III. HAEMATOLOGY

III. HAEMATOLOGICAL STUDIES ON S. MOSSAMBICA AFTER EXPOSURE TO NATURAL PISCICIDE

1. Introduction :

As pointed out in the introduction chapter there are very few studies on the haematology of fish under stress environment. Therefore, the present investigation on the haematology of fresh water fish, S. mossambica during toxicity of natural piscicide from the leaves of L. eriocephalus was undertaken.

2. General behaviour of fish :

The fish exposed to natural piscicide, present in the ethanol extract of the leaves of L. eriocephalus changed their behaviour when compared to the controls. At lower concentrations (20 and 40 ppm) the behaviour of the fish was not changed. But after exposure to 80 ppm ethanol extract the fish showed high excitability and quick responses to different types of external stimuli like light, touch, etc. Their behaviour was remarkably changed at higher concentrations of the piscicide. At highest concentration of 200 ppm the fish swam sluggishly, restricted to the corners of the aquaria, lost their equilibrium, turned on one side along their long axis and finally remained upside down posture at the bottom of the aquaria. The opercular movements were increased around 90-110 per minute in the experimental animals whereas it ranged from 60-75 per minute in the controls. The fishes were slipery to touch,

indicating the hypersecretion of the mucous. The gills were covered with thick mucous and blood clots indicating bleeding in the gills.

3. Haematological observations :

i) Total R.B.C. (TRBC) :

Many changes were noted in the blood parameters of the exposed fish. The changes in the total erythrocyte count using Neubaur's haemocytometer were recorded which have given in Table No.1 and have shown graphically in Graph No.1. From Table No.1 it is evident that total erythrocyte count showed decrease starting from the 12 hours exposure onwards. The percentage decrease after exposure to 20 ppm was 5.91%, 6.25%, 6.58%, 7.81%, 8.72%, 8.77%, 9.68% and 10.28% after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. After exposure to 40 ppm the per cent decrease was 7.50%, 7.81%, 9.24%, 9.68%, 10.90%, 11.91% and 15.52% after 12, 24, 36, 48, 60, 72 and 84 hrs, respectively. At 80 ppm concentration per cent decrease was 9.67% after 12 hrs, 11.49%, after 24 hrs, 11.91% after 36 hrs, 12.50% after 48 hrs, 13.70% after 60 hrs and 14.10% after 72 hrs. At 120, 160 and 200 ppm the values of the per cent decrease were 11.83%, 15.26%, 18.06% after 12 hrs; 12.18%, 15.93% and 18.75% after 24 hrs; whereas 12.53%, 16.30% and 19.43% after 36 hrs. After exposure to 120 ppm concentration the per cent decrease of TRBC was 14.06% after 48 hrs and it was 14.64% after 60 hrs At 160 ppm the per cent decrease in the TRBC was 17.18% after 48 hrs. At 200 ppm concentration it was 19.43% after 36 hrs. The fish died at higher concentrations therefore, we could not record the TRBC number upto 96 hrs in all the doses.

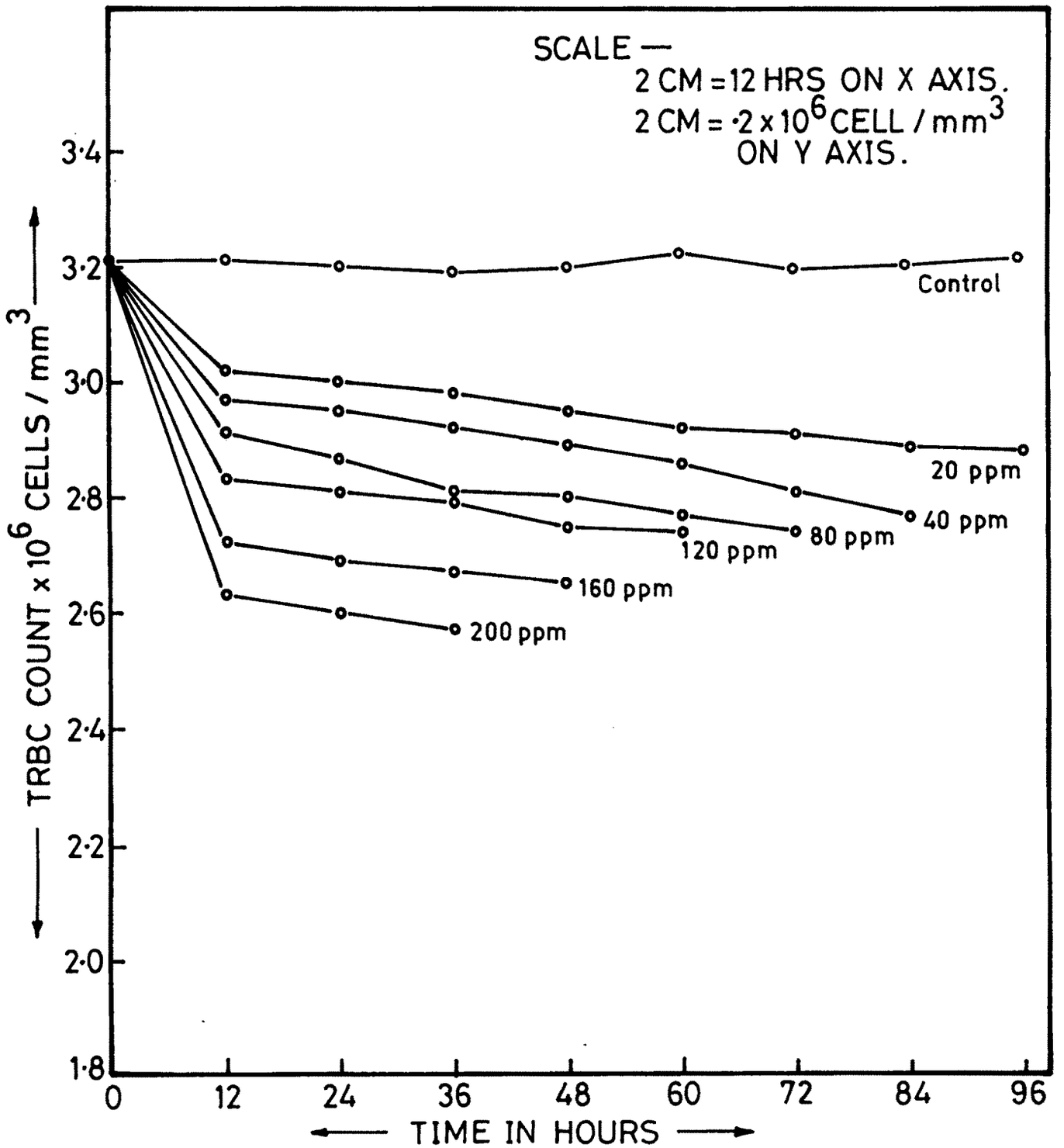
TABLE - 1

Haematological observations on the blood of Sarotherodon mossambica during intoxication due to natural piscicide in the ethanol extract of leaves of L. eriocephalus.

