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Pesticide Induced Haematological Alterations in a Fresh Water Fish *Saccobranchus fossilis*

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Studies on piscian haematology especially on the blood chemistry as compared to other vertebrates remains obscure till this time. Studies on various haematological parameters were made by various authors, but systematic and detailed studies on blood biochemical changes are very rare. FIELD et al. (1943) studied certain inorganic elements, organic compounds, and vitamins in the blood of carp and trout. HAYAMA AND KUWABARA (1962) and MAZEAND (1969) studied the effects of organophosphate pesticides on the cholinesterase activity of fish blood. GRANT AND MEHRLE (1970, 1973) made chronic toxicological studies of endrin on serum electrolytes of the gold fish (Carassius auratus) and rainbow trout (Salmo gairdnerii).

A review of the literature on piscian haematology, reveals that very little work has been done so far on the alteration of blood constituents following chronic pesticide poisoning in fresh water fishes. Therefore, the effect of chlordane (an organochlorine pesticide) on the hematopactic system of a fresh water teleost fish, Saccobranchus fossilis has been studied.

MATERIALS AND METHODS

The fish S. fossilis, commonly known as 'singii', belongs to the order Cypriniformes and family Saccobranchidae and is easily available in the ponds of this region. The size of the fish varies from 190 - 220 mm (average 200 mm) and the weight varies from 50 - 70 g (average 60 g). The fishes of both 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 transferred into the experimental tanks. The solutions of pesticide were made by adopting the dilution technique (STANDARD METHODS, 13th edition, 1971). The sublethal concentration, i.e. 0.12 mg/L of chlordane as obtained

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by VERMA et al. (1977a and b), was taken for long term exposure of S. fossilis.

Living specimens of S. fossilis were kept in big cemented aquariums of about 2000-L capacity. To avoid the effect of starvation on any of the haematological parameters, fishes were provided with an artificial diet and the water was renewed in all tanks at five days interval. Blood was collected by the method of STEUCKE AND SCHOETTGER (1967) by severing the caudal peduncle. The blood was collected into vials containing disodium salt of EDTA as anticoagulant for all parameters except chloride, for which heparinised blood was taken. In other vials, the blood sample was allowed to clot in an incubator at 37°C, serum was collected and stored in a refrigerator for subsequent analysis. However, blood for total red blood cell count, WBC, haemoglobin and clotting time was taken directly following the method of WINTROBE (1967).

Red blood cells (RBC) and white blood corpuscles (WBC) were counted by Neubauer double haemocytometer using Hayem's and Tuerk's solutions as diluting fluid respectively. Haemoglobin (Hb) was measured by Sahli's haemometer, Packed cell volume (PCV) or hematocrit value by Wintrobe's Method (3,000 rev/min for 1 h), erythrocyte sedimentation rate (ESR) by Westergen tube Method, clotting time (CT) by Lee and White method, while mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (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.

Chemical methods for serum analysis, inspite of certain limitations, gave good reproducibility and accuracy of the results. For determination of glucose, Nelson-Somogi method; for lactate, Barker and Summerson method; for plasma proteins, Micro-Kjeldahl method; for non-protein nitrogen, Folin-Wu method; for Cholesterol, Zak's method; for sodium, Weinback method; for potassium, Looney and Dyer method; for magnesium, Titan yellow method; for calcium, Clark-Collip modification of the Kramer-Tisdall method; for chloride, Schales and Schales method; and the phosphorus and iron were determined as given by OSER (1965) in "Hawk's Physiological Chemistry". The values so obtained are given in Table 2.

RESULTS AND DISCUSSION

Since blood takes part either directly or indirectly in all the biochemical processes of the body, it is but natural to expect alterations in it when exposed to environmental toxicants.

