IV. ENZYMOLOGY

IV. ENZYMOLOGICAL STUDIES ON ALKALINE PHOSPHATASE IN SOME TARGET ORGANS OF S. MOSSAMBICA AFTER EXPOSURE TO NATURAL PISCICIDE

1) INTRODUCTION :

Heavy metals and synthetic pesticides are known to produce changes in acid and alkaline phosphatase activity in the target organs of fish. But there is very little information on the effect of natural piscicides on these enzymes in important organs like liver, kidney, gill and brain of the fish. The present investigation deals with the variations in the activity of alkaline phosphatase in the above mentioned organs of S. mossambica during natural piscicide intoxication.

2) ENZYMOLOGICAL OBSERVATIONS :

i) Liver :

a) Biochemical Observations :

Alkaline phosphatase activity in terms of μ mol p-nitrophenol phosphate per gram tissue wet weight showed changes in relation to the impact of natural piscicide. The variations in the activity occured from 4630 ± 75 μ moles in the liver of the control fish to 6280 ± 148 μ moles in the experimental fish after exposure to 200 ppm for 36 hrs. The changes in alkaline phosphatase activity are compiled in Table No.5 and they are shown graphically in Graph No. 5.

TABLE - 5

Alkaline phosphatase activity in the liver of S. mossambica after exposure to the ethanol extract of leaves of L. eriocephalus.

Time intervals of intoxication. (Hrs)	Alkaline phosphatase activity at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	4630 ± 75	4630 ± 75	4630 ± 75	4630 ± 75	4630 ± 75	4630 ± 75	4630 ±75
12	4630 ± 98	4810 ± 85 (3.88%)	4932 ± 113 (6.52%)	5270 ± 121 (13.82%)	5590 ± 136 (20.74%)	5780 ± 129 (24.83%)	5992 ± 137 (29.41%)
24	4640 ± 35	4919 ± 92 (6.01%)	4980 ± 109 (7.32%)	5350 ± 135 (15.30%)	5615 ± 130 (21.01%)	5832 ± 133 (25.68%)	6132 ± 122 (32.21%)
36	4635 ± 42	4998 ± 87 (7.83%)	5132 ± 112 (10.72%)	5380 ± 119 (16.07%)	5685 ± 122 (22.65%)	5920 ± 121 (27.72%)	6280 ± 148 (35.49%)
48	4640 ± 64	5032 ± 102 (8.44%)	5198 ± 105 (12.02%)	5415 ± 126 (16.70%)	5715 ± 131 (23.16%)	6020 ± 126 (29.74%)	-
60	4645 ± 51	5116 ± 94 (10.13%)	5210 ± 112 (12.16%)	5485 ± 123 (18.08%)	5790 ± 138 (24.65%)	-	-
72	4656 ± 11	5185 ± 97 (11.50%)	5285 ± 108 (13.65%)	5520 ± 120 (18.70%)	-	-	-
84	4648 ± 54	5209 ± 98 (12.06%)	5315 ± 98 (14.35%)	-	-	-	-
96	4653 ± 61	5280 ± 109 (13.47%)	-	-	-	-	-

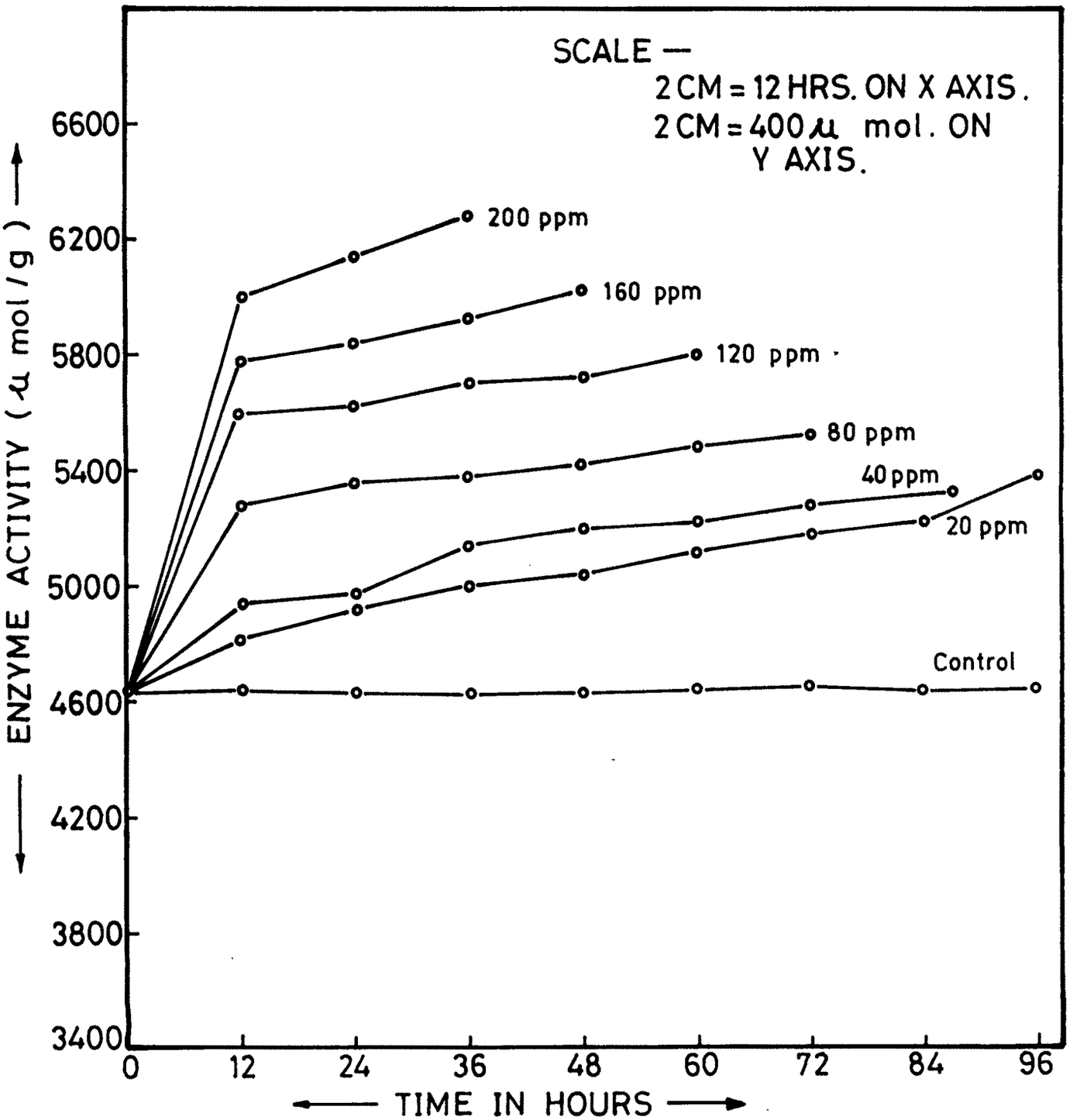
N.B. :

a) Alkaline phosphatase activity is expressed in terms of μ mol p-nitrophenol phosphate per gm tissue wet weight.

b) Figures in the brackets indicate per cent increase in alkaline phosphatase activity.

c) Average length of fish = 19 cm.

d) Average weight of fish = 45 gm.



GRAPH NO. 5 — ALKALINE PHOSPHATASE ACTIVITY IN THE LIVER OF S. MOSSAMBICA AFTER EXPOSURE TO ETHANOL EXTRACT OF LEAVES OF L. ERIOCEPHALUS.

Alkaline phosphatase activity in the liver was increased with the increasing concentration of ethanol extract and with the increase in duration of the intoxication. The enzyme activity in terms of μ moles/gm of the control fish remained more or less constant in all the experimental sets showing 4630 ± 98 after 12 hrs, 4640 ± 35 after 24 hrs, 4635 ± 42 after 36 hrs, 4640 ± 64 after 48 hrs, 4645 ± 51 after 60 hrs, 4656 ± 11 after 72 hrs, 4648 ± 54 after 84 hrs and 4653 ± 61 after 96 hrs.

After exposure to 20 ppm these activities were 4810 ± 85, 4919±92, 4998 ± 87, 5032 ± 102, 5116 ± 94, 5185 ± 97, 5209 ± 98 and 5280 ± 109 after 12, 24, 36, 48, 60, 72, 84, and 96 hrs, respectively. After exposure to 40 ppm the values of the enzyme activity were 4932 ± 113, 4980 ± 109 5132 ± 112, 5198 ± 105, 5210 ± 112, 5285 ± 108 and 5315 ± 98 for 12, 24, 36, 48, 60,72 and 84 hrs,respectively.

Whereas after exposure to 80 ppm enzyme activities were 5270±121, 5350 ± 135, 5380 ± 119, 5415 ± 126, 5485 ± 123 and 5520 ± 120 after 12, 24, 36, 48, 60 and 72 hrs,respectively. After exposure to 120 ppm these values were 5590 ± 136, 5615 ± 130, 5685 ± 122, 5715 ± 131 and 5790±138 for 12, 24, 36, 48 and 60 hrs, respectively.

