CHAPTER 3

OBSERVATIONS

III. OBSERVATIONS

1) Observations on Leaf Extract of Lasiosiphon Eriocephalus :

The dried leaves were powdered and the extraction was carried in three different solvents, benzene, chloroform and ethanol, respectively and the three extracts obtained, their percentage composition and weight in grams is shown in the following table (No.2)

Table No. 2

The percentage composition of compounds extracted in benzene, chloroform and ethanol of Lasiosiphon eriocephalus

No.	Material	Solvent	Weight in Gm.	Percentage %
1	Shade dried leaves	-	250.000	-
2	Benzene extract	Benzene	2.102	0.84
3	Chloroform Extract	Chloroform	3.703	1.84
4	Ethanol extract	Ethanol	8.312	3.35
5	Residue after extraction	-	235.823	94.33

2) Phytochemical Observations :

A) TLC Observations :

TLC was carried out on the plates coated with silica gel G using chloroform : ethanol (85 : 15) solvent system. The three extracts (benzene, chloroform and ethanol) were simultaneously spotted on the plates and chromatographs were developed by exposing the plates in Iodine chamber. The

results of this experiment have been recorded in the Fig. No.1 and the components observed in each extract and their Rf values have been summarized in Table No.3.

Table No. 3

The components and their Rf values of benzene, chloroform and ethanol extracts of Lasiosiphon eriocephalus leaves at 21.6°C

No.	Extract	Compon-nents	Distance travelled		Rf values
			Components cm	Solvent cm	
I	Benzene extract	B_1	10.0	17.5	0.57
		B_2	13.5	17.5	0.77
II	Chloroform extract	C_1	6.0	17.5	0.34
		C_2	8.0	17.5	0.45
		C_3	10.8	17.5	0.61
		C_4	12.5	17.5	0.71
		C_5	14.0	17.5	0.8
III	Ethanol extract	E_1	6.5	17.5	0.37
		E_2	10.5	17.5	0.6

B) Melting Point :

The melting pointof Benzene extract = 80°C

The melting point of Chloroform extract = 100°C

The melting point of ethanol extract = 118 to 120°C

FIG. NO. 1

Showing TLC of Benzene (BE) Chloroform (CE) and ethanol extract (EE) of L.eriocephalus leaves.

TLC of extracts of L.eriocephalus Leaves.

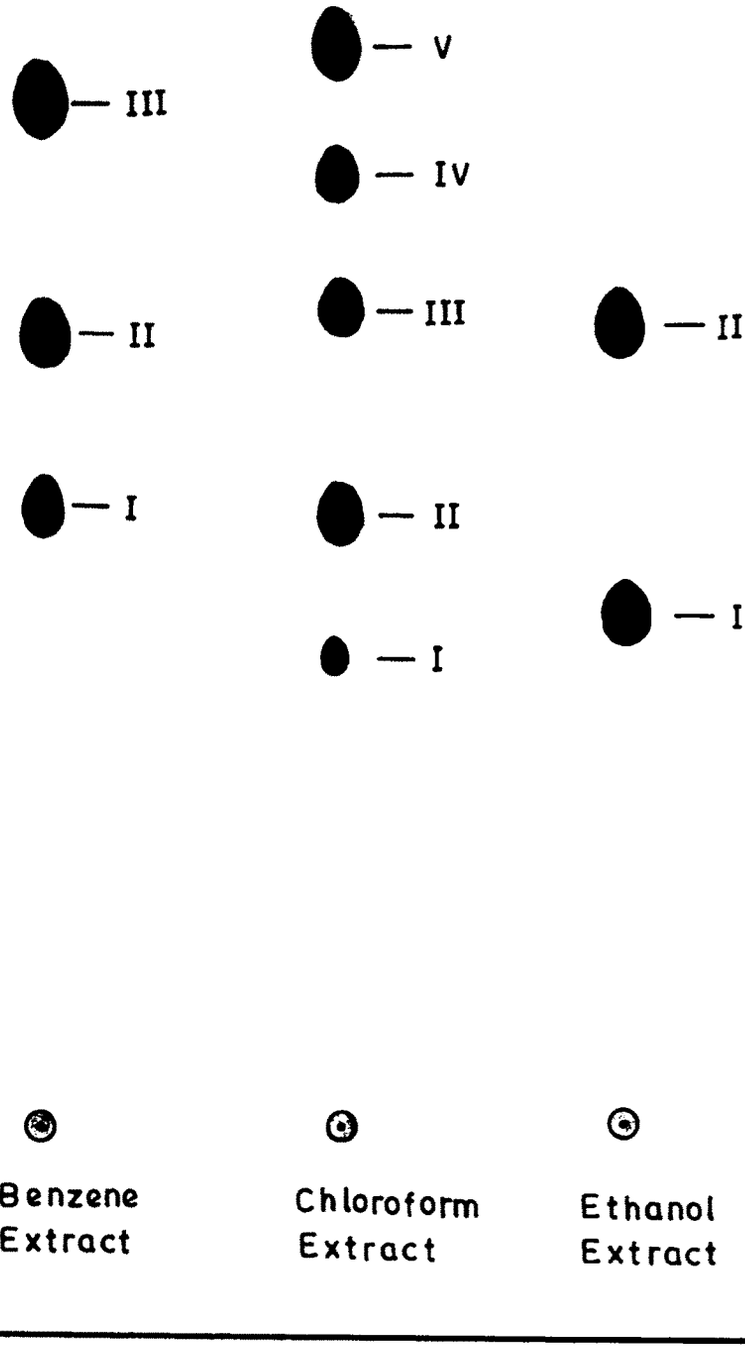


Fig. No. 1

C) UV Spectral Observation :

UV spectra of the three extracts (benzene, chloroform and ethanol) showed intense peaks at λ max in between 190 nm to 300 nm. Fig. Nos.2,3 and 4 show the UV spectra for the benzene soluble components, chloroform soluble components and ethanol soluble components respectively.

UV spectra of all the three components in the benzene extract showed identical intense peaks at λ 240. Chloroform extract contained five components. The UV spectrum of first component showed maximum peak values at λ 240 and 260, the second component's peaks were at 240 and 260, the third components also showed λ_{max} at 240 and 260 nm and fourth and fifth components showed their peak values at 240 and 260 nm. The ethanol soluble components were two. First component showed its maximum peak value at λ 210 nm and second at λ 260 nm.; whereas the second component showed only one peak only at λ_{max} 210 nm.

D) NMR Spectral Observations :

Fig.No.5 shows the NMR spectrum of benzene soluble components in CCl_4 with TMS as internal standard scanned on Perkin-Elmer 90 MH_z R32. spectrophotometer. The values of the chemical shift expressed in δ ppm are given. The spectrum shows unresolved multiplet at 0.9, a singlet at 1.25, δ 1.6 (unresolved) multiplet and a singlet at δ 2.0.

Fig.No.6 shows the NMR spectrum of chloroform soluble components in CCl_4 with TMS as internal standard scanned on Perkin-Elmer 90 MH_z Spectrophotometer. The values of the chemical shift expressed in δ ppm are given. The spectrum shows identical pattern to that of the spectrum of benzene soluble components except a singlet at δ 2.0, which is absent in chloroform soluble components.

FIG. NO. 2

Showing UV spectrum of Benzene extract (BE) of

L.eriocephalus leaves.

U V SPECTRUM OF BENZENE EXTRACT OF

Lasiosiphon eriocephalus (Decaisne) .