TABLE 1

Alterations in haematological parameters on exposure to chlordane in S. fossilis

Parameters*	Control	15 days	30 days	45 days	60 days
Clotting time (Sec)	125.0 ± 5.0	115.0 ± 8.0	110.0 ± 4.0	90.0 ± 6.0	85.0 ± 5.0
Haemoglobin (g/dl)	14.4 ± 1.1	15.2 ± 1.0	14.9 ± 1.1	16.3 ± 1.1	17.0 ± 1.2
RBC ($\times 10^6$/cmm)	3.5 ± 0.3	4.5 ± 0.2	4.0 ± 0.2	5.0 ± 0.3	5.9 ± 0.2
WBC ($\times 10^3$/cmm)	3.9 ± 0.2	4.1 ± 0.2	4.3 ± 0.3	4.3 ± 0.2	4.4 ± 0.2
ESR (mm/h)	1.1 ± 0.4	0.8 ± 0.3	1.1 ± 0.4	0.8 ± 0.2	0.5 ± 0.2
PCV (%)	38.5 ± 1.1	42.0 ± 1.1	40.5 ± 1.0	43.5 ± 1.1	44.5 ± 1.2
MCH (pg)	40.7 ± 1.1	33.8 ± 1.1	37.7 ± 1.0	32.5 ± 1.1	28.4 ± 1.1
MCHC (g/dl)	37.4 ± 1.1	36.2 ± 1.1	36.8 ± 1.0	37.1 ± 1.1	38.2 ± 1.1
MCV (fl)	108.8 ± 1.2	93.3 ± 1.2	102.5 ± 1.2	86.7 ± 1.2	74.4 ± 1.2

*Values are mean ± S.E. (10 observations).

TABLE 2

Alterations in certain organic and inorganic components induced by chlordane in *S. fossilis*

Parameters*	Control	15 days	30 days	45 days	60 days
Glucose (mg/dl)	88.7 ± 1.4	92.4 ± 1.5	102.3 ± 1.1	118.4 ± 1.3	124.5 ± 1.5
Lactate(mg/dl)	18.4 ± 1.4	34.3 ± 1.4	40.1 ± 1.5	30.1 ± 1.4	25.2 ± 1.5
Protein (g/dl)	5.2 ± 1.2	5.0 ± 1.2	4.0 ± 1.2	3.8 ± 1.2	3.8 ± 1.2
Total phosphorus (mg/dl)	60.6 ± 1.4	62.4 ± 1.5	64.6 ± 1.3	63.9 ± 1.5	63.8 ± 1.5
Non-protein nitrogen (mg/dl)	58.4 ± 1.5	59.2 ± 1.5	64.5 ± 1.5	63.4 ± 1.6	68.5 ± 1.5
Cholesterol (mg/dl)	375.5 ± 1.4	332.2 ± 1.4	298.1 ± 1.4	246.5 ± 1.5	210.4 ± 1.5
Sodium (mg/dl)	295.0 ± 1.5	310.5 ± 1.6	354.0 ± 1.5	340.1 ± 1.6	360.2 ± 1.5
Potassium (mg/dl)	6.3 ± 1.0	6.8 ± 1.0	7.2 ± 1.0	8.4 ± 1.0	8.3 ± 1.1
Magnesium (mg/dl)	1.3 ± 1.0	1.7 ± 1.0	2.4 ± 1.0	3.3 ± 1.0	3.1 ± 1.0
Calcium (mg/dl)	12.9 ± 1.1	13.4 ± 1.1	13.9 ± 1.1	15.2 ± 1.2	15.8 ± 1.1
Iron (mg/dl)	26.3 ± 1.3	32.5 ± 1.2	36.8 ± 1.3	46.5 ± 1.3	50.4 ± 1.2
Chloride (mg/dl)	278.0 ± 1.6	298.0 ± 1.5	312.0 ± 1.5	340.0 ± 1.0	382.0 ± 1.5

*Values are mean ± S.E. (3 observations)