i) TRBC NUMBER

Time intervals of intoxication. (Hrs)	TRBC count at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	3.21 ± 0.26	3.21 ± 0.26	3.21 ± 0.26	3.21 ± 0.26	3.21 ± 0.26	3.21 ± 0.26	3.21 ± 0.26
12	3.21 ± 0.19	3.02 ± 0.20 (-5.91%)	2.97 ± 0.07 (-7.50%)	2.91 ± 0.07 (-9.67%)	2.83 ± 0.04 (-11.83%)	2.72 ± 0.05 (-15.26%)	2.63 ± 0.16 (-18.06%)
24	3.20 ± 0.28	3.0 ± 0.11 (-6.25%)	2.95 ± 0.05 (-7.81%)	2.87 ± 0.09 (-11.49%)	2.81 ± 0.04 (-12.18%)	2.69 ± 0.08 (-15.93%)	2.60 ± 0.05 (-18.75%)
36	3.19 ± 0.30	2.98 ± 0.17 (-6.58%)	2.92 ± 0.06 (-9.24%)	2.81 ± 0.16 (-11.91%)	2.79 ± 0.08 (-12.53%)	2.67 ± 0.04 (-16.30%)	2.57 ± 0.06 (-19.43%)
48	3.20 ± 0.15	2.95 ± 0.16 (-7.81%)	2.89 ± 0.14 (-9.68%)	2.80 ± 0.04 (-12.50%)	2.75 ± 0.06 (-14.06%)	2.65 ± 0.03 (-17.18%)	-
60	3.22 ± 0.18	2.93 ± 0.09 (-8.72%)	2.86 ± 0.06 (-10.90%)	2.77 ± 0.06 (-13.70%)	2.74 ± 0.03 (-14.64%)	-	-
72	3.19 ± 0.15	2.91 ± 0.17 (-8.77%)	2.81 ± 0.08 (-11.91%)	2.74 ± 0.12 (-14.10%)	-	-	-
84	3.20 ± 0.17	2.89 ± 0.14 (-9.68%)	2.77 ± 0.15 (-15.52%)	-	-	-	-
96	3.21 ± 0.76	2.88 ± 0.07 (-10.28%)	-	-	-	-	-

N.B. :
a) Figures indicate TRBC count x 10^6 = No. of RBCs /mm³
b) Figures in the brackets indicate per cent decrease in TRBC.
c) Average length of fish = 14 cm.
d) Average weight of fish = 35 gm.



GRAPH NO. 1 — CHANGES IN THE TRBC COUNT IN S. MOSSAMBICA DURING INTOXICATION DUE TO NATURAL PISCICIDE IN THE LEAVES OF L. ERIOCEPHALUS.

ii) Total W.B.C. (TWBC) :

The changes in the total white blood cells count using Neubaur's haemocytometer were recorded which have given in Table No.2 and have been shown graphically in Graph No. 2.

From Table No.2 it is evident that total white blood cell count showed increase starting from the 12 hours exposure onwards. The per cent increase after exposure to 20 ppm was 3.79%, 4.27%, 4.61%, 5.59%, 6.37%, 7.79%, 9.40% and 10.02% after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. After exposure to 40 ppm the per cent increase was 6.62%, 7.73%, 8.90%, 9.22%, 9.68%, 10.18% and 10.62% after 12, 24, 36, 48, 60, 72 and 84 hrs, respectively. At 80 ppm concentration per cent increase was 7.19% after 12 hrs, 9.26% after 24 hrs, 9.99% after 36 hrs, 10.54% after 48 hrs, 10.81% after 60 hrs and it was 11.54% after 72 hrs. At 120, 160 and 200 ppm the per cent increase values were 8.70%, 9.55% 10.02% after 12 hrs; 9.72%, 10.88% and 11.11% after 24 hrs; whereas 10.20%, 11.29% and 11.90% after 36 hrs. After exposure to 120 ppm concentration the per cent increase was 10.92% after 48 hrs and it was 11.04% after 60 hrs. At 160 ppm concentration the per cent increase in the TWBC was 11.63% after 48 hrs. At 200 ppm concentration it was 11.90% after 36 hrs. We could not record TWBC number upto 96 hrs in all the doses, because some of the exposed fish died at higher doses of the ethanol extract.

iii) Clotting time :

The changes in the clotting time using capillary tube method were recorded which have given in Table No.3 and have shown graphically in Graph No.3.