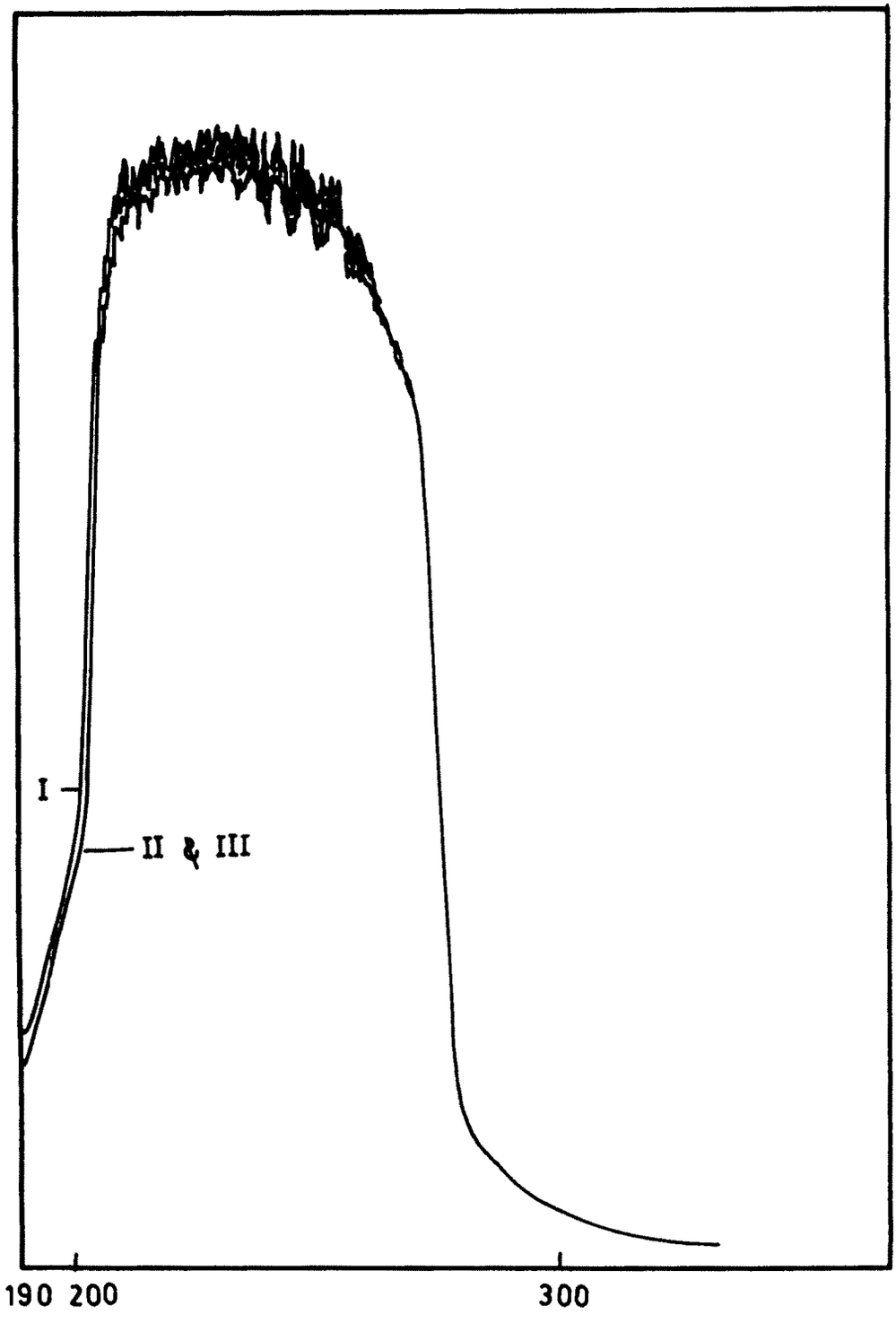


Fig. No. 2

FIG. NO. 3

Showing UV spectra of chloroform extract (CE)

of L.eriocephalus leaves.

U V SPECTRUM OF CHLOROFORM EXTRACT OF *Lasiosiphon eriocephalus* (Decaisne).

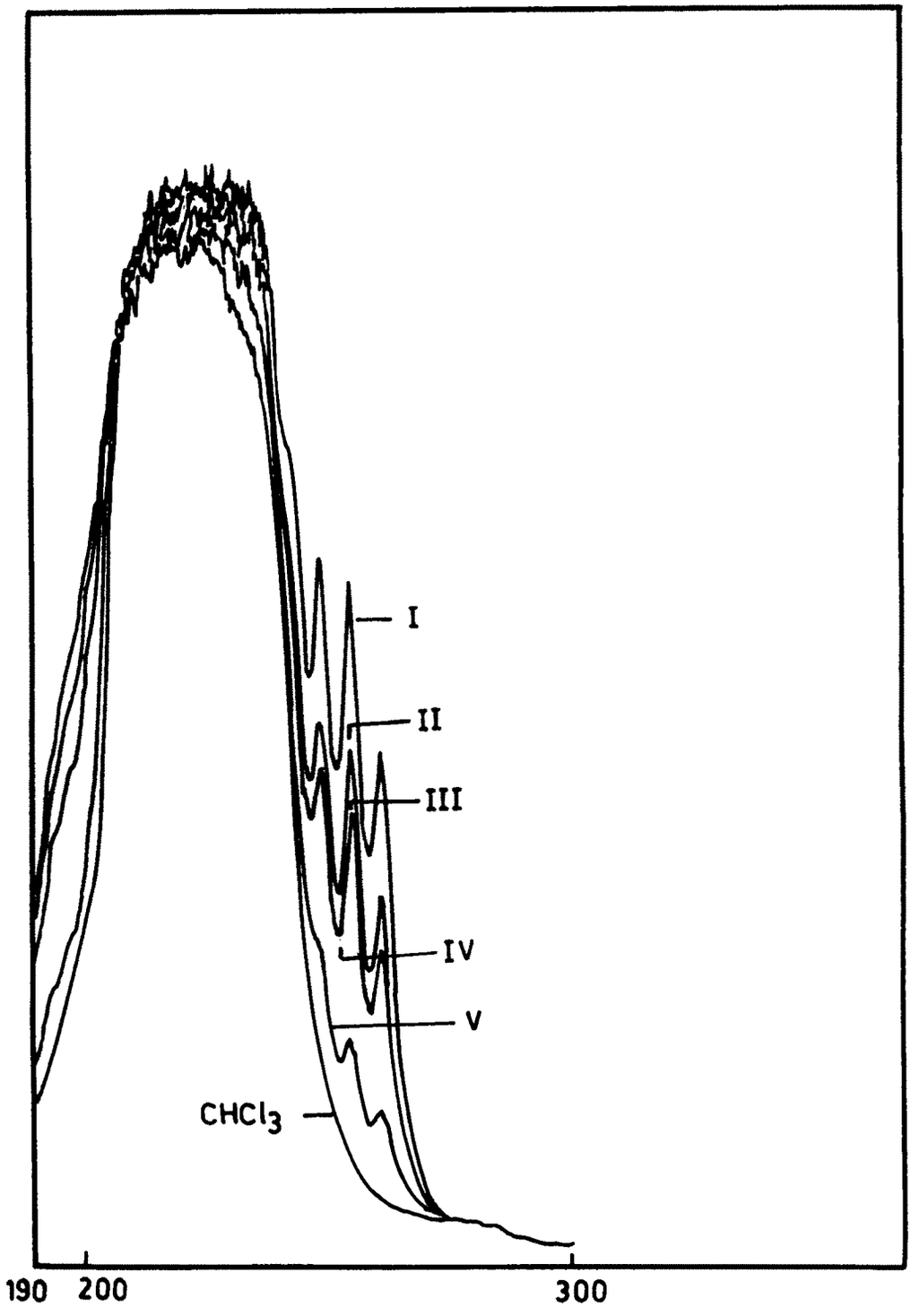


Fig. No. 3

FIG. NO. 4

Showing UV spectrum of ethanol extract (EE)

of L.eriocephalus leaves.

U V SPECTRUM OF ETHANOL EXTRACT OF

Lasiosiphon eriocephalus (Decaisne).