After exposure to 160 ppm the estimated enzyme activities were 5780 ± 129, 5832 ± 133, 5920 ± 121 and 6020 ± 126 after 12, 24, 36 and 48 hrs, respectively. In the highest concentration of 200 ppm enzyme activities were 5992 ± 137, 6132 ± 122 and 6280 ± 148 for 12, 24 and 36 hrs, respectively.

Thus the enzyme activity was increased by 35.49% at the highest concentration after 36 hrs than the control.

b) Histoenzymological Observations :

The histoenzymological localization and changes in the activity of alkaline phosphatase in the liver of S. mossambica were very distinctly observed during natural piscicide intoxication. The histoenzymological data on staining reactions of alkaline phosphatase in the liver are recorded according to the visually estimated intensity and shade with one plus representing the controlled activity and ++, +++, ++++ signs representing the moderate, intense and very intense activity respectively. Alkaline phosphatase activity showed distinct alteration during natural piscicide intoxication which have been summarised in Table No.6. The localization and alterations in the activity of alkaline phosphatase in the liver are illustrated microphotographically in Plate No.3, figs. 1 to 6.

In treated fish the alkaline phosphatase activity was distinctly observed in certain cells and surrounding the blood vessels and sinusoids. The enzyme activity at these sites was slightly increased after exposure to 20 ppm (Plate No.3, fig.2) which went on increasing in 40 ppm (Plate No.3, fig.3), in 80 ppm (Plate No.3, fig.4), in 120 ppm (Plate No.3, fig.5) and reaching very intense in 200 ppm (Plate No.3, fig.6).

ii) Kidney :

a) Biochemical Observations :

Alkaline phosphatase activity in terms of μ mol p-nitrophenol phosphate per gram kidney tissue wet weight showed changes in relation to impact of natural piscicide. The variations in the activity in the kidney occured from 5035 ± 126 of the control fish to 6916 ± 124 in the experimental fish after exposure to 200 ppm for 36 hrs. The changes in the

TABLE - 6

Histoenzymological observations on alkaline phosphatase activity in the liver of *S. mossambica* after exposure to the ethanol extract of leaves of *L. eriocephalus*.

Time intervals of intoxication.	Alkaline phosphatase activity at different concentrations of ethanol extract													
	Cells surrounding blood vessels and sinusoids :							: Hepatocytes						
(Hrs.)	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)	: Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
12	+	+	+±	+±	++	++±	+++	+	+	+	+	+	+	++
24	+	+±	+±	+±	++	++±	+++±	+	+	+	+	++	++	++±
36	+	+±	+±	+±	++±	+++	+++⁺	+	+	+	+±	++	++	++⁺
48	+	+±	+±	+±	++±	+++	-	+	+	+	+±	++±	+++	-
60	+	+±	+±	++	++⁺	-	-	+	+	+	+±	++⁺	-	-
72	+	+±	+±	++	-	-	-	+	+	+	++	-	-	-
84	+	+±	++	-	-	-	-	+	+	+±	-	-	-	-
96	+	++	-	-	-	-	-	+	+	-	-	-	-	-

N.B. :

+ = Activity in controlled tissues.
++ = Moderate reaction.
+++ = Intense reaction.
++++ = Very intense reaction.
± = Doubtful activity.
- = Activity was not recorded.

PLATE NO. 3, Figs. 1 to 6

(Histoenzymological alterations in the liver of S. mossambica after exposure to the natural piscicide in the ethanol extract of leaves of L. eriocephalus)

Fig. No. 1 : Alkaline phosphatase activity in the liver of control fish. X 200.

Fig. No. 2 : Alkaline phosphatase activity in the liver of fish exposed to 20 ppm for 96 hrs. X 200.

Fig. No. 3 : Alkaline phosphatase activity in the liver of fish exposed to 40 ppm for 84 hrs. X 320.

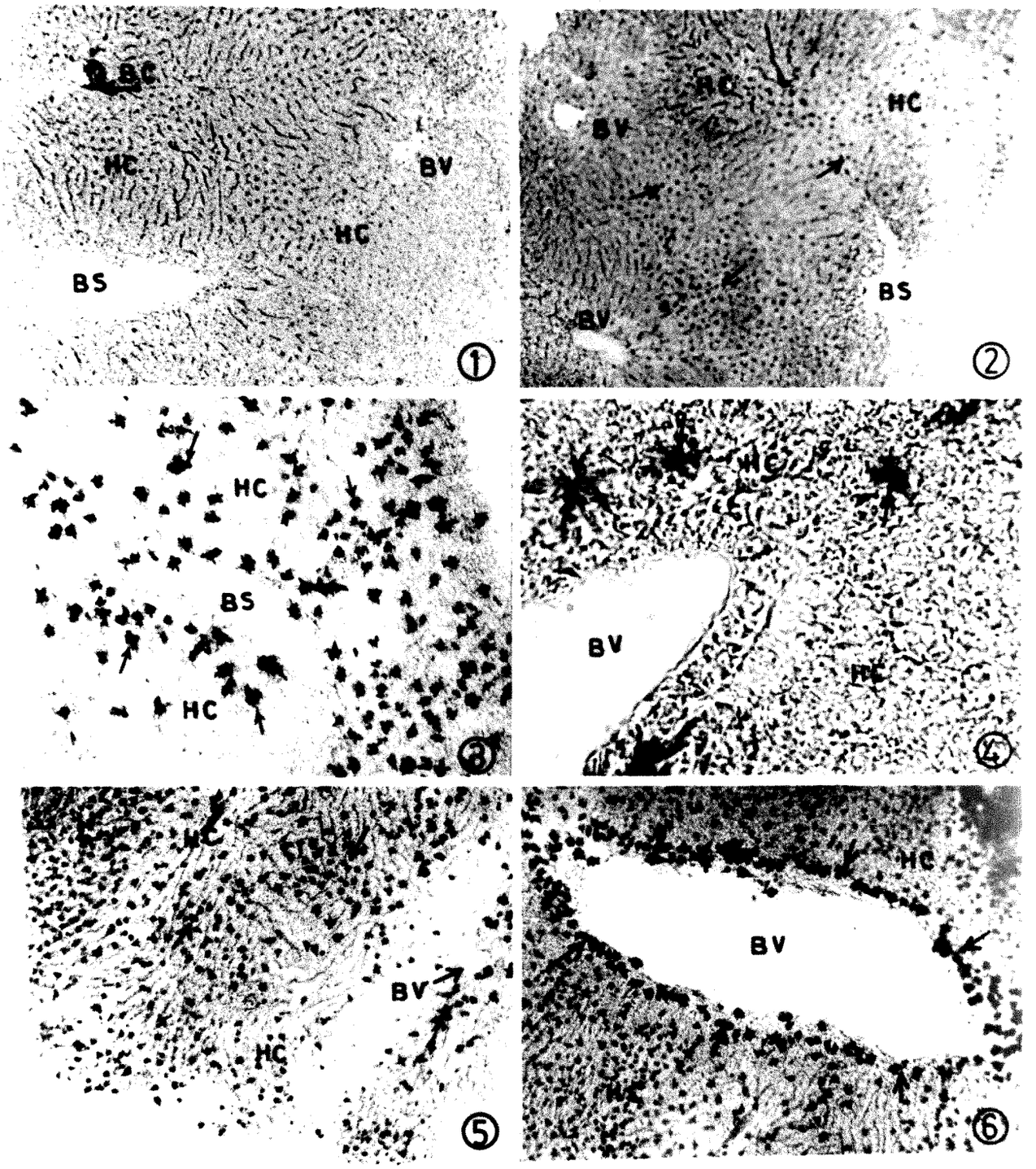
Fig. No. 4 : Alkaline phosphatase activity in the liver of fish exposed to 80 ppm for 72 hrs. X 400.

Fig. No. 5 : Alkaline phosphatase activity in the liver of fish exposed to 120 ppm for 60 hrs. X 200.

Fig. No. 6 : Alkaline phosphatase activity in the liver of fish exposed to 200 ppm for 36 hrs. X 200.

Abbreviations : BC - Blood cells.
BV - Blood vessel.
BS - Blood sinusoid.
HC - Hepatocytes.

PLATE No. 3



alkaline phosphatase activity are compiled in Table No.7 and they are shown graphically in Graph No.6.