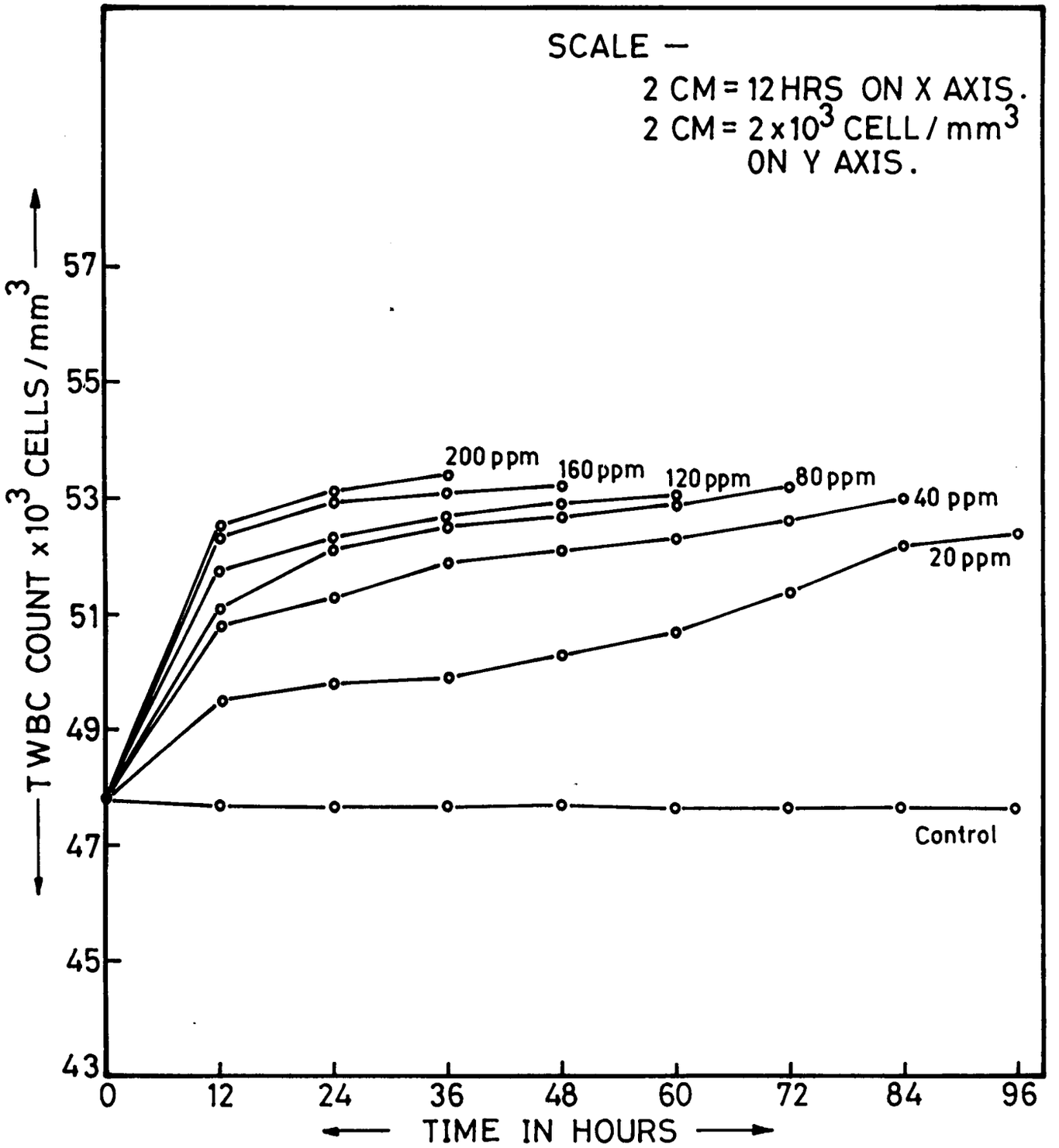
TABLE - 2

Haematological observations on the blood of Sarotherodon mossambica during intoxication due to natural piscicide in the ethanol extract of leaves of L. eriocephalus.

ii) TWBC NUMBER

Time intervals of intoxication. (Hrs)	TWBC count at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	47.70 ± 5.16	47.70 ± 5.16	47.70 ± 5.16	47.70 ± 5.16	47.70 ± 5.16	47.70 ± 5.16	47.70 ± 5.16
12	47.70 ± 4.62	49.51 ± 5.36 (3.79%)	50.86 ± 5.16 (6.62%)	51.13 ± 3.45 (7.19%)	51.85 ± 4.21 (8.70%)	52.26 ± 4.12 (9.55%)	52.48 ± 4.19 (10.02%)
24	47.69 ± 4.67	49.73 ± 4.26 (4.27%)	51.38 ± 4.96 (7.73%)	52.11 ± 3.58 (9.26%)	52.33 ± 4.08 (9.72%)	52.88 ± 4.32 (10.88%)	52.99 ± 4.03 (11.11%)
36	47.72 ± 5.63	49.92 ± 4.31 (4.61%)	51.97 ± 4.22 (8.90%)	52.49 ± 3.19 (9.99%)	52.59 ± 4.39 (10.20%)	53.11 ± 4.25 (11.29%)	53.40 ± 4.27 (11.90%)
48	47.70 ± 4.68	50.37 ± 4.58 (5.59%)	52.10 ± 4.33 (9.22%)	52.73 ± 3.11 (10.54%)	52.11 ± 4.13 (10.92%)	52.25 ± 4.39 (11.63%)	-
60	47.72 ± 4.86	50.76 ± 3.87 (6.37%)	52.34 ± 4.29 (9.68%)	52.88 ± 3.27 (10.81%)	52.99 ± 4.03 (11.04%)	-	-
72	47.71 ± 4.72	51.43 ± 4.19 (7.79%)	52.57 ± 4.25 (10.18%)	53.22 ± 3.58 (11.54%)	-	-	-
84	47.72 ± 4.65	52.21 ± 4.83 (9.40%)	52.79 ± 4.12 (10.62%)	-	-	-	-
96	47.69 ± 4.82	52.47 ± 4.42 (10.02%)	-	-	-	-	-

N.B. :
a) Figures indicate TWBC count x 10^3 = No. of WBCs /mm^3
b) Figures in the brackets indicate per cent increase in TWBC.
c) Average length of fish = 16 cm.
d) Average weight of fish = 39 gm.



GRAPH NO. 2 – CHANGES IN THE TWBC COUNT IN S. MOSSAMBICA DURING INTOXICATION DUE TO NATURAL PISCICIDE IN THE LEAVES OF L. ERIOCEPHALUS.

From Table No.3 it is evident that clotting time showed decrease starting from 12 hours exposure onwards. The per cent decrease after exposure to 20 ppm was 2.57%, 3.43%, 4.84%, 5.42%, 6.25%, 6.28%, 6.59% and 8.00%, after 12, 24, 36, 48, 60, 72, 84, 96 hrs, respectively. After exposure to 40 ppm concentration per cent decrease in the clotting time of blood was 8.57%, 9.16%, 10.82%, 11.42%, 12.50%, 14.28% and 14.61% after 12, 24, 36, 48, 60, 72, 84 hrs, respectively. At 80 ppm concentration per cent decrease was 11.71% after 12 hrs, 13.18% after 24 hrs, 14.52% after 36 hrs, 14.85% after 48 hrs, 16.19% after 60 hrs and 16.28% after 72 hrs. At 120, 160 and 200 ppm the per cent decrease values were 16.85%, 18.00%, 22.57%, after 12 hrs; 18.05%, 18.62%, and 24.06% after 24 hrs; whereas 19.65%, 20.51% and 25.64% after 36 hrs. After exposure to 120 ppm concentration the per cent decrease was 19.71% after 48 hrs and it was 20.73% after 60 hrs. At 160 ppm the percentage decrease in the clotting time was 21.14% after 48 hrs. At 200 ppm concentration it was 25.64% after 36 hrs. The fish died at higher concentrations therefore, we could not record clotting time upto 96 hrs in all the doses.

iv) Haemoglobin :(THb) :

The mean values of the haemoglobin percentage by using Sahli's and Wong's methods were recorded which have given in the Table No.4 and have shown graphically in Graph No.4.

From Table No.4 it is evident that haemoglobin percentage showed decrease starting from the 12 hrs exposure onwards. The per cent decrease after exposure to 20 ppm was 1.70%, 1.62%, 2.52%, 2.79%, 3.50%, 3.96% 4.67% and 4.85%, after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively.