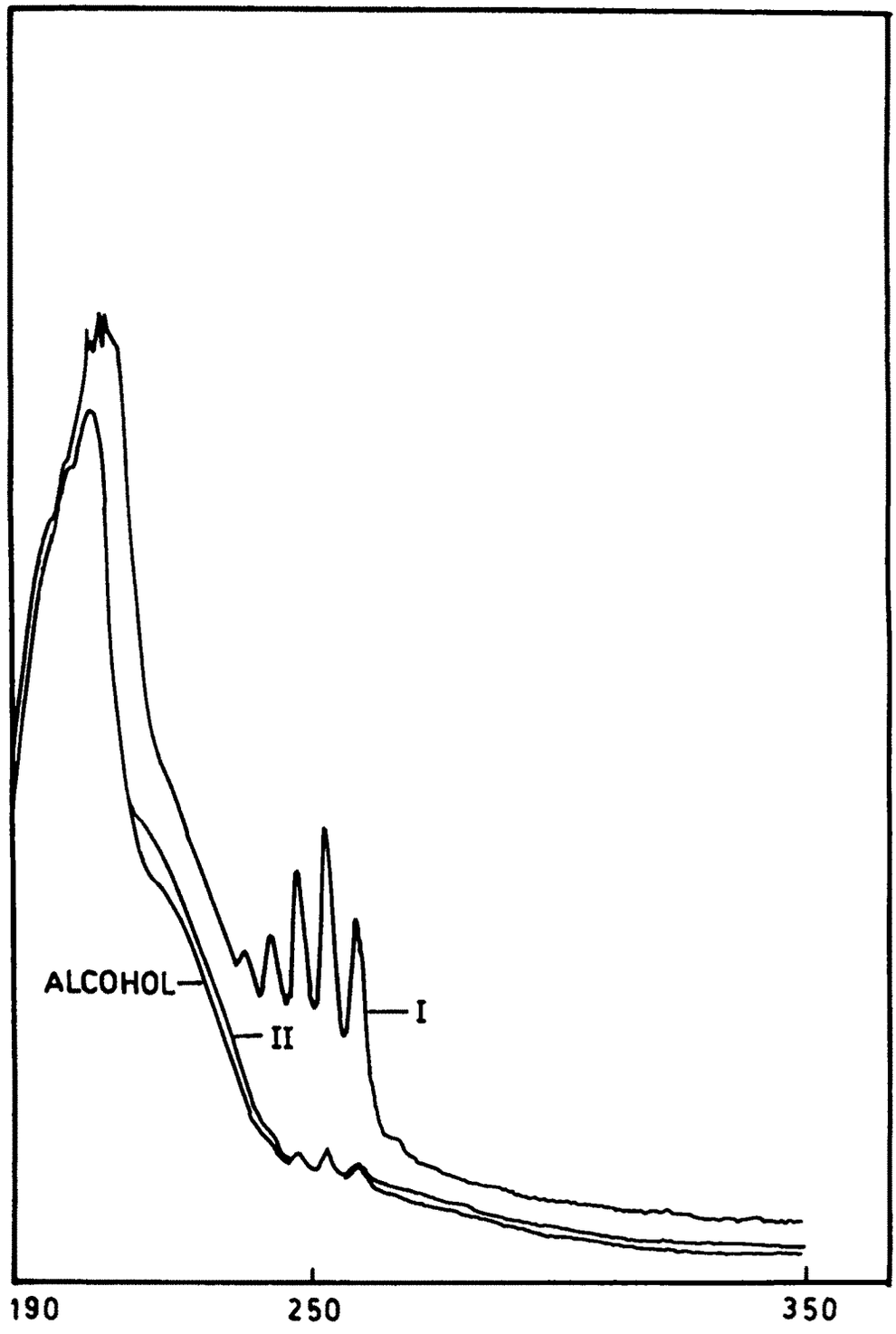


Fig. No. 4

FIG. NO. 5

Showing NMR spectrum of Benzene extract (BE)

of L.eriocephalus leaves.

NMR SPECTRUM OF BENZENE EXTRACT OF Lasiosiphon eriocephalus (Decaisne)

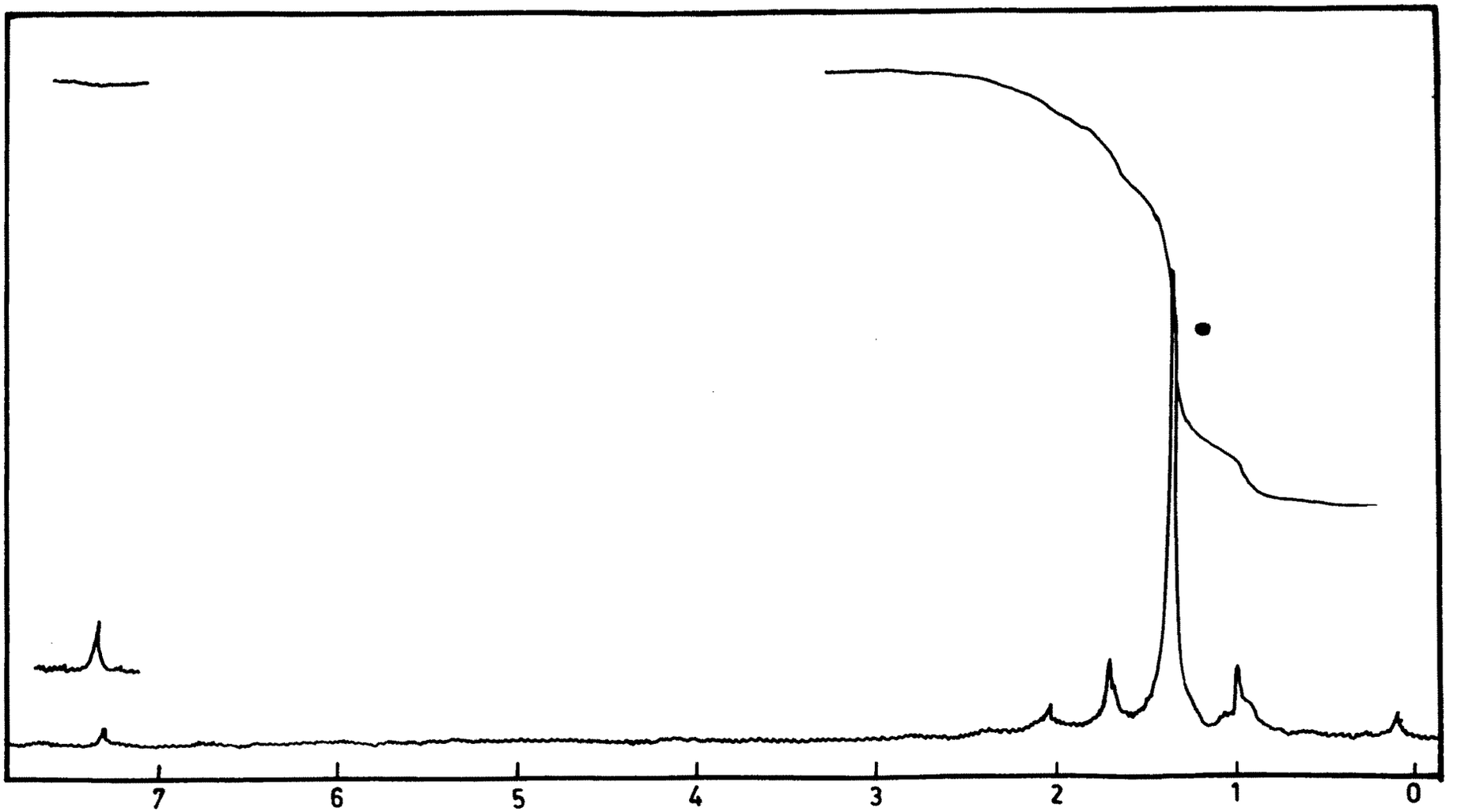


Fig. No. 5

FIG. NO. 6

Showing NMR spectrum of chloroform extract (CE)

of *L. eriocephalus* leaves.

NMR SPECTRUM OF CHLOROFORM EXTRACT OF Lasiosiphon eriocephalus (Decaisne)