Alkaline phosphatase activity in the kidney was increased with the increasing concentration of ethanol extract of L. eriocephalus leaves and with the increase in duration of intoxication. The enzyme activity in the control fish remained more or less constant in all the experimental sets showing 5035 ± 140, after 12 hrs, 5014 ± 97 after 24 hrs, 5026 ± 117 after 36 hrs, 5032 ± 54 after 48 hrs, 5041 ± 79 after 60 hrs, 5020 ± 135 after 72 hrs, 5052 ± 103 after 84 hrs and 5037 ± 80 after 96 hrs. After exposure to 20 ppm these activities were 5437 ± 47, 5687 ± 144, 5819 ± 122, 5887 ± 69, 5964 ± 117, 6015 ± 76, 6078 ± 151 and 6113 ± 64 after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. After exposure to 40 ppm the values of the enzyme activity were 5645 ± 79, 5912 ± 102, 6127 ± 43, 6275 ± 148, 6327 ± 58, 6409 ± 44 and 6486 ± 105 for 12, 24, 36, 48, 60, 72 and 84 hrs,respectively. Whereas after exposure to 80 ppm enzyme activities were 5832 ± 112, 5948 ± 87, 6183 ± 103, 6296 ± 113, 6339 ± 135 and 6492 ± 118 after 12, 24, 36, 48, 60 and 72 hrs,respectively. After exposure to 120 ppm these values were 6032 ± 137, 6215±53, 6307 ± 135, 6478 ± 58 and 6524 ± 114 for 12, 24, 36, 48 and 60 hrs, respectively. After exposure to 160 ppm the estimated enzyme activities were 6394 ± 159, 6502 ± 116, 6658 ± 69 and 6686 ±108 after 12, 24, 36 and 48 hrs,respectively. In the highest concentration of 200 ppm enzyme activities were 6574 ± 143, 6898 ± 85 and 6916 ± 124 for 12, 24 and 36 hrs,respectively.

Thus, the enzyme activity was increased by 37.60% at the highest concentration after 36 hrs than the control.

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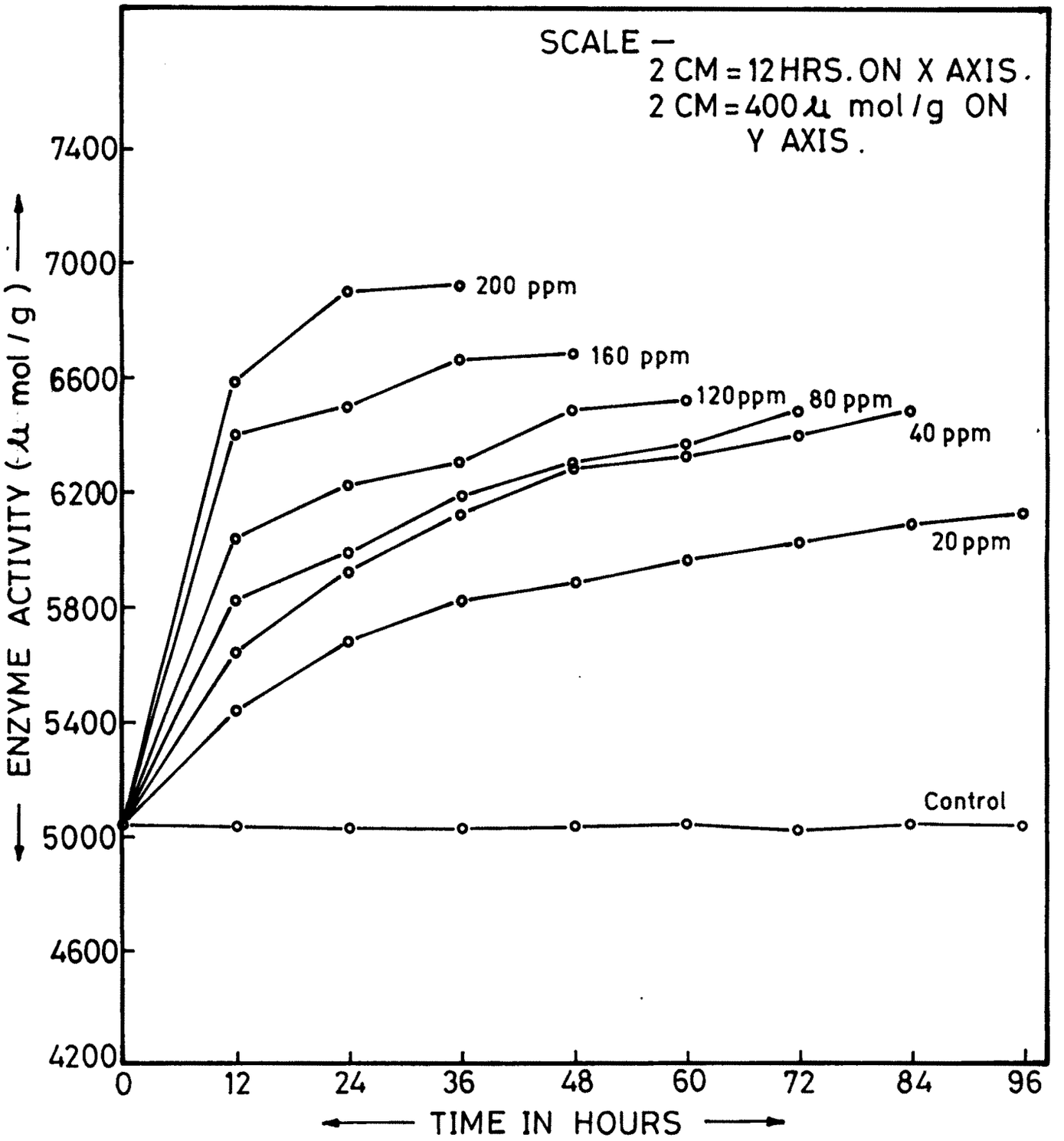
TABLE - 7

Alkaline phosphatase activity in the kidney of S. mossambica after exposure to the ethanol extract of leaves of L. eriocephalus.

Time intervals of intoxication. (Hrs)	Alkaline phosphatase activity at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	5035 ± 126	5035 ± 126	5035 ± 126	5035 ± 126	5035 ± 126	5035 ± 126	5035 ± 126
12	5035 ± 140	5437 ± 47 (7.98%)	5645 ± 79 (12.11%)	5832 ± 112 (15.82%)	6032 ± 137 (20.39%)	6394 ± 159 (26.99%)	6574 ± 143 (30.56%)
24	5014 ± 97	5687 ± 144 (13.42%)	5912 ± 102 (17.90%)	5948 ± 87 (18.62%)	6215 ± 53 (23.95%)	6502 ± 116 (29.67%)	6898 ± 85 (37.57%)
36	5026 ± 117	5819 ± 122 (15.77%)	6127 ± 43 (21.90%)	6183 ± 103 (23.02%)	6307 ± 135 (25.48%)	6658 ± 69 (32.47%)	6916 ± 124 (37.60%)
48	5032 ± 54	5887 ± 69 (16.99%)	6275 ± 148 (24.70%)	6296 ± 113 (25.11%)	6478 ± 58 (28.73%)	6686 ± 108 (32.86%)	-
60	5041 ± 79	5964 ± 117 (18.30%)	6327 ± 58 (25.51%)	6339 ± 135 (25.94%)	6524 ± 114 (29.41%)	-	-
72	5020 ± 135	6015 ± 76 (19.82%)	6409 ± 44 (27.66%)	6492 ± 118 (29.33%)	-	-	-
84	5052 ± 103	6078 ± 151 (20.30%)	6486 ± 105 (28.38%)	-	-	-	-
96	5037 ± 80	6113 ± 64 (21.36%)	-	-	-	-	-

N.B. :
a) Alkaline phosphatase activity is expressed in terms of μ mol p-nitrophenol phosphate per gm tissue wet weight.
b) Figures in the brackets indicate per cent increase in alkaline phosphatase activity.
c) Average length of fish = 14 cm.
d) Average weight of fish = 38 gm.



GRAPH NO. 6 — ALKALINE PHOSPHATASE ACTIVITY IN THE KIDNEY OF S. MOSSAMBICA AFTER EXPOSURE TO ETHANOL EXTRACT OF LEAVES OF L. ERIOCEPHALUS.

b) Histoenzymological Observations :

The histoenzymological localization and changes in the activity of alkaline phosphatase in the kidney of S. mossambica were very distinctly observed during natural piscicide intoxication. The histoenzymological data on staining reactions in the kidney are recorded according to the visually estimated intensity and shade with one plus representing the controlled activity and ++, +++, ++++ signs representing the moderate, intense and very intense activity which have been recorded in Table No.8. The localization and alterations in alkaline phosphatase in kidney during natural piscicide intoxication are illustrated microphotographically in Plate No.4, figs. 1 to 8.

The enzyme activity in the kidney of controlled fish was observed maximum in the proximal tubules, moderate in the glomeruli and very less in the distal tubules. The brush borders of the proximal tubules showed maximum activity. The cytoplasm of the tubular cells showed activity in the form of diffused black granules. The intensity of staining of the granules and their number was more towards the brush border. The nuclei did not show enzyme activity. The enzyme activity was in the form of a black ringaround the brush border in the distal tubules.