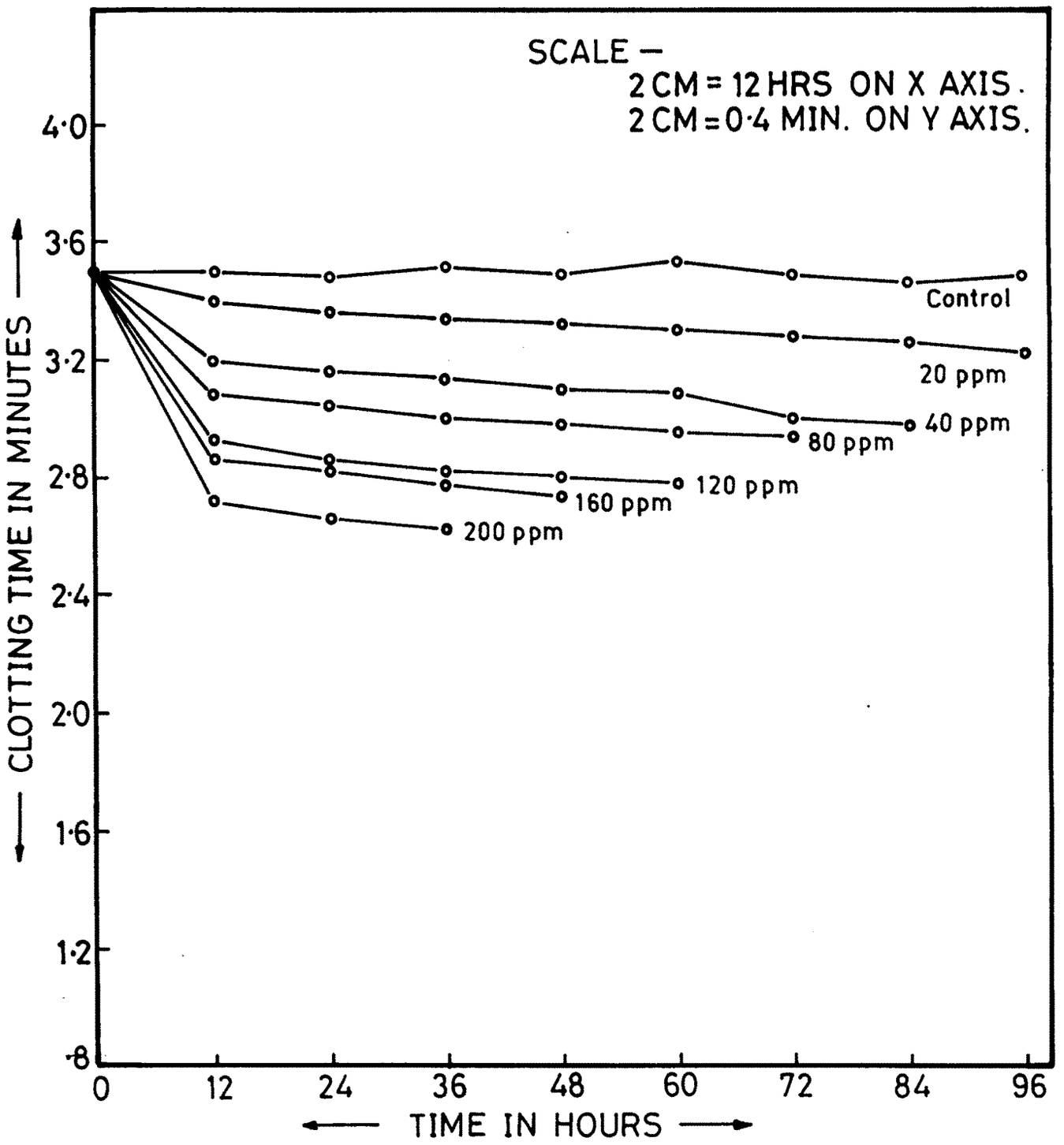
TABLE - 3

Haematological observations on the blood of Sarotherodon mossambica during intoxication due to natural piscicide in the ethanol extract of leaves of L. eriocephalus.

iii) Clotting time

Time intervals of intoxication. (Hrs)	Clotting time at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	3.50 ± 0.32	3.50 ± 0.32	3.50 ± 0.32	3.50 ± 0.32	3.50 ± 0.32	3.50 ± 0.32	3.50 ± 0.32
12	3.50 ± 0.26	3.41 ± 0.19 (-2.57%)	3.20 ± 0.19 (-8.57%)	3.09 ± 0.16 (-11.71%)	2.51 ± 0.15 (-26.85%)	2.87 ± 0.13 (-18.00%)	2.71 ± 0.14 (-22.57%)
24	3.49 ± 0.34	3.37 ± 0.16 (-3.43%)	3.17 ± 0.12 (-9.16%)	3.03 ± 0.12 (-13.18%)	2.86 ± 0.09 (-18.05%)	2.84 ± 0.10 (-18.62%)	2.65 ± 0.09 ((-24.06%)
36	3.51 ± 0.27	3.34 ± 0.32 (-4.84%)	3.13 ± 0.14 (-10.82%)	3.00 ± 0.12 (-14.52%)	2.82 ± 0.10 (-19.65%)	2.79 ± 0.09 (-20.51%)	2.61 ± 0.21 (-25.64%)
48	3.50 ± 0.33	3.31 ± 0.12 (-5.42%)	3.10 ± 0.09 (-11.42%)	2.98 ± 0.19 (-14.85%)	2.81 ± 0.18 (-19.71%)	2.76 ± 0.12 (-21.14%)	-
60	3.52 ± 0.31	3.30 ± 0.16 (-6.25%)	3.08 ± 0.11 (-12.50%)	2.95 ± 0.10 (-16.19%)	2.79 ± 0.09 (-20.73%)	-	-
72	3.50 ± 0.28	3.28 ± 0.22 (-6.28%)	3.00 ± 0.10 (-14.28%)	2.93 ± 0.12 (-16.28%)	-	-	-
84	3.49 ± 0.25	3.26 ± 0.13 (-6.59%)	2.98 ± 0.12 (-14.61%)	-	-	-	-
96	3.50 ± 0.23	3.22 ± 0.14 (-8.00%)	-	-	-	-	-

a) Figures indicate clotting time in minutes.
b) Figures in the brackets indicate per cent decrease in clotting time.
c) Average length of fish = 18 cm.
d) Average weight of fish = 40 gm.



GRAPH NO. 3 — CHANGES IN THE CLOTTING TIME IN S. MOSSAMBICA DURING INTOXICATION DUE TO NATURAL PISCICIDE IN THE LEAVES OF L. ERIOCEPHALUS.

