V. GENERAL SUMMARY & CONCLUDING REMARKS

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1. GENERAL SUMMARY

The brief survey of toxicological research on fish species showed that many investigators have found target systems, organs, tissues and cells, as well as the nature of changes produced in affected animals and the toxic actions of many pesticides, detergents and plant toxins. Although, very scanty information on the effect of natural piscicides especially of plant origin on the haematological parameters and enzymes of many target organs is available. Therefore, an extensive project on the impact of natural piscicide of leaves of L. eriocephalus on the important haematological parameters like TRBC, TWBC, clotting time and THb and on non-lysosomal enzyme-alkaline phosphatase in the liver, kidney, gill and brain had undertaken. The reasons that led us to take up the present investigation have been elaborately described in the first introduction chapter. During investigation widely accepted and well-known haematological methods have been employed in assessing toxic effect of natural piscicide. In case of enzymological study, both histoenzymological as well as biochemical assay methods were employed to find out the distribution and alteration in the alkaline phosphatase enzyme in the liver, kidney, gill and brain during intoxication to fish. Histoenzymological results have been provided in tabular form and supplemented by many microphotographs also. Bio-statistical observations on enzyme study have

been summarised in tables and represented by graphical illustrations also. The piscicide induced morphological changes in the blood cells are given in many coloured microphotographs.

The following is a brief summary of the results obtained in the present investigatioin :

A) Haematological Study

i) TRBC :

The total RBC count of control fish S. mossambica, $3.21 \times 10^6/mm^3$ remained more or less constant throughout the exposure period. After exposure of fish to natural piscicide, the TRBC count decreased with increase in dose concentration and duration of intoxication. Thus at 20 ppm concentration, TRBC count (No. x $10^6/mm^3$) was 3.02 after 12 hrs, 3.0 after 24 hrs, 2.98 after 36 hrs, 2.95 after 48 hrs, 2.93 after 60 hrs, 2.91 after 72 hrs, 2.89 after 84 hrs and 2.88 after 96 hrs. Similar decreasing counts were recorded after exposure to 40, 80, 120, 160 and 200 ppm also, at above mentioned time intervals.

ii) TWBC :

The total WBC count of control fish S. mossambica was 47.70 x $10^3/mm^3$ and it remained more or less the same during 96 hrs period. This count in exposed fish was found increased and such rise was dependent on dose concentration and on duration of intoxication. The values of counts after exposure to different concentrations including 20, 40, 80, 120, 160 and 200 ppm were 49.51, 50.86, 51.13, 51.85, 52.26 and 52.48 x $10^3/ mm^3$ after 12 hrs intoxication, respectively. Similarly, such rise in TWBC was observed after 24, 36, 48, 60, 72, 84 and 96 hrs intoxication.

iii) Clotting Time :

The clotting time in control fish S. mossambica was 3.50 min, which remained practically the same throughout the experimental period (96 hrs) in this group. But in exposed fish, clotting time of this fish decreased constantly with increase in concentration of the natural piscicide. Duration of intoxication also influenced on this haematological parameter. Long was the intoxication period less was the clotting time of blood and vice versa. Thus, at highest dose of 200 ppm after 36 hrs clotting time was decreased about 25.64% than the control fish. Similar observations were found at other concentrations also.

iv) THb :

The haemoglobin percentage in the control fish S. mossambica was 11.13 gm/100 ml of blood and it ranged about this concentration throughout the experiment in this group. The haemoglobin percentage in exposed fish was decreased with increase in concentration of piscicide. The duration of intoxication also influenced the haemoglobin content of the fish. In general, higher concentration of piscicide and longer duration of intoxication reduced the haemoglobin percentage of fish e.g. at 200 ppm, THb percentage decrease was 10.69%, 11.08% and 11.70% after 12, 24 and 36 hrs, respectively, showing the dose and duration dependent decrease during natural piscicide intoxication in this fish. Such results were observed at 160, 120, 80, 40 and 20 ppm concentrations also.

v) Morphology of blood cells :

Abnormal sizes and shapes of erythrocytes were commonly observed in the present study. In many cases, loosened chromatin material, vacuole formation and deposition of black pigment on the surface of RBC were observed in treated fish. The changes in nuclear shape, staining and size were also observed in RBCs and WBCs, at higher concentrations of the piscicide.

The decrease in total mature RBCs seems to be due to erythropoitic response and due to haemolysis as indicated by Anonymous (1952) and Chopra et al. (1958). The increase in TWBC number from 47.70 to 53.40 x $10^3/mm^3$ was just contradictory to TRBC number in treated fish. Such leucocytosis may suggest inflamation in tissues and may be to cope up with the removal of cell debris of necrosed tissues under the toxicant stress. The morphological changes in blood cells were supposed as immediate effect of the natural piscicide. The exact significance of decrease in clotting time of blood during natural piscicide intoxication cannot drawn with the available information. The decrease in THb percentage pointed out the respiratory stress at higher concentration of piscicide and after longer exposure of fish. Therefore, after exposure to higher concentrations of piscicide increased opercular movements were observed in the present intestigation.