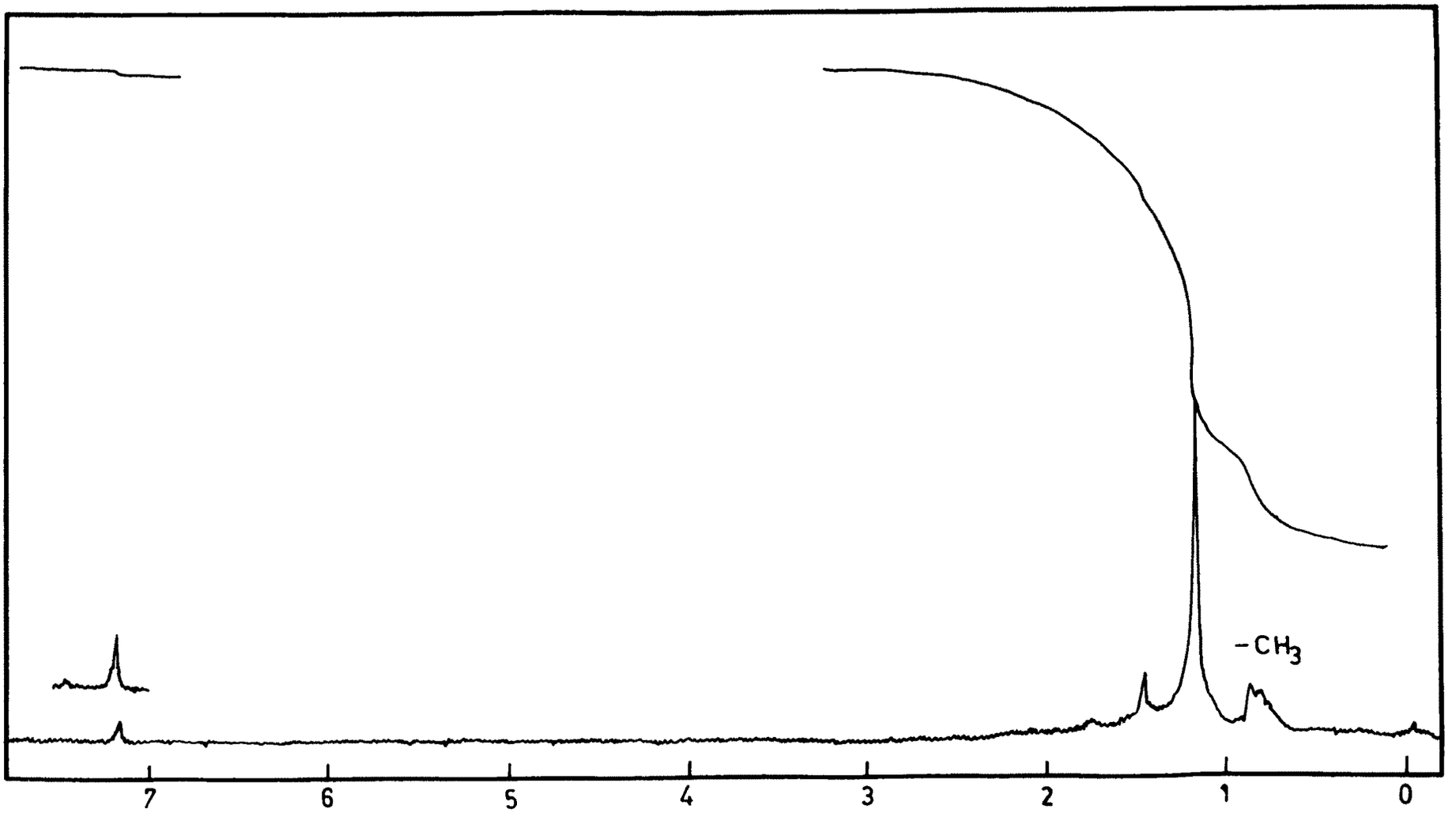


Fig. No. 6

FIG. NO. 7

Showing NMR spectrum of Ethanol extract (EE)

of L. eriocephalus leaves.

NMR OF ETHANOL EXTRACT OF Lasiosiphon eriocephalus (Decaisne)

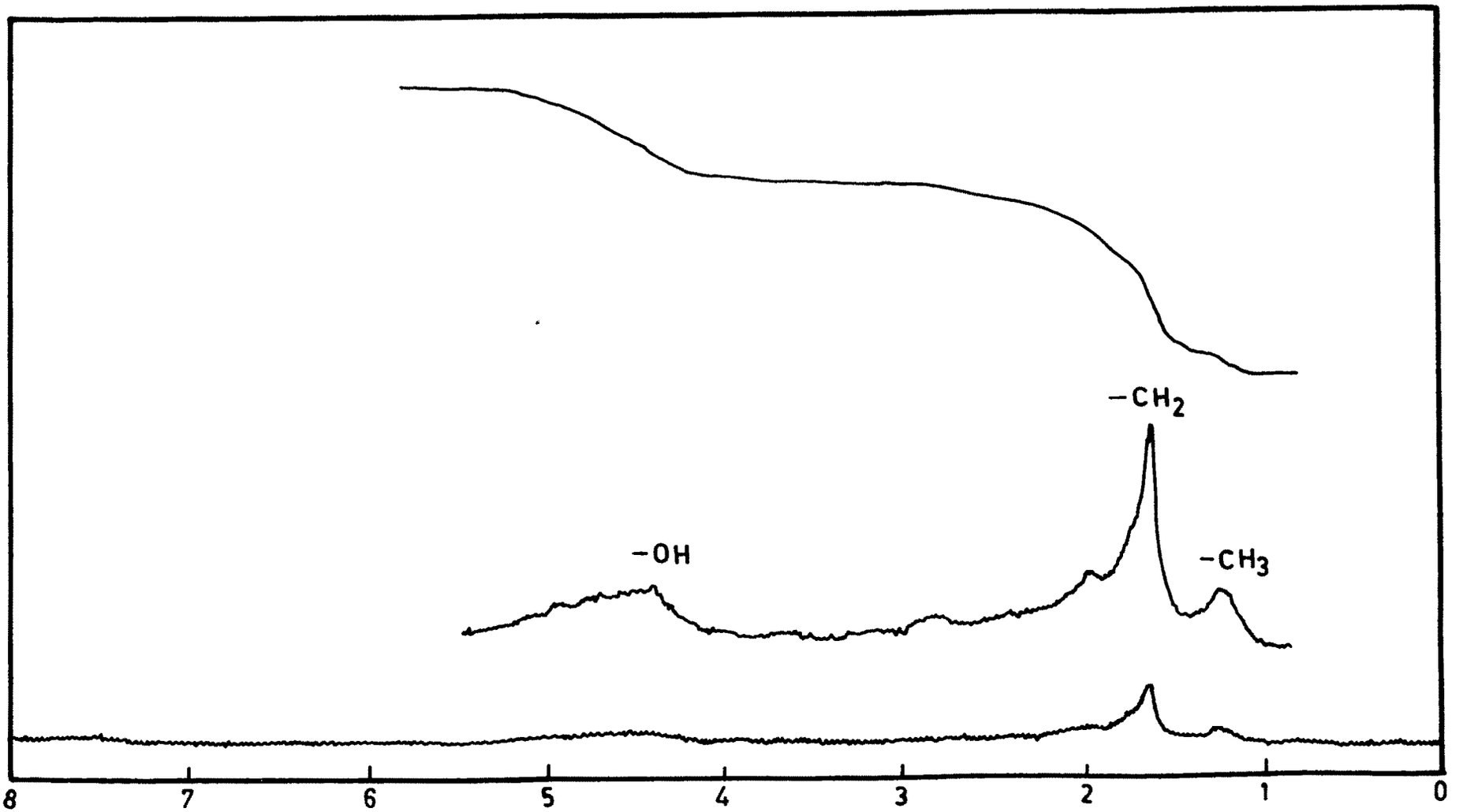


Fig. No. 7

Fig.No.7 shows the NMR spectrum of ethanol soluble components in Trifluroacetic acid (TFA) scanned on Perkin-Elmer 90 MH_z spectrophotometer The values of the chemical shift expressed in δ ppm are given. The spectrum shows four peaks a multiplet (unresolved) at δ 1.2 - 1.3, a multiplet (unresolved) at δ 1.5 - 1.65, a singlet at δ 2.0 and a multiplet at δ 4.2 - 5.0.

E) IR Spectral Observations :

IR spectra were scanned on PERKIN-ELMER-783 U.K. in nujol.

1) The benzene extract (Fig.No.8) showed stretching band at 1850 cm^{-1} and a second stretching band at 1740 cm^{-1}.

2) The chloroform extract (Fig.No.9) showed a broad band at 3400 - 3500 cm^{-1} and a band at 1708 cm^{-1}.

3) The ethanol extract (Fig.No.10) showed stretching broad band at 3500 cm^{-1} and a second band between 1700-1720 cm^{-1}.

F) Atomic Absorption Spectrophotometric Observation :

The benzene, chloroform and ethanol extracts were analysed for inorganic ions on Atomic Absorption Spectrophotometer (Perkin Elmer,3030 USA). The observations are summarized in the Table No.4.

3) The Experimental Procedure :

The fishes were kept in rectangular glass acquaria (25 liter capacity) and were acclimatized to the laboratory conditions for a week in the conditions similar to their natural habitat. Special care was taken to maintain the same DO, pH, temperature and hardness of the water. The feeding was stopped before 24 hrs. of the commencement of the experiment. The fishes were not fed during the test period. For each experiment 10 - 20 fishes were

FIG. NO. 8

Showing IR spectrum of Benzene extract (BE)

of L. eriocephalus leaves.