In treated fish the alkaline phosphatase activity was distinctly observed in brush border of proximal convulated tubules and glomeruli of kidney and it was less in distal tubules. The enzyme activity was slightly increased after exposure to 20 ppm (Plate No.4, fig.2) which went on increasing in 40 ppm (Plate No.4, fig.3), in 80 ppm (Plate No.4, fig.4), in 120 ppm (Plate No.4, figs.5 and 6), in 160 ppm (Plate No.4, fig.7) and reaching very intense in 200 ppm (Plate No.4, fig.8).

TABLE - 8

Histoenzymological observations on alkaline phosphatase activity in the kidney of *S. mossambica* after exposure to the ethanol extract of leaves of *L. eriocephalus*.

Time intervals of intoxication.	Alkaline phosphatase activity at different concentrations of ethanol extract																				
	Proximal tubules							Distal tubules							Glomeruli						
(Hrs.)	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
12	+	+	+±	+±	+⁺	++±	+++	+	+	+	+	+	+	+	+	+	+	+	±±	++	++
24	+	+±	+±	++	++	+++	+++±	+	+	+	+	+	+±	++	+	+	+±	+±	++	++±	+++
36	+	+±	++	++±	++±	+++±	++++	+	+	+	+	+	+±	++	+	+	+±	++	++	++±	+++
48	+	+±	++±	++±	++±	+++±	–	+	+	+	+	+±	++	–	+	+	+±	++	++	+++	–
60	+	+±	++±	++±	+++	–	–	+	+	+	+	+±	–	–	+	+±	++	++	++±	–	–
72	+	++	++±	+++	–	–	–	+	+	+	+	–	–	–	+	+±	++	++	–	–	–
84	+	++	+++	–	–	–	–	+	+	+	–	–	–	–	+	+±	++	–	–	–	–
96	+	++	–	–	–	–	–	+	+	–	–	–	–	–	+	+±	–	–	–	–	–

N.B. :
+ = Activity in controlled tissues.
++ = Moderate reaction.
+++ = Intense reaction.
++++ = Very intense reaction.
± = Doubtful activity.
– = Activity was not recorded.

PLATE NO. 4, Figs. 1 to 8

(Histoenzymological alterations in the kidney of S. mossambica after exposure to the natural piscicide in the ethanol extract of leaves of L. eriocephalus)

Fig. No. 1 : Alkaline phosphatase activity in the kidney of control fish. X 150.

Fig. No. 2 : Alkaline phosphatase activity in the kidney of fish exposed to 20 ppm for 96 hrs. X 150.

Fig. No. 3 : Alkaline phosphatase activity in the kidney of fish exposed to 40 ppm for 96 hrs. X 200.

Fig. No. 4 : Alkaline phosphatase activity in the kidney of fish exposed to 80 ppm for 72 hrs. X 400.

Fig. No. 5 : Alkaline phosphatase activity in the kidney of fish exposed to 120 ppm for 48 hrs. X 150.

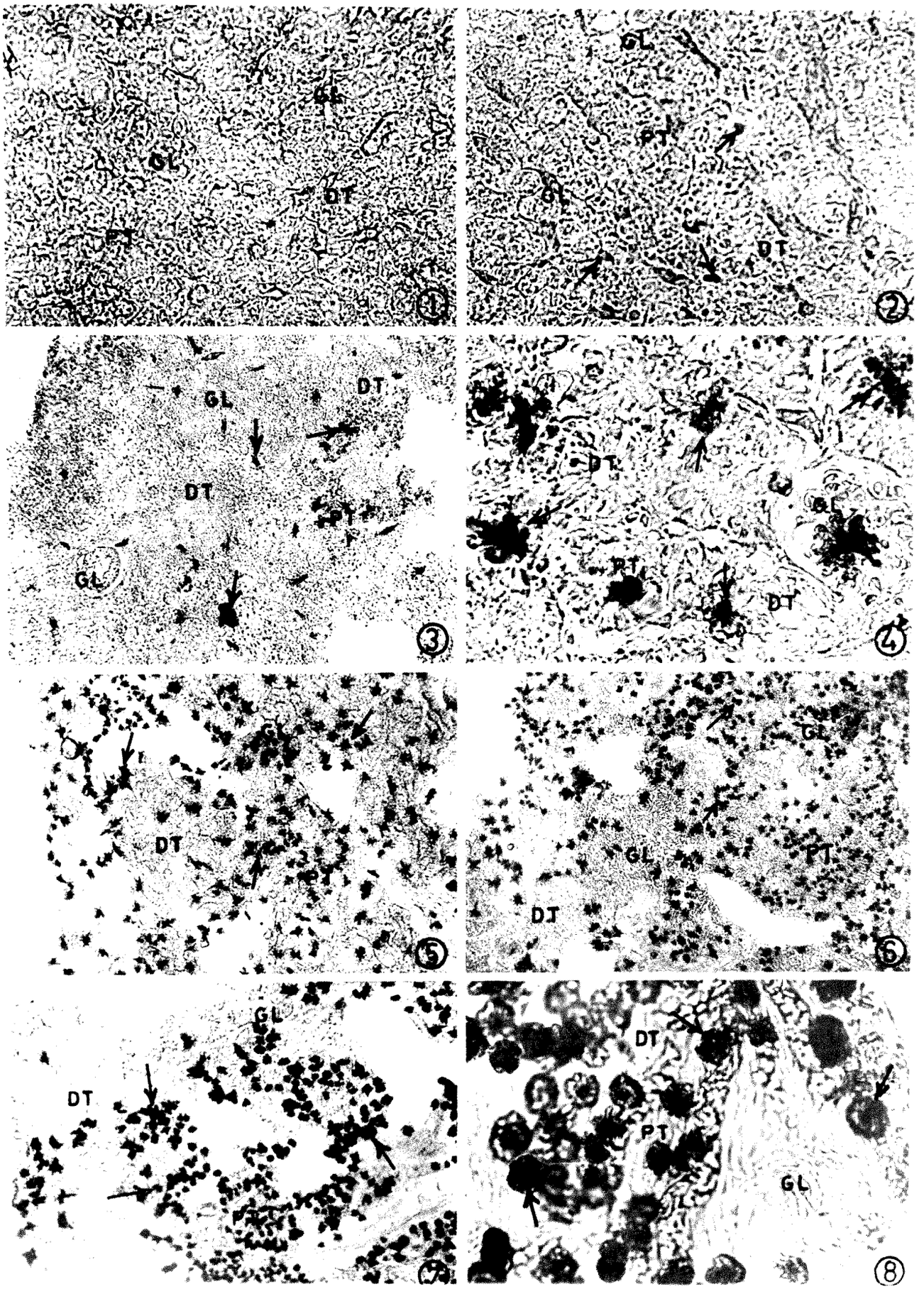
Fig. No. 6 : Alkaline phosphatase activity in the kidney of fish exposed to 120 ppm for 60 hrs. X 150.

Fig. No. 7 : Alkaline phosphatase activity in the kidney of fish exposed to 160 ppm for 48 hrs. X 150.

Fig. No. 8 : Alkaline phosphatase activity in the kidney of fish exposed to 200 ppm for 36 hrs. X 300.

Abbreviatioins : GL - Glomeruli.
DT - Distal tubules.
PT - Proximal tubules.

PLATE No. 4


GL
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iii) Gill :

a) Biochemical Observations :

Alkaline phosphatase activity in terms of μ mol p-nitrophenol phosphate per gram gill tissue wet weight showed changes in relation to impact of natural piscicide. The variations in the activity occured from 3142 ± 51 in the gill of control fish to 4764 ± 86 in the experimental fish after exposure to 200 ppm for 36 hrs. The changes in the alkaline phosphatase activity are compiled in Table No.9 and they are shown graphically in Graph No.7.