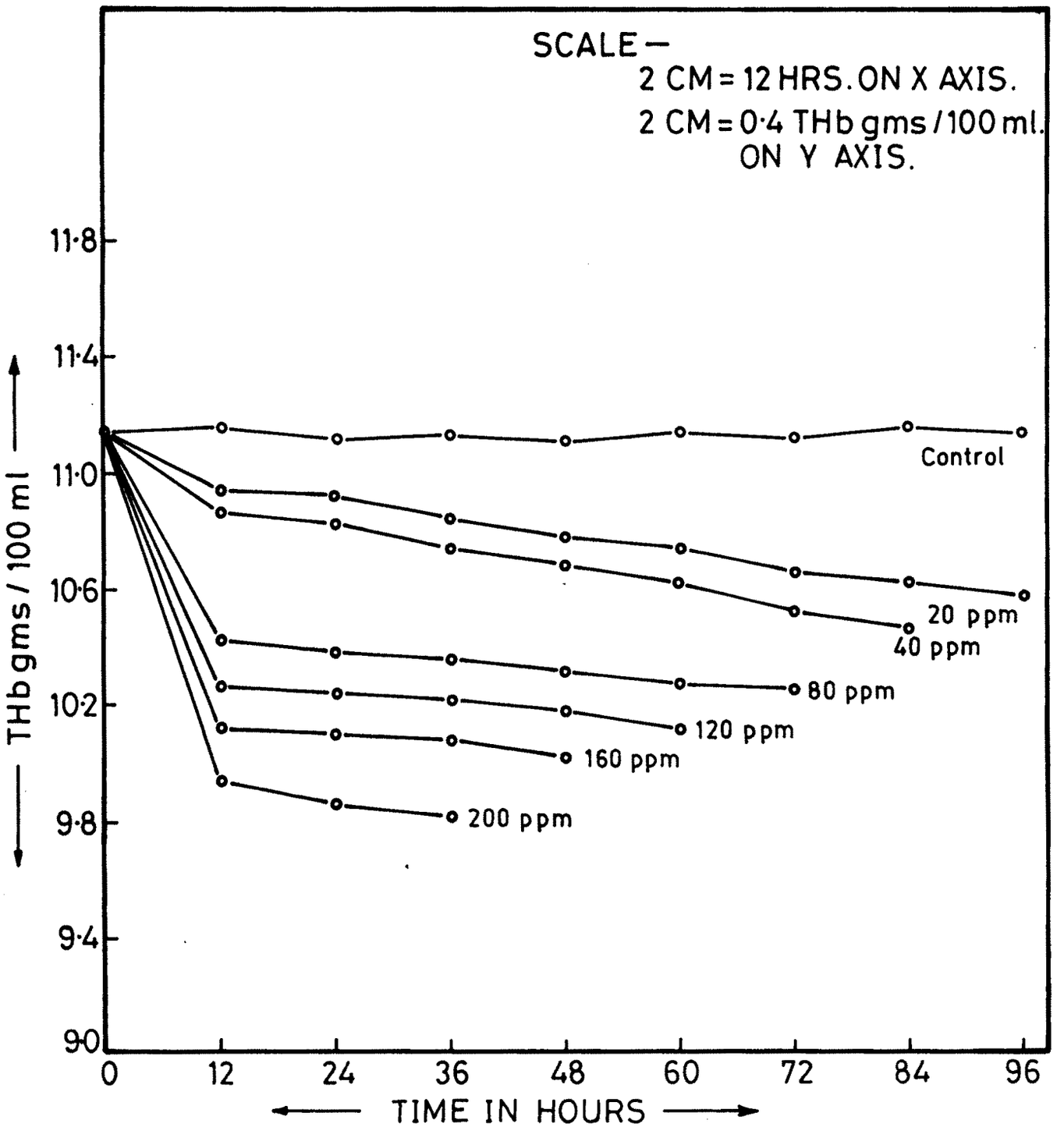
TABLE - 4

Haematological observations on the blood of Sarotherodon mossambica during intoxication due to natural piscicide in the ethanol extract of leaves of L. eriocephalus.

iv) Haemoglobin Percentage

Time intervals of intoxication. (Hrs)	Clotting time at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	11.13 ± 0.29	11.13 ± 0.29	11.13 ± 0.29	11.13 ± 0.29	11.13 ± 0.29	11.13 ± 0.29	11.13 ± 0.29
12	11.13 ± 0.22	10.94 ± 0.14 (-1.70%)	10.87 ± 0.14 (-2.33%)	10.41 ± 0.19 (-6.43%)	10.27 ± 0.22 (-7.72%)	10.12 ± 0.18 (-9.07%)	9.94 ± 0.11 (-10.69%)
24	11.10 ± 0.26	10.92 ± 0.18 (-1.62%)	10.82 ± 0.12 (-2.52%)	10.38 ± 0.26 (-6.48%)	10.23 ± 0.19 (-7.83%)	10.09 ± 0.21 (-9.09%)	9.87 ± 0.16 (-11.08%)
36	11.11 ± 0.23	10.83 ± 0.25 (-2.52%)	10.74 ± 0.24 (-3.33%)	10.36 ± 0.17 (-6.75%)	10.21 ± 0.12 (-8.10%)	10.08 ± 0.16 (-9.27%)	9.81 ± 0.21 (-11.70%)
48	11.10 ± 0.27	10.79 ± 0.17 (-2.79%)	10.69 ± 0.18 (-3.69%)	10.33 ± 0.11 (-6.93%)	10.18 ± 0.18 (-8.28%)	10.03 ± 0.12 (-9.63%)	-
60	11.12 ± 0.24	10.73 ± 0.11 (-3.50%)	10.61 ± 0.19 (-4.58%)	10.29 ± 0.19 (-7.46%)	10.13 ± 0.13 (-8.90%)	-	-
72	11.11 ± 0.29	10.67 ± 0.19 (-3.96%)	10.52 ± 0.21 (-5.31%)	10.26 ± 0.28 (-7.65%)	-	-	-
84	11.13 ± 0.20	10.61 ± 0.16 (-4.67%)	10.47 ± 0.24 (-5.92%)	-	-	-	-
96	11.12 ± 0.28	10.58 ± 0.18 (-4.85%)	-	-	-	-	-

N.B. :
a) Figures indicate THb gms/100 ml.
b) Figures in the brackets indicate per cent decrease in THb.
c) Average length of fish = 15 cm.
d) Average weight of fish = 36 gm.



GRAPH NO. 4 — CHANGES IN THE THb PERCENTAGE IN S. MOSSAMBICA DURING INTOXICATION DUE TO NATURAL PISCICIDE IN THE LEAVES OF L. ERIOCEPHALUS .