IR OF BENZENE EXTRACT OF Lasiosiphon eriocephalus (Decaisne)

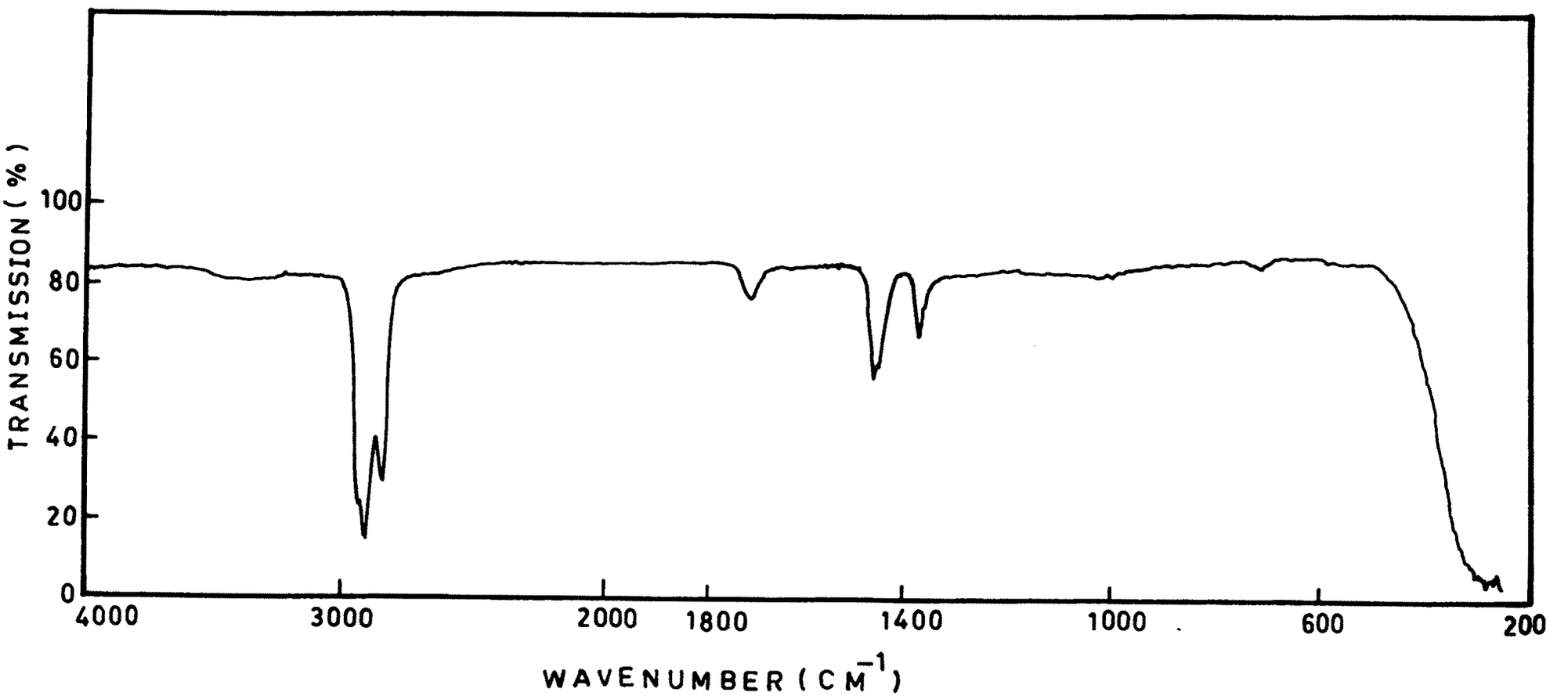


Fig. No. 8

FIG. NO. 9

Showing IR spectrum of Chloroform extract (CE)

of L. eriocephalus leaves.

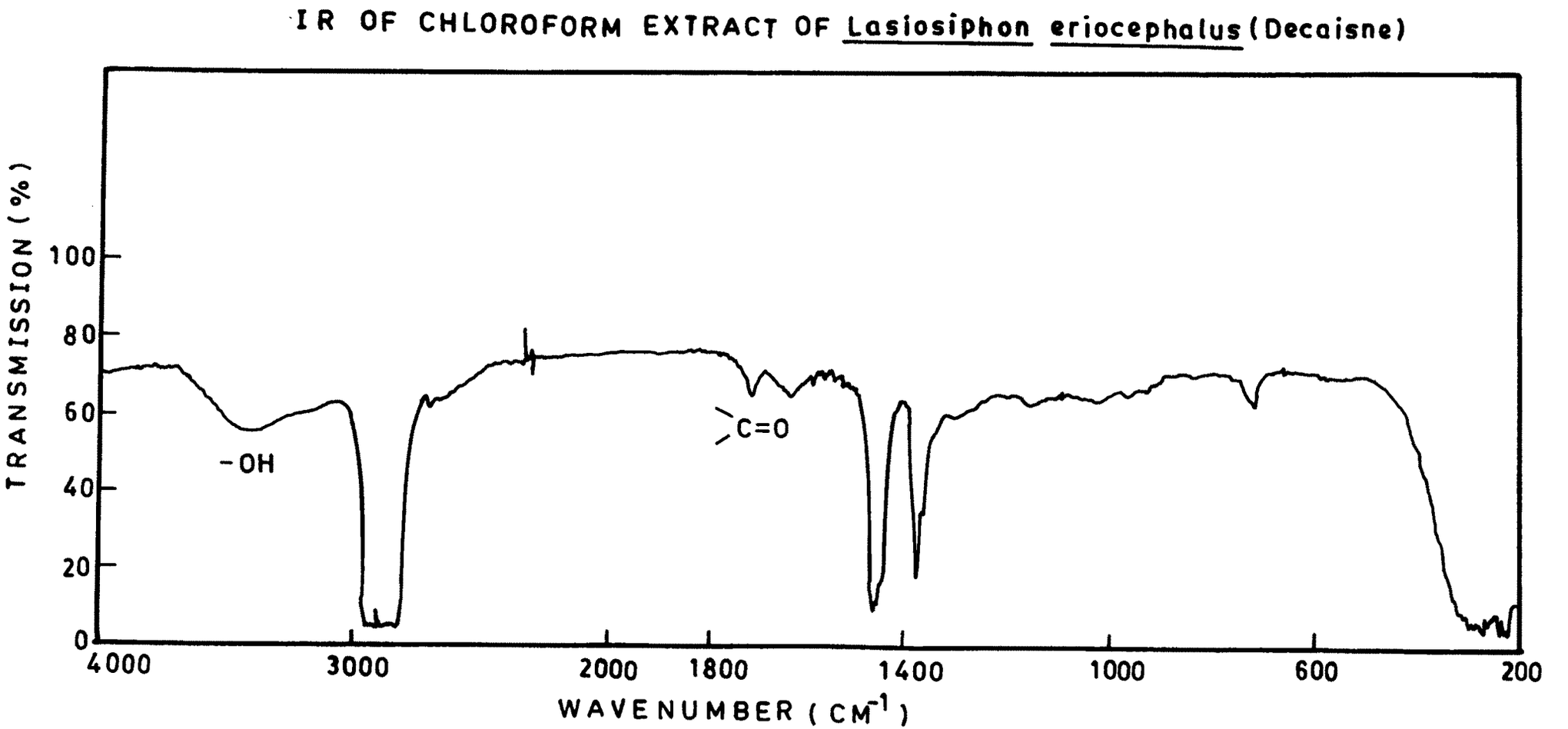

IR OF CHLOROFORM EXTRACT OF Lasiosiphon eriocephalus (Decaisne)
TRANSMISSION (%)
100
80
60
40
20
0
-OH
C=O
4000
3000
2000
1800
1400
1000
600
200
WAVENUMBER (CM-1)

Fig. No. 9

FIG. NO. 10

Showing IR spectrum of Ethanol extract (EE)

of L. eriocephalus leaves.