Alkaline phosphatase activity in the gill was increased with the increasing concentration of ethanol extract of leaves of L. eriocephalus and with the increase in duration of intoxication. The enzyme activity in the control fish remained more or less constant in all the experimental sets showing 3142 + 48 after 12 hrs, 3160 ± 82 after 24 hrs, 3125 ± 67 after 36 hrs, 3154 ± 40 after 48 hrs, 3146 ± 97 after 60 hrs, 3158 ± 76 after 72 hrs, 3139 ± 61 after 84 hrs and 3147 ± 85 after 96 hrs. After exposure to 20 ppm these activities were 3308 ± 51, 3414 ± 40, 3497 ± 92, 3544 ± 54, 3589 ± 76, 3613 ± 45, 3697 ± 71 and 3728 ± 63 after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. After exposure to 40 ppm the values of the enzyme activity were 3485 ± 64, 3516 ± 56, 3589 ± 84, 3664 ± 69, 3708 ± 28, 3781 ± 36 and 3824 ± 72 for 12, 24, 36, 48, 60, 72 and 84 hrs, respectively. Whereas after exposure to 80 ppm enzyme activities were 3618 ± 43, 3854 ± 76, 3935 ± 49, 4018 ± 85, 4186 ± 44 and 4294 ± 57 after 12, 24, 36, 48, 60 and 72 hrs, respectively. After exposure to 120 ppm these values were 3787 ± 66, 3931 ± 83,

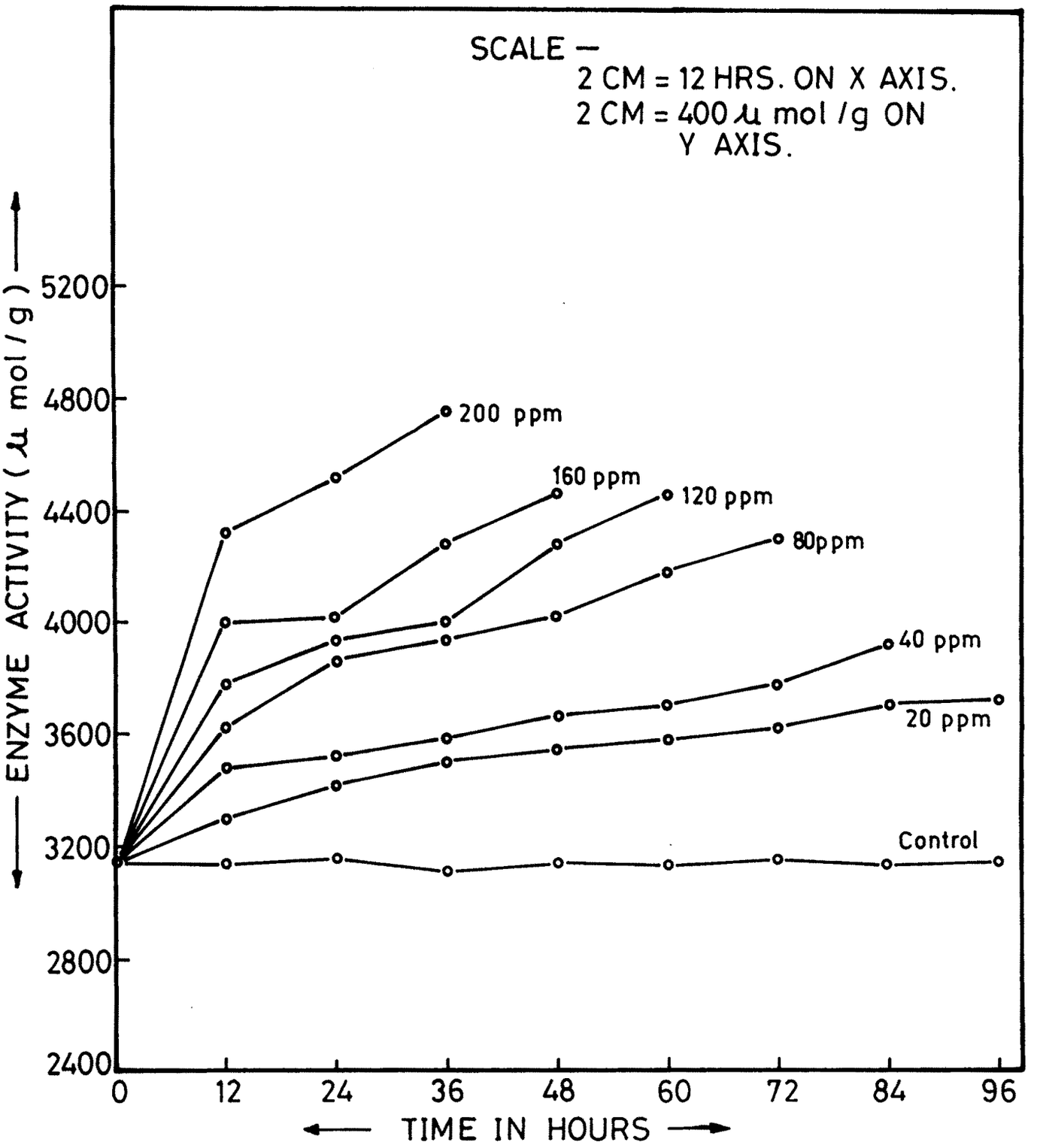
TABLE - 9

Alkaline phosphatase activity in the gill of *S. mossambica* during intoxication due to ethanol extract of leaves of *L. eriocephalus*.

Time interval of intoxication. (Hrs)	Alkaline phosphatase activity at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	3142 ± 51	3142 ± 51	3142 ± 51	3142 ± 51	3142 ± 51	3142 ± 51	3142 ± 51
12	3142 ± 48	3308 ± 51 (5.28%)	3185 ± 64 (10.90%)	3618 ± 43 (15.14%)	3787 ± 66 (20.52%)	3992 ± 78 (27.05%)	4317 ± 98 (37.39%)
24	3160 ± 82	3414 ± 40 (8.03%)	3516 ± 56 (12.26%)	3854 ± 76 (21.96%)	3931 ± 83 (24.39%)	4019 ± 59 (27.18%)	4521 ± 73 (43.06%)
36	3125 ± 67	3497 ± 92 (11.90%)	3589 ± 84 (14.84%)	3935 ± 49 (25.92%)	3992 ± 52 (27.74%)	4286 ± 48 (37.15%)	4764 ± 86 (52.44%)
48	3154 ± 40	3544 ± 54 (12.36%)	3664 ± 69 (16.16%)	4018 ± 85 (27.39%)	4276 ± 63 (35.57%)	4465 ± 77 (41.56%)	-
60	3146 ± 97	3589 ± 76 (14.08%)	3708 ± 28 (17.86%)	4186 ± 44 (33.05%)	4454 ± 53 (41.57%)	-	-
72	3158 ± 76	3613 ± 45 (14.40%)	3781 ± 36 (19.72%)	4294 ± 57 (35.97%)	-	-	-
84	3139 ± 61	3697 ± 71 (17.68%)	3824 ± 72 (21.82%)	-	-	-	-
96	3147 ± 85	3728 ± 63 (18.46%)	-	-	-	-	-

N.B. :

a) Alkaline phosphatase activity is expressed in terms of μ mol p-nitrophenol phosphate per gm tissue wet weight.
b) Figures in the brackets indicate per cent increase in alkaline phosphatase activity.
c) Average length of fish = 15 cm.
d) Average weight of fish = 39 gm.



GRAPH NO.7 — ALKALINE PHOSPHATASE ACTIVITY IN THE GILL OF S. MOSSAMBICA AFTER EXPOSURE TO ETHANOL EXTRACT OF LEAVES OF L. ERIOCEPHALUS.

3992 ± 52, 4276 ± 63 and 4454 ± 53 for 12, 24, 36, 48 and 60 hrs, respectively. After exposure to 160 ppm the enzyme activities were 3992±78, 4019 ± 59, 4286 ± 48 and 4465 ± 77 after 12, 24, 36 and 48 hrs, respectively. In the highest concentration of the 200 ppm enzyme activities were 4317 ± 98, 4521 ± 73 and 4764 ± 86 after 12, 24 and 36 hrs, respectively.

Thus the enzyme activity was increased by 52.44% at the highest concentration after 36 hrs than the control.

b) Histoenzymological Observations :

The histoenzymological localization and changes in the enzyme activities in the gill of S. mossambica were very distinctly observed during natural piscicide intoxication. The histoenzymological data on staining reactions in the gill are recorded according to the visually estimated intensity and shade with one plus representing the controlled activity and ++, +++, ++++ signs representing the moderate, intense and very intense activity which have been recorded in Table No.10. The localization and alterations in alkaline phosphatase activity in gill during natural piscicide intoxication are illustrated microphotographically in Plate No.5, figs. 1 to 8.

In control fish the alkaline phosphatase activity was localized prominantly in pilar cells of the secondary lamellae and moderate in cartilage tissue of gills.

In treated fish the alkaline phosphatase activity was distinctly increased in pillar cells and in cartilage. At lower concentrations of natural piscicide the enzyme activity was slightly increased after exposure to 20 ppm (Plate No.5, figs.1,2) which went on increasing in 40 ppm

TABLE - 10

Histoenzymological observations on alkaline phosphatase activity in the gill of *S. mossambica* after exposure to the ethanol extract of leaves of *L. eriocephalus*.

Time intervals of intoxication. (Hrs.)	Alkaline phosphatase activity at different concentrations of ethanol extract													
	Pillars cells of secondary lamellae							Cartilage						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
12	+	+	+	+±	++	++±	+++±	+	+	+	+	+	+	+±
24	+	+	+±	++	++±	+++	++++	+	+	+	+	+	+±	++
36	+	+	+±	++±	+++	+++±	++++	+	+	+	+	+±	+±	++
48	+	+±	+±	+++	+++±	++++	-	+	+	+	+±	+±	++	-
60	+	+±	+±	+++±	++++	-	-	+	+	+±	+±	++	-	-
72	+	+±	++	+++±	-	-	-	+	+	+±	++	-	-	-
84	+	+±	++	-	-	-	-	+	+	++	-	-	-	-
96	+	++	-	-	-	-	-	+	+	-	-	-	-	-

N.B. :

+ = Activity in controlled tissues.
++ = Moderate reaction.
+++ = Intense reaction.
++++ = Very intense reaction.
± = Doubtful activity.
- = Activity was not recorded.