After exposure to 40 ppm the per cent decrease was 2.33%, 2.52%, 3.33% 3.69%, 4.58%, 5.31% and 5.92% after 12, 24, 36, 48, 60, 72 and 84 hrs, respectively. At 80 ppm concentration per cent decrease was 6.43% after 12 hrs, 6.48% after 24 hrs, 6.75% after 36 hrs, 6.93% after 48 hrs, 7.46% after 60 hrs and it was 7.65% after 72 hrs. At 120, 160 and 200 ppm the per cent decrease values were 7.72%, 9.07%, 10.69% after 12 hrs; 7.83%, 9.09% and 11.08% after 24 hrs; whereas 8.10%, 9.27% and 11.70% after 36 hrs. After exposure to 120 ppm concentration the per cent decrease was 8.28% after 48 hrs and it was 8.90% after 60 hrs. At 160 ppm concentration the per cent decrease in the THb was 9.63% after 48 hrs. At 200 ppm concentration it was 11.70% after 36 hrs. The fish died at higher concentrations therefore, we could not record THb upto 96 hrs in all the doses.

v) Morphological observations on blood cells :

Throughout the 96 hrs of exposure period, at all the concentrations many interesting changes were recorded in the morphology of different peripheral blood cells. The major morphological changes observable under light microscope are summarised in the following paragraphs and have been microphotographically illustrated in Plate Nos. 1 and 2.

20 ppm exposure :

The number of RBC was slightly decreased. The staining of the nuclei was deepened. WBC were intensely stained (Plate No.1, fig.2) when compared with the control fish (Plate No.1, fig.1).

40 ppm exposure :

RBC formed rouleaux or elongated strands (Plate No.1, fig.3). Their

PLATE NO. 1, Figs. 1 to 8

(Morphological changes in blood cells induced after exposure of fish S. mossambica to natural piscicide in the ethanol extract of leaves of L. eriocephalus)

Fig.No. 1 : Blood smear of control fish.
Note normal shape and size of RBC. X 240.

Fig.No. 2 : Blood smear of treated fish after exposure to 20 ppm for 96 hrs. Note enhanced staining of the nuclei of RBC and deep pink stain of WBC. X 240.

Fig.No. 3 : Blood smear of treated fish after exposure to 40 ppm after 24 hrs. Note rouleaux or elongated strands of RBC and enhanced staining of their nuclei X 240.

Fig.No. 4 : Blood smear of treated fish after exposure to 40 ppm for 84 hrs. Note small vacuoles in the cytoplasm of few RBCs. X 240.

Fig.No. 5 : Blood smear of treated fish after exposure to 80 ppm for 24 hrs. Note intensely stained RBCs and large, many vacuoles in their cytoplasm. X 240.

Fig.No. 6 : Blood smear of treated fish after exposure to 80 ppm for 48 hrs. Note intensely stained RBCs and very large vacuoles in their cytoplasm. X 240.

Fig.No. 7 : Blood smear of treated fish after exposure to 80 ppm for 72 hrs. Note intensely stained cytoplasm of RBCs. Interestingly some RBCs show binucleated condition. Also note enhanced size and staining of WBCs. X 240.

Fig.No. 8 : Blood smear of treated fish after exposure to 120 ppm for 60 hrs. Note enlarged size of vacuoles and reduction of the RBCs. Also note increase in the number of WBCs in their size. X 240.

nuclei showed slightly enhanced staining. Cytoplasm of few RBCs showed small vacuoli (Plate No.1, fig. 4). WBC number was found elevated.

80 ppm exposure :

RBCs were stained intensely (Plate No.1, figs. 5,6,7). Their cytoplasm contained many vacuoles. In some RBCs binucleated condition was observed. The size of the WBC was increased (Plate No.1, fig. 7). They were stained deeply.

120 ppm exposure :

The vacuoles in the RBCs were enlarged and their nuclear size was reduced. The number of WBC was increased (Plate No.1, fig.8).

160 ppm exposure :

The nuclear and cellular hypotrophy (Plate No.2, fig.3) and bursting occurred (Plate No.2, fig. 4). Most of the RBCs contained many large vacuoles (Plate No.2, figs. 1,2). The WBC number was increased.

200 ppm exposure :

Many pathological changes were occurred in the peripheral blood cells when compared to the controls. Some of the RBCs were hypochromic (Plate No.2, figs. 5,6,7) and had clumped chromatin material. Some of the RBCs had ragged appearance and a few were anucleate. In some RBCs darkly stained material was deposited at the circumference (Plate No.2, fig.6). Haemolysis was distinctly observed (Plate No.2, figs. 7, 8). WBC number was increased enormously (Plate No.2, fig.8).

PLATE NO. 2, Figs. 1 to 8

(Morphological changes in blood cells induced after exposure of fish S. mossambica to natural piscicide in the ethanol extract of leaves of L. eriocephalus)

Fig. No. 1 : Blood smear of treated fish after exposure to 160 ppm for 12 hrs. Note cytoplasm of RBCs filled with many large vacuoles and reduction in their nuclear size. X 240.

Fig. No. 2 : Blood smear of treated fish after exposure to 160 ppm for 24 hrs. Note changed shape of RBCs. X 240.

Fig. No. 3 : Blood smear of treated fish after exposure to 160 ppm for 36 hrs. Note spindle shaped RBCs and hypotrophy of their cytoplasm and nuclei. X 240.

Fig. No. 4 : Blood smear of treated fish after exposure to 160 ppm for 48 hrs. Note nuclear and cellular hypotrophy and bursting of RBCs. X. 240.

Fig. No. 5 : Blood smear of treated fish after exposure to 200 ppm for 12 hrs. Note many hypochronic RBCs. X 240.

Fig. No. 6 : Blood smear of treated fish after exposure to 200 ppm for 24 hrs. Note darkly stained material on the surface of RBCs. X 240.

Fig. No. 7 : Blood smear of treated fish after exposure to 200 ppm for 36 hrs. Note ragged and anucleate RBCs. Also note the clumping of nuclear material in them. X 240.

Fig. No. 8 : Blood smear of treated fish after exposure to 200 ppm for 36 hrs. Note haemolysed RBCs and increased number of intensely stained WBCs. X 240.

4. DISCUSSION :

Behaviour of fish :

The behavioural responses of the fishes in relation to the toxicity of natural piscicide studied in the present investigation were similar to those recorded by the earlier workers on different plants toxins (Bhuyan, 1967, 1969; Chakraborty et al., 1972; Harold, 1987; Bhosale, 1988). The changes due to piscicide in the leaves of L. eriocephalus in the behaviour of S. mossambica were first time recorded by Harold (1987). He noted the hypersensitivity and disturbed responses to external stimuli of this fish. Similar responses have also been noted in R. danicohius (Nayak and Madhyastha, 1980) after exposure to DDT. The increased production of mucous was noted in R. daniconius exposed to SLS (Madhyastha and Nayak, 1979) and to the commercial detergent, point (Nayak and Madhyastha, 1980).