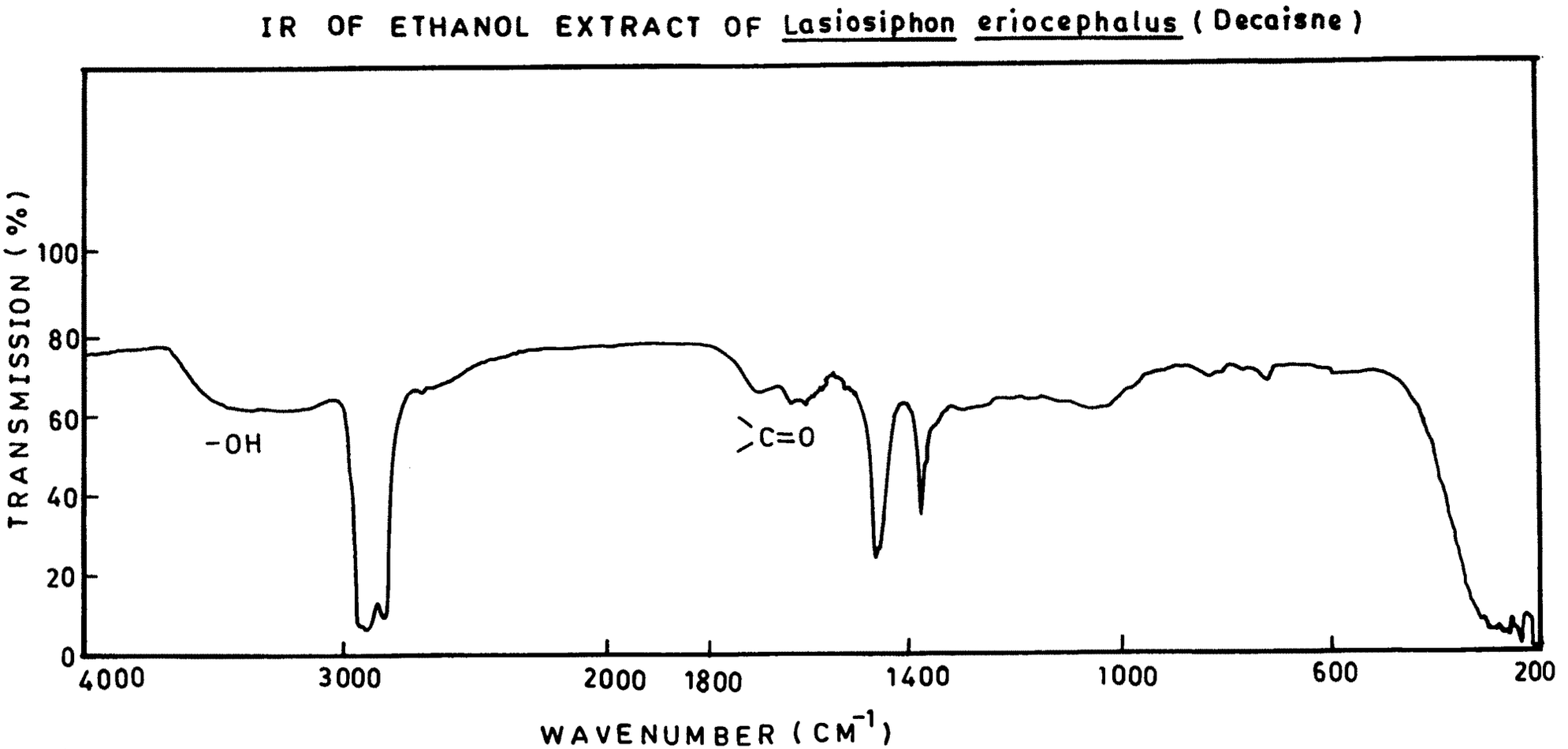

IR OF ETHANOL EXTRACT OF Lasiosiphon eriocephalus (Decaisne)
TRANSMISSION (%)
100
80
60
40
20
0
-OH
>C=O
4000
3000
2000
1800
1400
1000
600
200
WAVENUMBER (CM-1)

Fig. No. 10

Table No. 4

Inorganic ions in the Benzene, Chloroform and Ethanol extracts of L.eriocephalus leaves

No.	Inorganic ions	Benzene extract			Chloroform extract			Ethanol extract		
		Readings in ppm	Mean	Std.	Readings in ppm	Mean	Std.	Readings in ppm	Mean	Std.
1	Fe	0.35			0.48			0.33		
		0.35	0.35	0.00	0.50	0.49	0.01	0.33	0.333	0.01
		0.36			0.49			0.33		
2	Ca	0.69			10.54			11.15		
		0.69	0.68	0.00	10.52	10.51	0.07	11.15	11.14	0.04
		0.69			10.57			11.08		
		0.68			10.42			11.17		
3	Zn	0.17			0.40			0.25		
		0.17	0.17	0.00	0.40	0.40	0.00	0.24	0.24	0.00
		0.17			0.41			0.24		
		0.17			0.40			0.24		
4	Cu	0.14			0.22			0.13		
		0.14	0.14	0.00	0.22	0.22	0.00	0.12	0.13	0.0 1
		0.14			0.22			0.14		
		0.14			0.22			0.13		
5	Co	0.08			0.07			0.04		
		0.07	0.07	0.01	0.06	0.07	0.01	0.05	0.05	0.01
		0.07			0.08			0.05		
		0.07			0.07			0.05		

transferred into a well cleaned test container. The DO, pH, temperature and hardness were measured before the addition of either crude powder or the leaf extract, and periodically at regular interval for a period of 24 hrs.

A. Experiment No. 1 :

The crude powder (80, 90 ..... 150 ppm) and the BE (10, 15 and 25 ppm) were diluted in separate test containers. Then the fishes were introduced and their per cent mortality was recorded. From the observations it seemed that there was no effect on the fish mortality on addition of the BE but the crude powder showed varied per cent mortality with the different concentrations.

B. Experiment No. 2 :

The second experiment using CE was performed in a similar manner to that for BE and their effects were recorded. There was zero mortality even after addition of higher concentration of CE. Here also the effect of crude powder was seen and varied per cent mortality was recorded with the different concentrations.

C. Experiment No. 3 :

The ethanol extract (EE) was water soluble hence various concentrations were prepared by using chlorine-free tap water and desired concentrations were made. Fig.Nos. 11 and 12 gives the DO values of the crude powder and of the ethanol extract respectively. Fig.Nos. 13 and 14 show the pH during experimental procedure for the crude powder and the extract (EE) respectively. Figs. 15 and 16 show values of the hardness for the crude powder and for the ethanol extract, whereas the Figs. 17(3 Hrs.), 18(6 Hrs.)

FIG. NO. 11

Showing dissolved oxygen values in mg/litre for different concentrations of the crude powder of L. eriocephalus leaves.

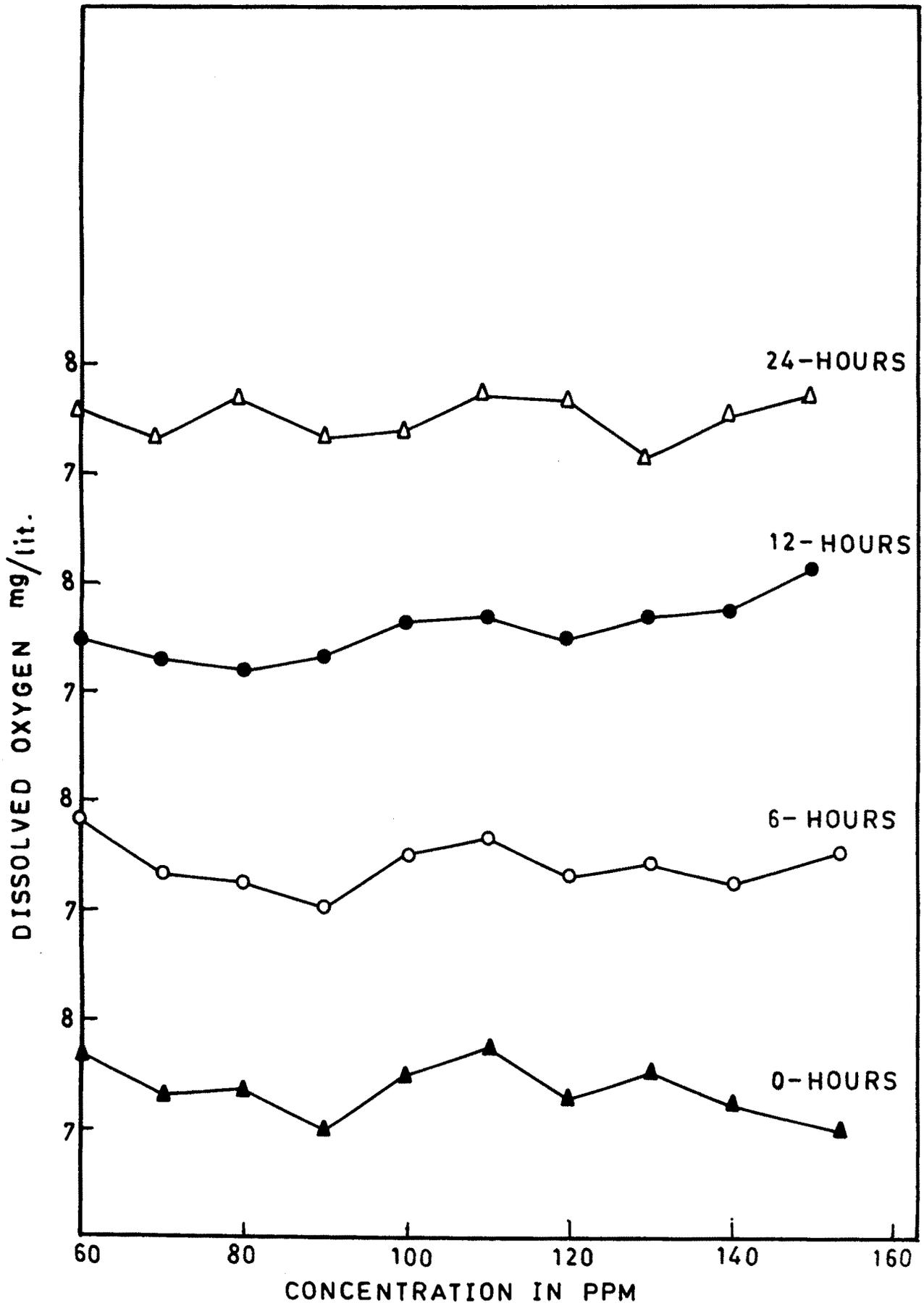

24-HOURS
12-HOURS
6-HOURS
0-HOURS
8
7
DISSOLVED OXYGEN mg/lit.
60
80
100
120
140
160
CONCENTRATION IN PPM

Fig. No. 11

FIG. NO. 12

Showing Dissolved oxygen values in mg/litre for different concentrations of the ethanol extract (EE) of L. eriocephalus leaves.

