and time interval. Similarly ESR did not show any significant increase or decrease in its value.

Significant elevation or reduction in haematological values of fishes exposed to different environmental toxicants have been reported by several workers. EISLER (1967) found that both methoxychlor and methyl-parathion reduced haematocrit values in northern puffers. Conversely, ANDREWS et al. (1966) observed an increase in haematocrit values of bluegills exposed to 0.05 mg/L of heptachlor for 4 h, but they returned to normal after 28 days. MOUNT AND PUTNICKI (1966) while investigating a fish kill due to endrin noted that the haematocrit values were lowered to half of the normal. ALLISON et al. (1964) examined the chronic effects of DDT on cutthroat trout, but could not detect any pathology due to the pesticide and likewise haematocrit values showed no difference between exposed and control fish. McKIM et al. (1970) noted a significant increase in RBC, haematocrit and haemoglobin values of brook trout (Salvelinus fontinalis) exposed to higher concentrations of Cu(II) after 6 and 21 days, but observed no significant variation at lower concentrations even after 337 days of treatment. CHRISTENSEN et al. (1972) noted an increase in RBC and a significant increase in haematocrit and haemoglobin values of brown bullhead (Ictalurus nebulosus) exposed to copper (II) at concentrations of 49 and 107 µg/L for 6 and 30 days of initial treatment, but no such increase was observed after 600 days at lower concentrations. Authors on the basis of the present findings, conclude that little increase in haematocrit and haemoglobin in the experi-

ment may be due to the catalyzing action of the chlordane on the incorporation of the body iron stores into haemoglobin, an effect that has been studied with mammals. McFADDEN (1965) found increased RBC production, greater Hb synthesis and decrease in available liver-iron stores in brook trout exposed to low concentrations of copper. LARSSON et al. (1976) noted that when winter flounders (Pleuronectes flesus L.) were exposed to 0.1, 1 and 10 mg Cd^{++}/L respectively for 15 days, Hb and haematocrit values showed a successive decrease from lower to higher cadmium concentration in the water. The decrease, however, was not statistically significant in the lowest cadmium concentration (0.1 mg Cd^{++}/L). A significant decrease in Hb and haematocrit at 5, 50 and 500 ppb of cadmium after 4 and 9 weeks has also been observed by LARSSON (1975) in flounders (P. flesus L.) and showed that it may be due to a decreased rate of production of and/or to an increased loss or destruction of RBC. CALABRESE et al. (1975) observed no significant difference between controls and Cd exposed winter flounder (Pseudopleuronectes americanus) for any haematological test, but significant reduction was observed in Hb, haematocrit and RBC at 10 ppb of mercury, They however, showed no significant difference in MCV, MCH and MCHC at either of the toxicant concentration. Conversely, SMITH et al. (1976) observed no significant differences in Hb and haematocrit values between control and Cd treated fish after 3 weeks period. VERMA et al. (1979) showed an increase in Hb RBC, WBC and PCV but decreased ESR and CT in S. fossilis after an interval of 15, 30, 45 and 60 days of chlordane exposure. They also showed a significant reduction of MCH and MCV but no significant variation was observed in MCHC. Authors here, noted that MCH decreased significantly ($P < 0.05$) at 17 and 70 µg/L after 60 days but only at 70 µg/L after 30 days. Similarly MCV decreases at 23 and 35 µg/L ($P < 0.01$), 70 µg/L ($P < 0.001$) in 30 days treatment, while significant decrease at 17 µg/L ($P < 0.05$), 35 and 70 µg/L ($P < 0.01$) was observed after 60 days treatment. Authors, here infer that chronic chlordane poisoning is characterised by dehydration, resulting in haemoconcentration and shrinkage of major blood cells as revealed by decreased MCV ($P < 0.001$) values. MCHC, however, showed no significant variation except at 35 µg/L after 60 days.

Differences in the hepatosomatic index (HSI) and gonadosomatic index (GSI), as derived by (Liver or Gonad wt./body wt.) x 100, were slight and non-significant. Specific gravity and pH value likewise, showed no significant differences. GRANT AND MEHRLE (1970) observed a significant difference ($P < 0.05$) in GSI of goldfish exposed to 430 µg endrin/kg body wt./day for 104 days, but observed no significant differences

in pH, HSI and GSI of rainbow trout fed 4.3, 14.5, 43.0 and 145.0 µg endrin/kg body wt/day for 163 days (GRANT AND MEHRLE 1973).

Hence, dramatic changes detected in most of the blood factors indicate that a relatively high degree of chemical stress has been imposed on the fish, which essentially compliments our previous findings with S. fossilis. Therefore, if we derive the toxicity index of chlordane for L. rohita on the basis of these haematological findings, we can say that 17 µg/L (1/12th of TL(50) of chlordane) is the 'no effect' concentration. However, this limit is still to be verified by still longer chronic studies.

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