TRBC Number :

The changes in number of blood cells have been reported in stress conditions by number of earlier investigators. The decrease in red blood cells was observed under metallic stress after exposure to mercury in A. dispar (Hilmy et al., 1980) in S. mossambica (Naidu et al., 1984) and H. fossilis (Banerjee, 1986) after exposure to lead in carp (Tishinora, 1982) or exposure of C. fasciatus to manganese (Agrawal and Srivastava, 1980) and under the stress of hexavalent chromium to C. batrachus (Agarwal et al., 1985).

The cellular hypertrophy, bursting and agglutination of erythrocytes have been noted under urea$_{\wedge}^{stress}$ in C. mrigala fingerlings (Srivastava and Srivastava, 1980).

Pesticide toxicity also induces increase or decrease in the blood cell counts. Exposure to alachlor resulted decrease in the RBC count and increase in leucocyte count in C. batrachus (Goel et al., 1984). In this fish the total erythrocyte count was also found decreased in the wake of increasing concentrations of BHC (Thakur and Pandey, 1989). Likewise a significant decrease in the TRBC count was recorded throughout the 28 days of exposure to a mixture of sodium lauryl sulfate, an active part of detergent and DDT in the fish R. daniconius (Madhyastha and Nayak, 1979).

Contrary to the above observations, Natrajan (1984) has noted an increase in the RBC count in the fresh water air breathing fish C. striatus on exposure to sublethal concentration of metasystox. Similarly, Nayak and Madhyasta (1980) observed a significant increase (< 0.05) in the number of immature red cells was observed in R. daniconius after 30 days exposure in a concentration of 100 ppm of detergent (Point). But they observed decrease in the mature red cells in this fish. According to them these changes might be attributed to stress and destruction of mature RBCs as evidenced by the significant (< 0.05) increase in the number of immature RBCs which might be due to an erythropoietic response caused by the detergent. These immature RBCs might be pushed into circulation to meet the additional requirement of oxygen demanded by the tissues. This observation is in accordance with the findings of McLeay (1973) and McLeay and Brown (1974) who observed the effect

of bleached kraft pulpmill effluent on the blood cells of Coho salmon (O. kistuch).

Different plant products also influence the number of blood cells in the fish. The vegetable oil effluent like aldrin (insecticide) and swascofix CD-38 affected the RBC number of C. batrachus after 30 days exposure. RBC count was found decreased with increased concentrations of these pollutants and time of exposure (Saxena and Bhatia, 1983). Saponin is one of the plant derived piscicides known to cause haemolysis in fishes (Anonymous, 1952; Chopra et al., 1958). But according to Ramanujam and Ratha (1979) although piscicidal componants are present in the fruits of Z. armatum, they do not have haemolytic properties in fish H. fossilis.

From the above discussion it is evident that except few cases the number of mature red blood cells decreases whereas the number of immature red blood cells in the circulation increases. To compensate this decrease the erythropoitic response in the head kidney was evident as revealed by an increase in the number of the precursor cells of the RBCs (Madhyasta and Nayak, 1979). In the histological observations on the peripheral blood cells, the decrease in the number and in the intensity of the reaction encountered was thought due to the presence of immature cells in the circulation. Thus, the present finding is in agreement with the observations of Blaxhall and Daisley (1973), Madhyasta and Nayak (1979) and Nayak and Madhyasdtha (1980). On this aspect, the differential red blood cell count would have given the accurate picture of the effect of this natural piscicide in the ethanol extract of the leaves of

L. eriocephalus on the haematological parameters of this fish and the view that a increase in the number of precursor cells of the myelocytic series in the head kidney would have been strengthened.

TWBC Number :

Total WBC count showed an increase in their number from 47.70 ± 4.62 to 53.40 ± 4.27 x $10^3/mm^3$ in treated fish. The changes observed in case of WBCs were just opposite to those of RBCs. In the present investigation the diferential count of WBCs was not observed.

Increase in the WBC count has been observed in the toxicity due to metals, pesticides, detergents, etc. Hilmy et al. (1980) observed greater concentration of leucocytes in response to mercury toxicity in the marine teleost A. dispar. Toshinora (1982) observed a lymphocytes decrease in the higher Pb dose. But Pb at 8 mg/l caused increase in neutrophils. Manganese poisoning evoked leucocytosis leading to an increase in the number of small lymphocytes (Agrawal and Srivastava, 1980). Alachlor resulted in a significant increase in the total leucocyte count in C. batrachus (Goel et al., 1984). According to them an increase in the number of leucocytes may be to cope up with the removal of cell debris of necrosed tissues under the toxicant stress. Leucocytosis has also been reported by Goel and Garg (1980) in C. punctatus when exposed to 2', 4' - diamino, 3'- aminoazobenzene (DAAB). Leucocytosis in pesticide toxicity may suggest inflamation to tissues. In the mixture of SLS + DDT caused significant increase in R. daniconious of TWBC, smudge cells, neutrophils and plasmocytes throughout the 28 days of exposure (Madhyastha and Nayak, 1979). They also observed that the monocytes

increased significantly only after 21 days of exposure which may be due to large scale destruction of the blood cells. There was comparative elevation of large lymphocytes in the experimental fish after 28 days exposure period whereas, the small lymphocytes exhibited a decrease. In R. daniconius, TWBC count, the neutrophils, monocytes and the thrombocytes were found increased whereas the small lymphocytes and eosinophils were decreased after exposure to detergent (Point) for 30 days.

The increase in the number of circulating neutrophils following BKME exposure was found in Coho salmon exposed to bleached Kraft pulpmill effluent for 25 days (McLeay, 1973). Both ACTH administration and stressfull stimuli have been reported to increase the circulating neutrophil counts in Salmonid fish (McLeay, 1973) or exposure to melachite green also led to increase in neutrophil count (Grizzle, 1977). The mammalian neutrophil is responsible for phagocytosis and disposal of foreign material or damaged tissues. McLeay and Brown (1976) assumed a similar role for the fish neutrophil and according to them the elevated neutrophil count in BKME exposed salmon suggest some tissue damage or entrance of foreign substance. Thus as suggested by these investigators and also by Nayak and Madhyastha (1980) the elevated WBC count in fish S.mossambica exposed to ethanol extract of the leaves of L. eriocephalus suggests damage by the natural piscicide in this extract or the entrance of some foreign material.