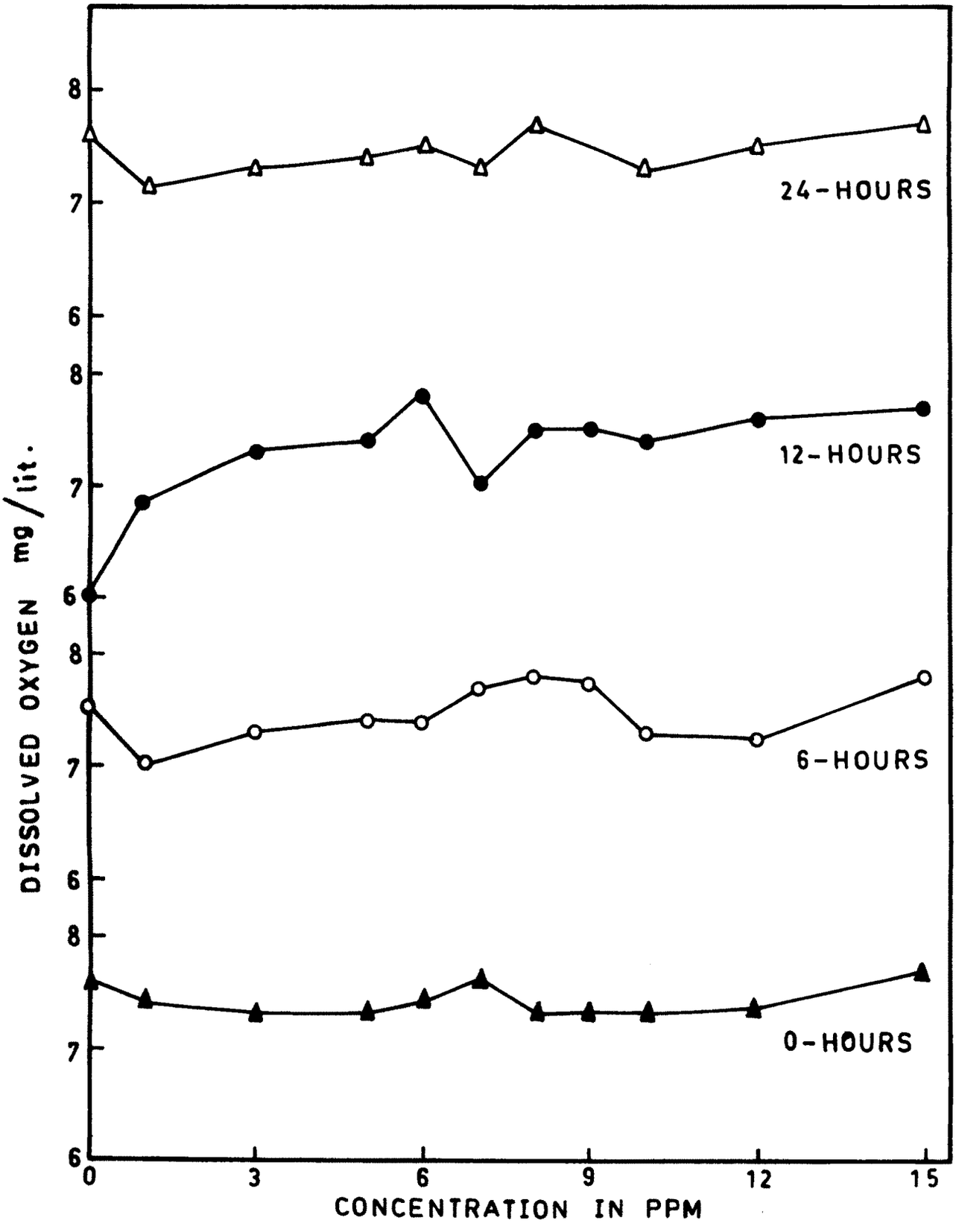

8
7
6
8
7
6
8
7
6
8
7
6
24-HOURS
12-HOURS
6-HOURS
0-HOURS
DISSOLVED OXYGEN mg/lit.
0
3
6
9
12
15
CONCENTRATION IN PPM

Fig. No. 12

FIG. NO. 13

Showing pH values for different Concentrations

of the crude powder of L. eriocephalus leaves

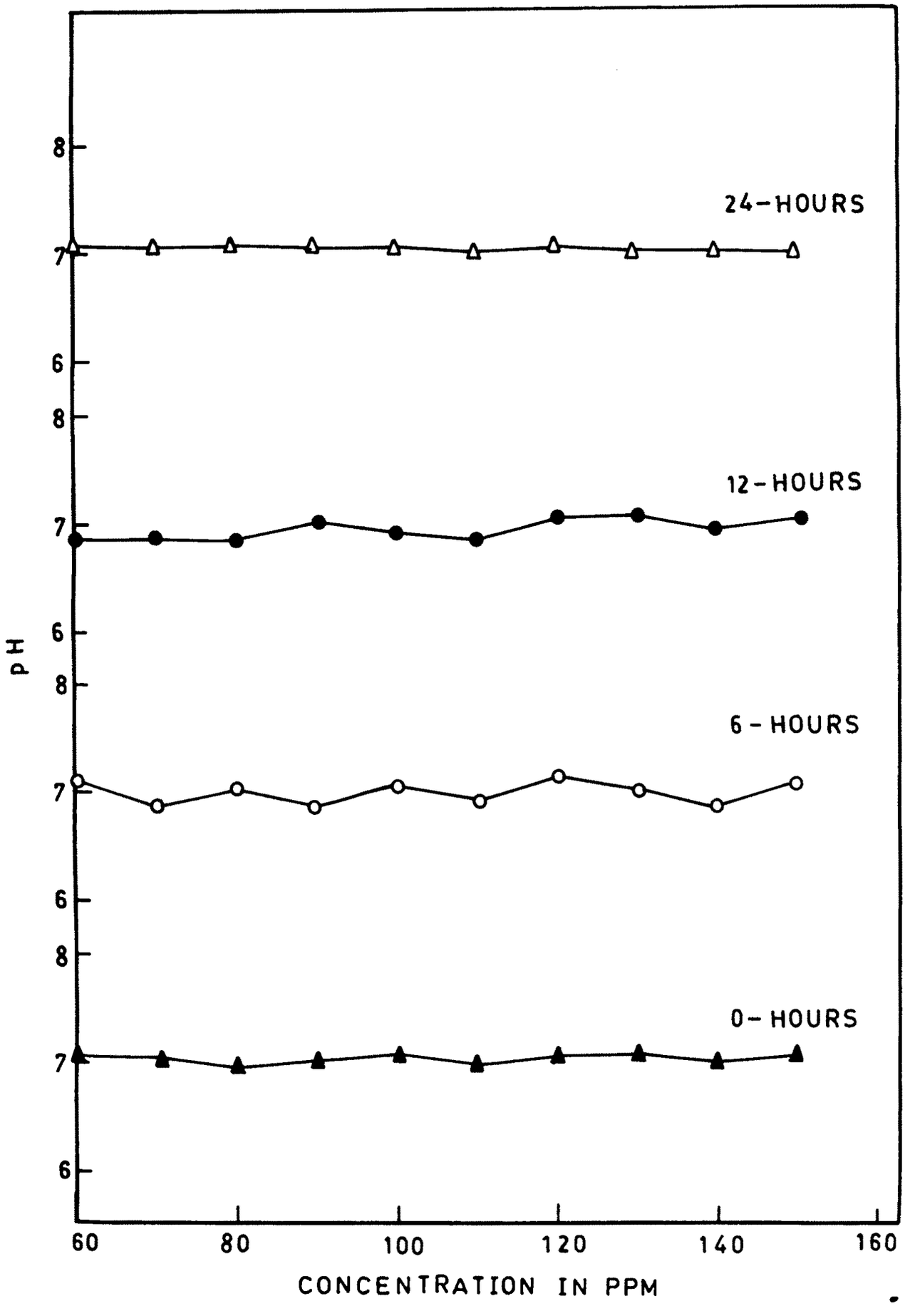

24-HOURS
12-HOURS
6-HOURS
0-HOURS
pH
8
7
6
60
80
100
120
140
160
CONCENTRATION IN PPM

Fig. No. 13

FIG. NO. 14

Showing pH values for different concentrations

of the ethanol extract (EE) of

L. eriocephalus leaves

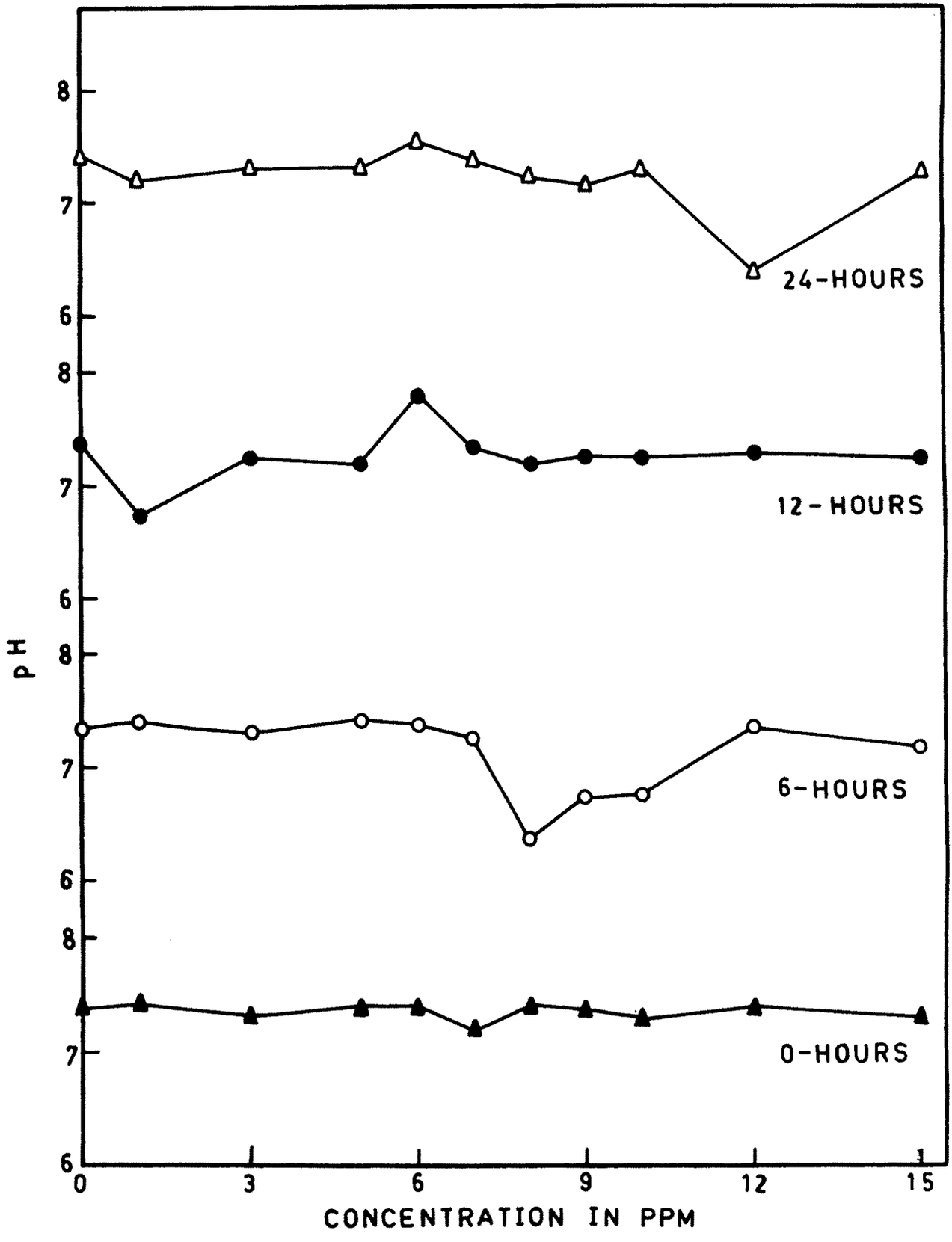