PLATE NO. 5, Fig. 1 to 8

(Histoenzymological alterations in the gills of S. mossambica after exposure to the natural piscicide in the ethanol extract of leaves of L. eriocephalus)

Fig. No. 1 : Alkaline phosphatase activity in the gill of fish exposed to 20 ppm for 84 hrs. X 200.

Fig. No. 2 : Alkaline phosphatase activity in the gill of fish exposed to 20 ppm for 96 hrs. X 300.

Fig. No. 3 : Alkaline phosphatase activity in the gill of fish exposed to 40 ppm for 84 hrs. X 300.

Fig. No. 4 : Alkaline phosphatase activity in the gill of fish exposed to 80 ppm for 72 hrs. X 300.

Fig. No. 5 : Alkaline phosphatase activity in the gill of fish exposed to 120 ppm for 60 hrs. X 600.

Fig. No. 6 : Alkaline phosphatase activity in the gill of fish exposed to 160 ppm for 48 hrs. X 600.

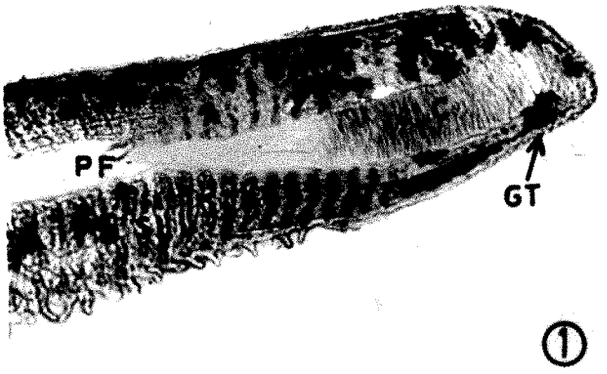
Fig. No. 7 : Alkaline phosphatase activity in the gill of fish exposed to 200 ppm for 24 hrs. X 300.

Fig. No. 8 : Alkaline phosphatase activity in the gill of fish exposed to 200 ppm for 36 hrs. X 600.

Abbreviations :

C	- Cartilage.	EP	- Epithelium.
AC	- Acidophil cells.	GT	- Gill tip.
BC	- Blood cells.	PF	- Primary filament.
PC	- Pillar cells.	SL	- Secondary lamella.

PLATE No. 5



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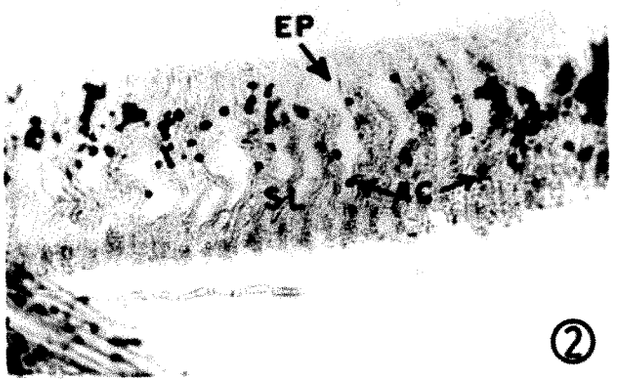

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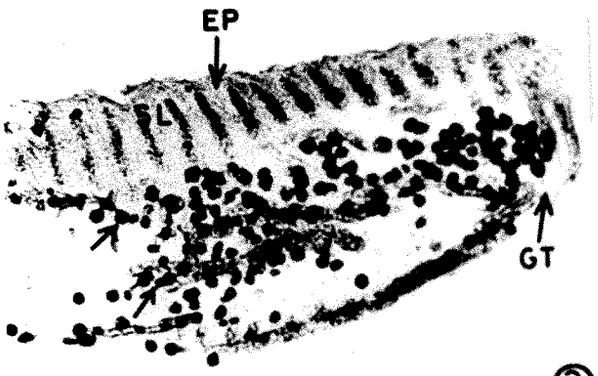

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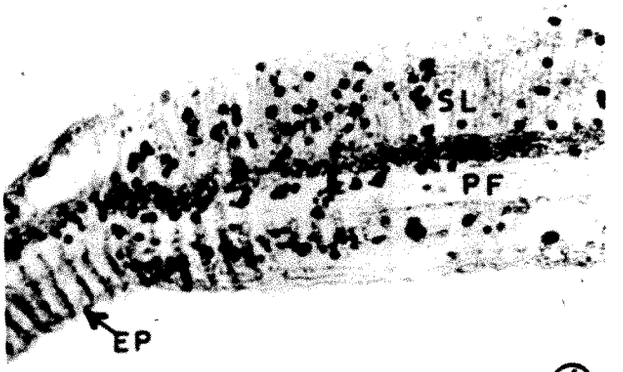

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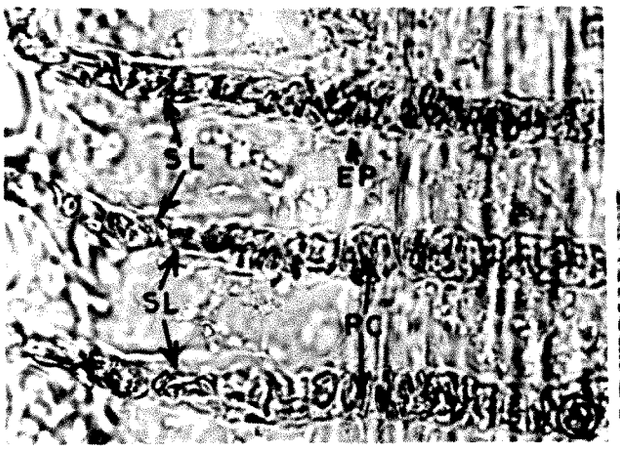

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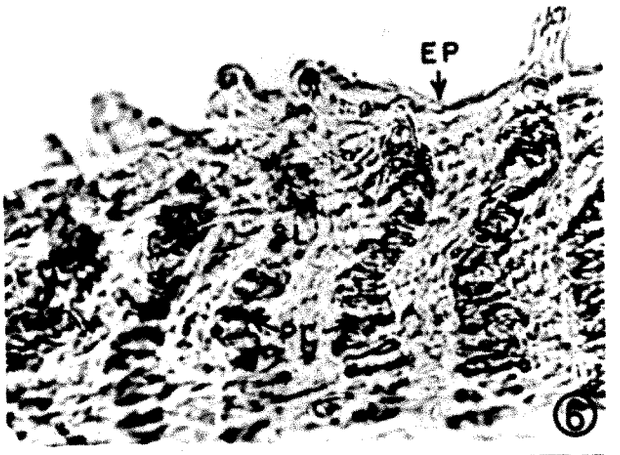

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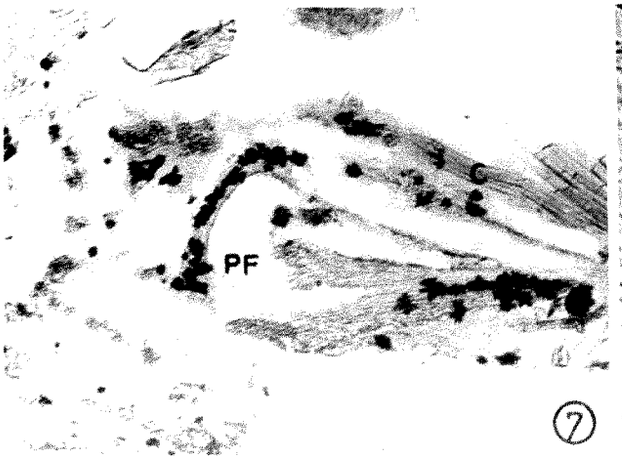

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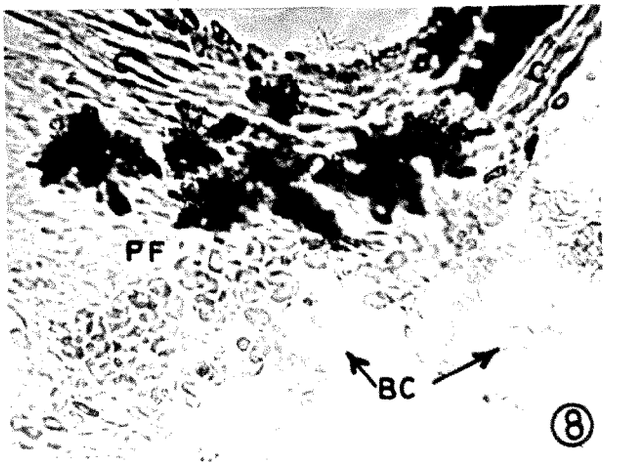

⑧

(Plate No.5, fig.3), in 80 ppm (Plate No.5, fig.4) in 120 ppm (Plate No.5, fig.5), in 160 ppm (Plate No.5, fig.6) and reaching very intense in 200 ppm. (Plate No.5, figs.7 and 8).

iv) Brain :

Alkaline phosphatase activity in terms of μ mol p-nitrophenol phosphate per gram brain tissue wet weight showed changes in relation to the impact of natural piscicide. The variations in the activity observed from 730 ± 37 in brain of the control fish to 946 ± 24 in the experimental fish after exposure to 200 ppm for 36 hrs. The changes in alkaline phosphatase activity are compiled in Table No.11 and they are shown graphically in Graph No.8.