Several haematological parameters were found either increased or decreased as a result of chronic chlordane poisoning in the fresh water teleost fish S. fossilis at an interval of 15, 30, 45 and 60 days treatment. Several factors like haemoglobin percentage, RBC, WBC and hematocrit value (PCV) were found increased while erythrocyte sedimentation rate (ESR) and clotting time were found decreased. Haemoglobin increased from 14.4 to 17.0 g/dl, RBC from 3.54×10^6 to 5.98×10^6/cmm, WBC from 3.92×10^3 to 4.42×10^3/cmm and PCV from 38.5 to 44.5 per cent. KHAN AND QAYYUM (1969) observed mean blood cell counts 3.24, 2.59, 2.57 and 2.73 millions/cmm in Ophiocephalus striatus, Ophiocephalus punctatus, Clarias batrachus and Heteropneustes fossilis, respectively. In the present investigation authors observed that the mean RBC count of S. fossilis is 3.45 millions/cmm which indicates that fish haematology fluctuates much on ecological conditions like age, sex, length, weight, maturity and seasonal variations. Other workers have, however, noted a significant reduction in hematocrit values of fishes exposed to environmental stimulations. EISLER (1967) found that both methoxychlor and methyl parathion produced this effect on northern puffers. Conversely, ANDREWS et al. (1966) observed an increase in hematocrit 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 hematocrit 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 treatment and hematocrit values likewise showed no difference between exposed and control fish. GILDERHUS (1966) also noted the same with the exposure of bluegills to sodium arsenite.

Authors observed, that out of the 12 blood constituents analysed, glucose, lactate, non-protein nitrogen, sodium, potassium, calcium, magnesium, iron and chloride increased while other two i.e. proteins and cholesterol decreased after exposure to pesticide. However, total phosphorus showed slight increase in its value. GRANT AND MEHRLE (1973) observed that nine out of the sixteen serum constituents analysed were significantly altered by forced swimming. STEVENS AND BLACK (1966) reported that forced swimming (for 5 min) of rainbow trout caused muscle lactate to peak immediately and the level in blood to increase more slowly, concurrent with lactate release from muscle. Authors, observed an initial increase in blood lactate, may be due to the increased excitation in initial stages but in 45 and 60 days treated fish decreased blood lactate have been

observed. This may be due to the reversal of the fish to its normal condition. Increased glucose level (hyperglycemia) after treatment with chlordane may be due to severe nephritis and pancreatic or hepatic disorders. GRANT AND MEHRLE (1973) also found a 50% increase in serum glucose level of trout, fed highest dietry dose of endrin. Plasma proteins were found decreased which may be due to renal excretion (albuminuria) or impaired protein synthesis or due to liver disorder. Non-protein nitrogen and total phosphorus were also found increased which in the blood plasma decreased (hypocholestremia) as the period of treatment increases which may be due to muscular exhaustion and some depletion of dissolved oxygen in experimental tanks.

The major serum electrolytes Na and Cl were highly correlated and tended to increase with increased time interval of treatment. Authors infer that osmoregulation in S. fossilis has been affected either directly by experimental chlordane solution. HOLMES AND McBEAN (1963) and HUSTON (1959) reported 9.4 and 4.4 per cent increase in serum sodium in rainbow trout on adaptation to saltwater respectively. GRANT AND MEHRLE (1970) reported that a high dose (430 ug/Kg body wt.) of endrin in goldfish caused Na and Cl loss and complete failure of osmoregulatory process. Therefore, authors assume that chlordane toxicosis is characterised by chronic dysfunction of osmoregulatory mechanism, mainfested by increased levels of the major electrolytes. EISLER AND EDMUNDS (1966) observed an increased concentration of Na in blood and decreased in the liver in northern puffers (Sphaeroides maculatus) a marine fish, exposed for 96 h to near lethal concentration of endrin. Their observations suggest a transfer of Na but not of K from tissues to blood by some means. Similar trend of increment of other electrolytes as Ca, Mg, K and Fe has been observed by authors in the present investigation. GRANT AND MEHRLE (1970, 1973) also reported other altered physiologic parameters of growth, reproduction, thyroid activity, intermediary metabolism and osmoregulation in gold fish (C. auratus) and rainbow trout (S. gairdnerii) chronically exposed to endrin diet. ISHIHARA et al. (1967) observed that pentachlorophenol, dimethylthiophosphate, endrin and phenyl mercuric acetate caused changes in the component ratio of serum proteins and lipoprotein in the blood of carp. LOCKHART et al. (1975) observed that rainbow trout (S. gairdnerii) exposed chronically to IMOLS-140, a synthetic high temperature lubricating oil also altered the serum constituents significantly. Similar alterations in serum contituent have also been observed in the present investigations.

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