B) Enzymological Study

Exposure of fish to synthetic piscicides commonly led to alterations of phosphatases and their accumulation points to tissue damage. Therefore, a non-lysosomal enzyme alkaline phosphatase was selected to

find out the impact and the extent of damage caused by the natural piscicide on the liver, kidney, gill and brain of fish, S. mossambica. In all these organs, the enzyme activity was found increased. The maximum increase after exposure of fish at 200 ppm for 36 hrs intoxication was 35.49%, 37.60% 52.44% and 27.66% in the liver, kidney, gill and brain respectively. The increase in enzyme activity was dose and duration dependeant in all these organs. The pattern of increase was linearly correlated with the dose of the piscicide and with the duration of intoxication also. The histoenzymological observations also supplemented the biostatistical observations on the enzyme activity. In hepatocytes of control fish alkaline phosphatase activity was in the form of small granules and distributed throughout the cells. In treated fish, alkaline phosphatase was found only in certain cells surrounding the blood vessels and sinusoids. In case of kidney, glomeruli, proximal tubules, distal tubules and collecting tubules all showed alkaline phosphatase activity. But after treatment, it was very distinct at brush borders of proximal tubules, moderate in glomeruli and weak in the distal tubules. This enzyme was localized in the primary and secondary gill lamellae, epithelium and cartilage of gill. In treated fish it was observed distinctly only in pillar cells of secondary lamellae and in cartilage.

In liver, it is likely that the treatment of natural piscicide might have induced hyperglycemia as a result of glycogenolysis and hence consequent increase of alkaline phosphatase activity was observed to meet energy requirements in order to counter the stress. This enzyme plays significant roles in cation transport, liberation of phosphoric acid from

its esters and transport of phosphate across the cell membranes of proximal tubules. The present study suspects the role of phosphate transport at proximal tubules and filtration role at glomeruli of alkaline phosphatase during piscicide stress condition in S. mossambica. However, the exact function of alkaline phosphatase at cellular level and its direct physiological role cannot be ascertained in the fish kidney. From the localization and variations under stress environment, the alkaline phosphatase enzyme in the fish gill seems to be involved in the coupling of phosphorylation as in case of kidney tubules as suggested by Bhatnagar (1983). The increase in alkaline phosphatase enzyme in the brain during natural piscicide intoxication cannot be explained on the basis of scanty information on this aspect. But some earlier investigators suppose that the toxication of synthetic chemicals on the central nervous system block the sulfhydryl groups of enzymes by these chemicals. However, this piscicide seems to activate alkaline phosphatase activity in the brain of this fish. But for conformation further investigations in this area are required.

2. CONCLUDING REMARKS

The aim of the present investigation was of two-folds and was concerned with the evaluation of the impact of natural piscicide in the ethanol extract of L. eriocephalus on haematological parameters including TRBC, TWBC, clotting time and THb and on alkaline phosphatase of liver kidney, gill and brain of freshwater teleost fish S. mossambica. It is hoped that the aims and objectives of the present investigation have satisfactorily been achieved.

Besides, the present investigation opens several avenues for further research in the field of toxicology, haematology and in the field of enzymology. Some of them have been cited below which may instigate further research :

1) While carrying out haematological experiments only four parameters were chosen but there are several other parameters like length and breadth of RBC and WBC and of their nuclei, PCV, ESR, MCV MCH, MCHC, etc., which can be studied during natural piscicide intoxication, the results of which would be useful to analyse toxic effect of this piscicide on the blood and other tissues of this fish.

2) Along with TRBC and TWBC counts, the differential counts of immature and mature red blood cells, small and large lymphocytes, neutrophils, thrombocytes, smudge cells can give the indications regarding erythropenia, leucopenia or erythropoisis and leucopoisis and can provide information on the extent of tissue damage during the intoxication.

3) Anaemic conditions and haemolysis have not been studied in the present investigation. Such information would be useful to know the chemical nature of the piscicide compound in the leaves of L. eriocephalus.

4) Generally, respiratory enzymes, LDH, SDH, PDH are studied during the stress environment. Similarly lysosomal enzymes like β-glucuronidase, acid phosphatase, esterase and arylsulfatase which are involved during lytic activities also studied in relation to toxicity.

The study of these enzymes would also provide better opportunity for evaluation of the impact of natural piscicide on the fish.

Inspite of these shortcomings, the author feels gratified that he has at least presented a preliminary information on alterations in haematological parameters and localization and alterations in alkaline phosphatase in liver, kidney, gill and brain of S. mossambica during natural piscicide intoxication. Such a study would be of crucial importance in the diagnosis of toxicity and in disclosure of metabolism in the stress environment. This will enable the environmental scientists to access the impact caused by other various natural piscicides. It will also provide guide line in order to recommend specific dose of this natural piscicide to eradicate this or other undesirable fish species from the nursary ponds prior to the pisciculture. These problems need to be thoroughly investigated.

The further studies are being carried out on some of the aforementioned aspects in this laboratory and the results of which will be published in due course of time.

" To make an end is to make a beginning

The end is where we start from "

- E.S. Eliot