Alkaline phosphatase activity in brain was increased with increasing concentration of ethanol extract of leaves of L. eriocephalus and with the increase in duration of the intoxication.

The enzyme activity in the control fish remained more or less constant in all the experimental sets showing 730 ± 32 after 12 hrs, 736 ± 13 after 24 hrs, 741 ± 36 after 36 hrs, 735 ± 46 after 48 hrs, 737 ± 26 after 60 hrs, 733 ± 28 after 72 hrs, 738 ± 25 after 84 hrs and 729 ± 34 after 96 hrs. After exposure to 20 ppm these activities were 763 ± 26, 779 ± 31, 786 ± 23, 794 ± 37, 802 ± 32, 817 ± 25, 825 ± 19 and 833 ± 24 after 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. After exposure to 40 ppm the values of enzyme activities were 789 ± 23, 802 ± 27, 813 ± 32, 821 ± 26, 834 ± 18, 846 ± 26 and 862 ± 39 after 12, 24, 36, 48, 60, 72 and 84 hrs, respectively. Whereas after exposure to

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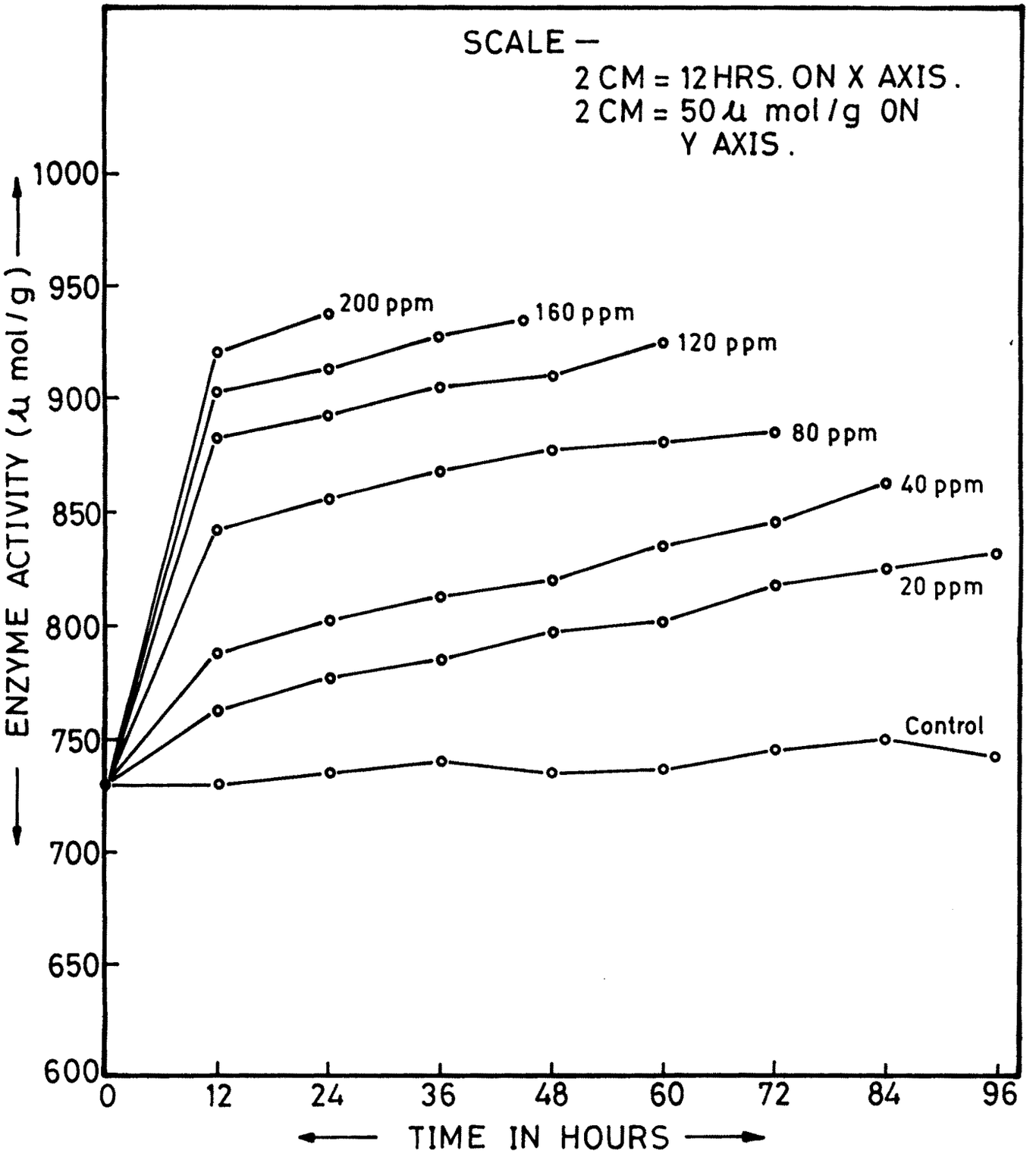
TABLE - 11

Alkaline phosphatase activity in the brain of *S. mossambica* after exposure to ethanol extract of leaves of *L. eriocephalus*.

Time intervals of intoxication. (Hrs)	Alkaline phosphatase activity at different concentrations of ethanol extract						
	Control	20 (ppm)	40 (ppm)	80 (ppm)	120 (ppm)	160 (ppm)	200 (ppm)
0	730 ± 37	730 ± 37	730 ± 37	730 ± 37	730 ± 37	730 ± 37	730 ± 37
12	730 ± 32	763 ± 26 (4.52%)	789 ± 23 (8.08%)	843 ± 31 (15.47%)	882 ± 29 (20.82%)	902 ± 32 (23.56%)	919 ± 41 (25.89%)
24	736 ± 13	779 ± 31 (5.84%)	802 ± 27 (8.96%)	856 ± 39 (16.30%)	893 ± 36 (21.33%)	912 ± 18 (23.91%)	937 ± 35 (27.30%)
36	741 ± 36	786 ± 23 (6.07%)	813 ± 32 (9.71%)	868 ± 35 (17.13%)	904 ± 18 (21.99%)	928 ± 49 (25.23%)	946 ± 24 (27.66%)
48	735 ± 46	794 ± 37 (8.02%)	821 ± 26 (11.70%)	877 ± 23 (19.13%)	910 ± 38 (23.80%)	925 ± 27 (25.85%)	-
60	737 ± 26	802 ± 32 (8.81%)	834 ± 18 (13.16%)	881 ± 19 (19.53%)	923 ± 29 (25.23%)	-	-
72	733 ± 28	817 ± 25 (11.25%)	846 ± 26 (15.41%)	885 ± 27 (20.73%)	-	-	-
84	738 ± 25	825 ± 19 (11.78%)	862 ± 39 (16.80%)	-	-	-	-
96	729 ± 34	833 ± 24 (14.09%)	-	-	-	-	-

N.B. :
a) Alkaline phosphatase activity is expressed in terms of μ mol p-nitrophenol phosphate per gm tissue wet weight.
b) Figures in the brackets indicate per cent increase in alkaline phosphatase activity.
c) Average length of fish = 17 cm.
d) Average weight of fish = 41 gm.



GRAPH NO. 8 — ALKALINE PHOSPHATASE ACTIVITY IN THE BRAIN OF S. MOSSAMBICA AFTER EXPOSURE TO ETHANOL EXTRACT OF LEAVES OF L. ERIOCEPHALUS .

80 ppm enzyme activities were 843 ± 31, 856 ± 39, 868 ± 35, 877 ± 23, 881 ± 19 and 885 ± 27 after 12, 24, 36, 48, 60 and 72 hrs, respectively. After exposure to 120 ppm the values were 882 ± 29, 893 ± 36, 904 ± 18 910 ± 38 and 923 ± 29 for 12, 24, 36, 48 and 60 hrs, respectively. After exposure to 160 ppm the estimated enzyme activities were 902 ± 32, 912 ± 18, 928 ± 49 and 925 ± 27 for 12, 24, 36 and 48 hrs, respectively. In the highest concentration of 200 ppm enzyme activities were 919 ± 41, 937 ± 35 and 946 ± 24 for 12, 24 and 36 hrs, respectively.