On the other hand, a decrease in the WBC count was observed after hexavalent chromium intoxication in C. batrachus (Agrawal et al., 1985). Exposure of rainbow trount S. gairdneri to melachite green at

1.35, 13.5 or 21.0 mg/l for 25-30 min or 42.0 or 72.0 mg/l for 5 min caused chronic leukopenia. Total leucocytes count was found declined (Hcauk and Bulkley, 1981). According to them such decrease was probably as a result of non-sepecific vertebrate stress syndrome rather than of a specific leucocytotoxic effect of this chemical. Similarly in C. striatus, the sublethal concentrations of metasystox decreased the WBC count (Natrajan, 1984). The toxicity due to vegetable oil effluent caused significant decrease in WBC count in C. batrachus (Saxena and Bhatia, 1983). The decrease in WBC count seems to be due to continuous stress of natural piscicide. The present observation is in accordance to Madhyastha and Nayak (1979) who suggested that stress is attributable to increase in the number of TWBCs.

Blood Cell Morphology :

In the present study, abnormal sizes and shapes of erythrocytes were commonly observed. These findings are in accordance with the findings of few previous investigagors. Bulkley (1977) who observed changes in the morphology of red blood cells of Coho salmon (O. kistuch) exposed to sublethal levels of total residual chlorine in municipal waste water. Madhyasta and Nayak (1980) observed many RBCs with vacuoles, micronucleus and dividing type of red blood cells. In many cases the chromatin material was very loosed and abundant in R. daniconius exposed to 100 ppm of a commercial detergent for 30 days. In this study, they also observed elongated to dumbell shaped RBCs and eccentric nucleus or two dark staining nuclei connected by chromatin material within a single cell. The significant decrease in the dimensions of the RBCs after

28 days of exposure to SLS + DDT in R. daniconius was found due to stress (Madhyastha and Nayak, 1979). A decrease in the RBC size has been reported in gold fish (Murray and Burton, 1979) and Ictalurus punctatus (Murray, 1980) maintained at different loading densities which caused fish density syndrome for RBC morphology. Tishinora (1982) also observed morphological changes in erythrocytes of a carp exposed to lead. Different abnormal shapes from spherical to oval of RBCs and the colour changes of nucleus from dark brown red to pink were reported by Srivastava and Sriwasthawa (1980) in the fingerlings of C. mrigala. Erythrocytes and their nuclei of C. punctatus showed hypochromasia, vacuolisation and deformities of shape in the exposure to Thimet 10 G (2.0 mg/l), Elsan 2% dust (10.0 mg/l) and Bazanon 5 G (14.0 mg/l). These changes were supposed as immediate effect of these pesticides (Banerjee and Verma, 1988).

As against above observations, Banerjee (1986) has noted that the exposure of heavy metal mercuric sulfate of 3.0 mg/l concentration had no effect on the length-breadth ratios of erythrocytes and eruthrocyte nucleus and as such there was no significant changes in erythrocyte and nuclear surface area in H. fossilis.

All the abnormalities observed in the present investigation are similar to the observations of Smith (1968) who observed many abnormal erythrocytes with distorted nuclei in the population of mature and old cells in salmonids, carp and catfishes after feeding diets deficient in folic acid but adequate in vitamin B_{12}.

Clotting time :(CT) :

According to Agrawal and Srivastava (1980) clotting time did not differ significantly from the control group in fresh water fish C. fasciatus when exposed to Mn. But increase in clotting time was observed during murcury toxicity in the marine teleost, A. dispar (Hilmy et al.,1980;1983). As against this the exposure of C. batrachus to sublethal concentration of aldrin (insecticide) and Swascofix CD-38 led to a decrease in the clotting time (Saxena and Bhatia, 1983). The results of the present investigation showed decreased clotting time similar to those observed by Saxena and Bhatia (1983) and Madhyastha and Nayak (1980;1985).Thus there are contraversial reports about this haematological parameter. In the present study, the clotting time decreases with the increase in concentration of ethanol extract of leaves of L. eriocephalus and with the increase in time of exposure. From these observations nothing positive conclusion can be drawn. More statistical information on many fishes about clotting time is required for critical discussion.

Haemoglobin (THb) :

Haemoglobin concentration is invariably studied under stress environment. In the present study Hb percentage declined from 11.13 $\pm$ 0.22 gm/100 ml of control to 9.81 $\pm$ 0.21 gm/100 ml of experimental fish when exposed to 200 ppm concentration of ethanol extract of L. eriocephalus leaves for 36 hrs, causing 11.70% decrease. This observation coinsides with the observation of Agrawal et al. (1985) who noted decrease in Hb in C. batrachus under the metallic stress of hexavalent chromium. Goel et al. (1982) in H. fossilis after melathion treatment and

Goel et al. (1984) in C. batrachus after alachlor treatment observed the significant fall in Hb content. Lead toxicity in C. batrachus also led to decrease in Hb% (Srivastava and Mishra, 1979). In C. batrachus exposure of various concentrations of BHC decreased Hb concentration (Thakur and Pandey, 1989). Similarly, in R. daniconius the treatment of mixture of SLS + DDT caused profound decrease in THb (Madhyastha and Nayak, 1979). The piscicide from plant origin (from the fruit of Z. armatum) also influenced the Hb concentration, which showed decrease with the increase in toxin dose and with the change of time (Ramanujam and Ratha, 1980). Feeding of T-2 toxin to rainbow trout, S. gairdneri depressed blood Hb concentration (Coffin and Combs, 1982).

As against above observations no significant decrease or increase in Hb was observed in C. fasciatus when exposed to manganese poisoning (Agrawal and Srivastava, 1980). In A. anguilla an increase in Hb was noted after exposure to pentachlorophenol (PCP) by Holmberg et al. (1972).

One thing was observed that whenever Hb concentration was reduced, it led to a microcytic hypochromic anaemia (Carla and Martelli, 1979). In lead toxicity also it led to erythropenia and anaemia in carp (Tishinora, 1982). Decrease in Hb in C. batrachus resulted in acute macrocytic anaemia due to hexavalent chromium poisoning (Agrawal et al., 1985). Anaemic condition has also been reported in fish H. fossilis after melathion treatment (Goel et al., 1982), in C. batrachus after alachlor toxicity (Goel et al., 1984) and in C. batrachus after exposure to BHC (Thakur and Pandey, 1989). Macrocytic anaemia is found associated

with leucocytosis suggesting inflamation in tissues. Erythropoiesis depression was another cause for anaemia in BHC intoxication (Thakur and Pandey, 1989). In some cases of toxicity haemolysis was also observed (Ramanujam and Ratha, 1980; Thakur and Pandey, 1989). Macrocytic anaemia also points to respiratory stress. Therefore, at higher doses increased number of opercular movements were observed in the present investigation.