Thus, the enzyme activity was increased by 27.66% at the highest concentration after 36 hrs than the control.

3. DISCUSSION :

The earlier investigations on the effects of pesticides on fish are concerned with the histopathological changes in the tissues especially in liver, kidney and gills. But alterations in alkaline and acid phosphatase are very common during such stress conditions. Their accumulation points to tissue damage which helps in the assessment of toxicity. Therefore, the present investigation on alkaline phosphatase during natural piscicide intoxication was undertaken. In the present study the increased activity of this enzyme in the experimental fish has been observed in all the sets. Biochemically estimated enzyme activities of liver, kidney, gill and brain were found increased with increase in concentration of the dose and with the increase in duration of intoxication.

Increased activity of alkaline phosphatase and acid phosphatase in the serum is reported in Heteropneustes and Channa on exposure to pesticides and aminoazodyes, respectively (Goel et al., 1982; Goel and Garg, 1980) and in C. batrachus on exposure to alachlor (Goel et al.,1984) According to them the altered pattern of serum enzymology indicated the distorted metabolism resulting from liver disfunction or from cellular injuries in different tissues of experimental fish.

During malathion toxicity, alkaline and acid phosphatase activity in the liver of the fish B. rerio was found inhibited markedly (Kaushal and Ansari, 1986). On the other hand alkaline phosphatase activity registered a rise in liver of S. mossambica following exposure to sumithion (Koundinya and Ramamurthi, 1982). They suggested that an increase in phosphatase activity could attribute to hyperglycemia. Similarly hyperglycemia has also been reported to stimulate alkaline phosphatase activity by Lather (1975). Koundinya and Ramamurthi (1979) found that exposure of S. mossambica to sumithion increased blood glucose content, hepatic phosphorylase activity and decreased hepatic glycogen concentration. Since phosphorylation of glucose is an energy requiring process, increase in phosphatase activity catalyses liberation of inorganic phosphates from phosphate esters like glycerophosphate, phenyl phosphate, etc. is justifiable. Fall in hepatic glycogen concentration and increased phosphorylase activity after exposure to sumithion suggested glycogen as the source of hyperglycemia in the treated fish. Hence, it is likely that treatment of sumithion induced hyperglycemia as a result of glycogenolysis and consequent increase of phosphatase activity to meet energy requirements in order to counter the stress.

Bostrom and Johansson (1972) and Holmberg et al. (1972) have concluded that exposure to PCP increased the aerobic metabolism and reduced the anaerobic breakdown of carbohydrates in the liver. They also observed a decrease in activity of lactate dehydrogenase in the liver of PCP exposed eels. Dando (1969) did not find any relationship between the glycolysis in muscle tissue and the activities of lactate dehydrogenases in the liver. In these experiments, there was a significant elevation of plasma inorganic phosphate after the PCP exposure. Hence as suggested by Koundinya and Ramamurthi (1979) and Holmberg et al.(1972) have concluded that the rise in plasma phosphate reflects the breakdown of high energy phosphates, which probably takes place due to PCP.

In the present study, liver enlargement after exposure to ethanol extract was noticed. According to Johnson (1968) the enlargement of liver has been reported as a common result of pesticide action. Holmberg et al. (1972) also observed a significant increase in the liver-somatic index. According to them this increase cannot only be explained by the decrease in body weight but must also and be mainly due to an enlargement of the liver.

In the present study, the activity of kidney alkaline phosphatase was found increased 37.60% than the control after exposure for 36 hrs to 200 ppm of ethanol extract of this natural piscicide. The increase in the activity was dependent on dose and time of intoxication. The widespread occurrence of this enzyme in the kidney indicated its some fundamental role in this organ as suggested by Kuo and Bhimenthal (1961). According to Kuo and Bhimenthal (1961) alkaline phosphatase has greater

capacity for cation transport. The different segments of nephron of vertebrate kidney have been attributed diverse functions i.e. filtration at the glomerulus, absorption of water, Na^+ ions, etc. and secretion of urea at the proximal tubules and absorption of water at the distal tubules. Concomitant with this functional variations there is always variations in the alkaline phosphatase activity in these different segments with reference to localization. Such difference in the localization of alkaline phosphatase activity due to intoxication in the natural piscicide has been observed in the present study also. The function of liberation of phosphoric acid from its esters has been attributed to this enzyme (Robson and Soames, 1924). Goldfischer et al. (1964) located alkaline phosphatase activity at the cell surfaces e.g. at the absorptive surfaces of proximal tubules of the kidney and suggested the function of transport of phosphate across the cell membranes. We confirm this observation, because alkaline phosphatase activity in the present study was found maximum in the proximal tubules, medium in the glomerulus and less in the distal tubules. In vitro studies of acid and alkaline phosphatase in the killifish by Jackim et al. (1973) have revealed marked differences in enzyme activities in the kidney following exposure to copper and mercury. Hence whatever be the function(s) of the alkaline phosphatase at cellular level, no direct physiological role can be assigned to the enzyme in the vertevrate kidney.

As against above, metal and endosulfan toxicity showed inhibition in the alkaline phosphatase activity in fish kidney. Rana (1974) observed inhibition in this enzyme after lead nitrate treatment. He noted practically no activity in proximal tubules but some positive reaction in the

basal epithelium in this treatment. Similarly, after exposure to the mercuric chloride, alkaline phosphatase activity in the kidney of C. punctatus was found inhibited (Sastry and Agrawal, 1977). However, they noted considerable strong activity along the brush border of the proximal tubules. In the cytoplasm of the tubular cells, the activity was located in the form of diffused black granules. The intensity of staining of the granules and their number was growing more towards the brush border and very few granules were distributed towards the extra-luminal cells. The nuclei showed very mild enzyme activity around the nuclear membrane. In the glomeruli very mild activity was located in the red blood cells. The distal tubules were less affected and showed comparatively only stronger alkaline phosphatase activity around the brush border. Hence a black ring was visible in the lumen of most of the distal tubules. In the cytoplasm of the tubular cells, the diffused granules were stained more darker than in the proximal tubules. The nuclei of the tubular cells and the glomeruli did not reveal any activity. Bhatnagar (1983) also observed a significant and maximum inhibition in alkaline phosphatase activity of kidney after exposure to sublethal concentration of endosulfan after 15 days. According to him the inhibition in the enzyme activity was due to uncoupling of phosphorylation.

Regarding the localization of enzyme activity, our results coincide with the observations of Sastry and Agrawal (1977). But instead of inhibition in the enzyme activity there we find a significant increase in it. Why such contradictory activity is observed? cannot explained. Whether, the natural piscicide of the leaves of L. eriocephalus does not interfer

in the coupling of phosphorylation which is supposed to be the cause for inhibition in endosulfan toxicity as suggested by Bhatnagar (1983).

As pointed out in the introductory chapter, the reports on alkaline phosphatase in the gill of fresh water teleost are very limited. There is not a single reference available on the effect of natural piscicide on the gill alkaline phosphatase. In the present study, it was found that the alkaline phosphatase activity was 3142 $\pm$ 48 µ mol p-nitrophenol phosphate per gram gill tissue wet weight of control fish which was found maximally increased to 4764 $\pm$ 86 in the experimental fish after exposure of fish to 200 ppm concentration of ethanol extract of the leaves of L. eriocephalus for 36 hrs. The response of alkaline phosphatase seems to be dose and duration dependent. From the variation under stress environment, the alkaline phosphatase enzyme in the gill seems to be involved in the coupling of phosphorylation as in case of kidney tubules as suggested by Bhatnagar (1983).

Pathogenesis of changes in the brain occuring in chemical intoxications is not yet fully explained. Some workers suppose that the toxication of these chemicals on the central nervous system consists in blocking the sulfhydryl groups of enzymes by the chemicals (Mukai 1972; Kozik et al., 1977). Besides such studies, extensive works on the toxic action of natural piscicides on the nervous system is wanting. For that reason, the present work on alkaline phosphatase activity in the brain following an experimental intoxication of fish by natural piscicide from the leaves of L. eriocephalus is very important.

Weakening or decrease in ACP, AChE and ATP-ase activity or an increase in TPP-ase and sometimes in NsE activity has been reported in the brain by earlier investigators (Kozik and Wygladalska, 1977; Kozik et al., 1977). The enzyme under study, alkaline phosphatase showed a significant increase in its activity in brain after exposure of fish to natural piscicide. From these results, it seems that this piscicide functions as an activator to alkaline phosphatase enzyme in the brain of the fish. But for confirmation, further investigations in this area would require. Furthermore, the observations of the present biochemical experiments must be supplimented by the application of histoenzymological methods, so that in which parts of the brain enzyme changes have occurred, would be confirmed.