8
7
6
24-HOURS
8
7
6
12-HOURS
8
7
6
6-HOURS
8
7
6
0-HOURS
pH
0
3
6
9
12
15
CONCENTRATION IN PPM

Fig. No. 14

FIG. NO. 15

Showing values of $CaCO_3$ hardness in mg/litre

for different concentrations of ethanol extract (EE)

of L. eriocephalus leaves.

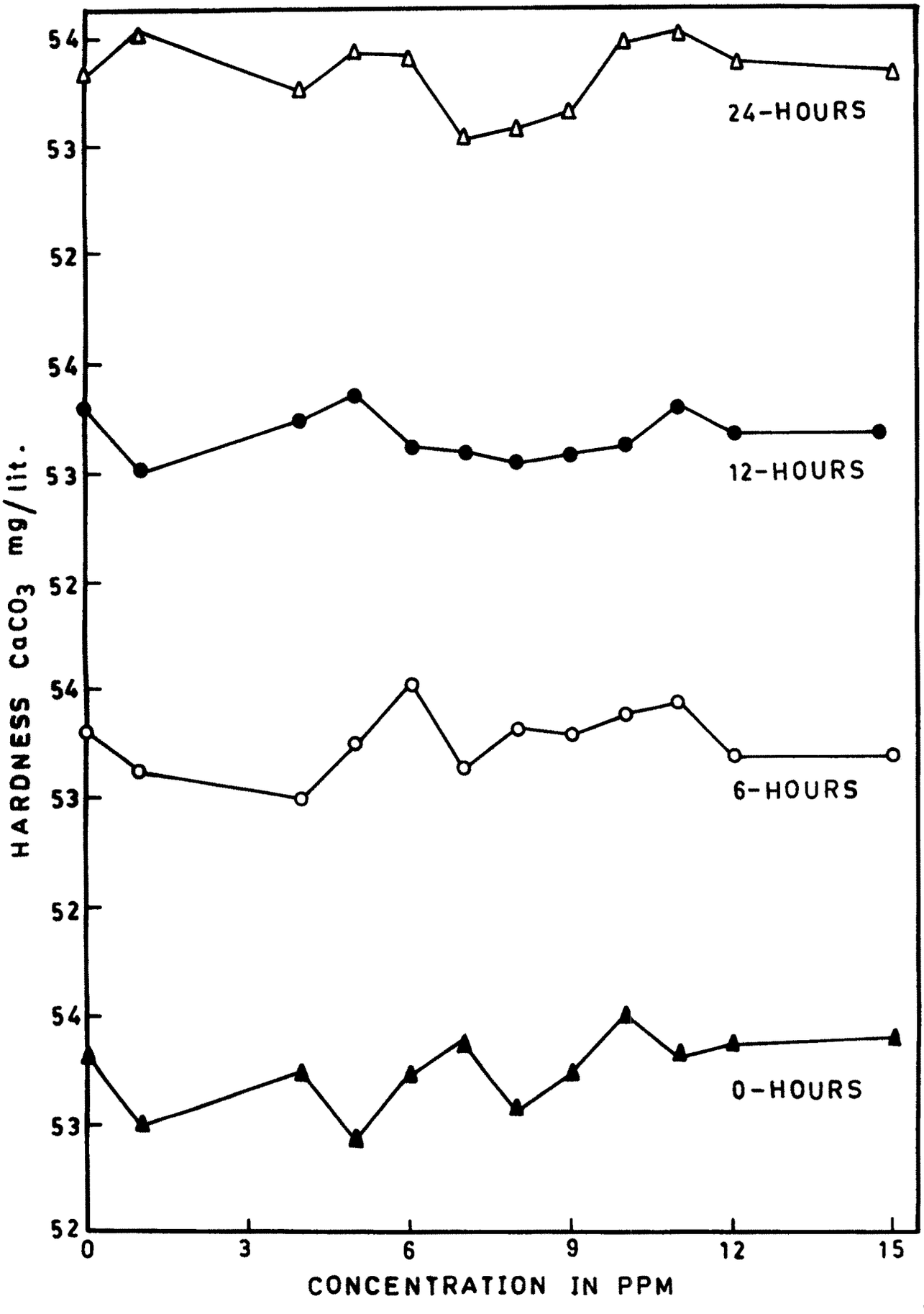

54
53
52
54
53
52
54
53
52
54
53
52
24-HOURS
12-HOURS
6-HOURS
0-HOURS
HARDNESS $CaCO_3$ mg/lit.
0
3
6
9
12
15
CONCENTRATION IN PPM

Fig. No. 15

FIG. NO. 16

Showing values of $CaCO_3$ hardness in mg/litre for different concentrations of crude powder of L. eriocephalus leaves.

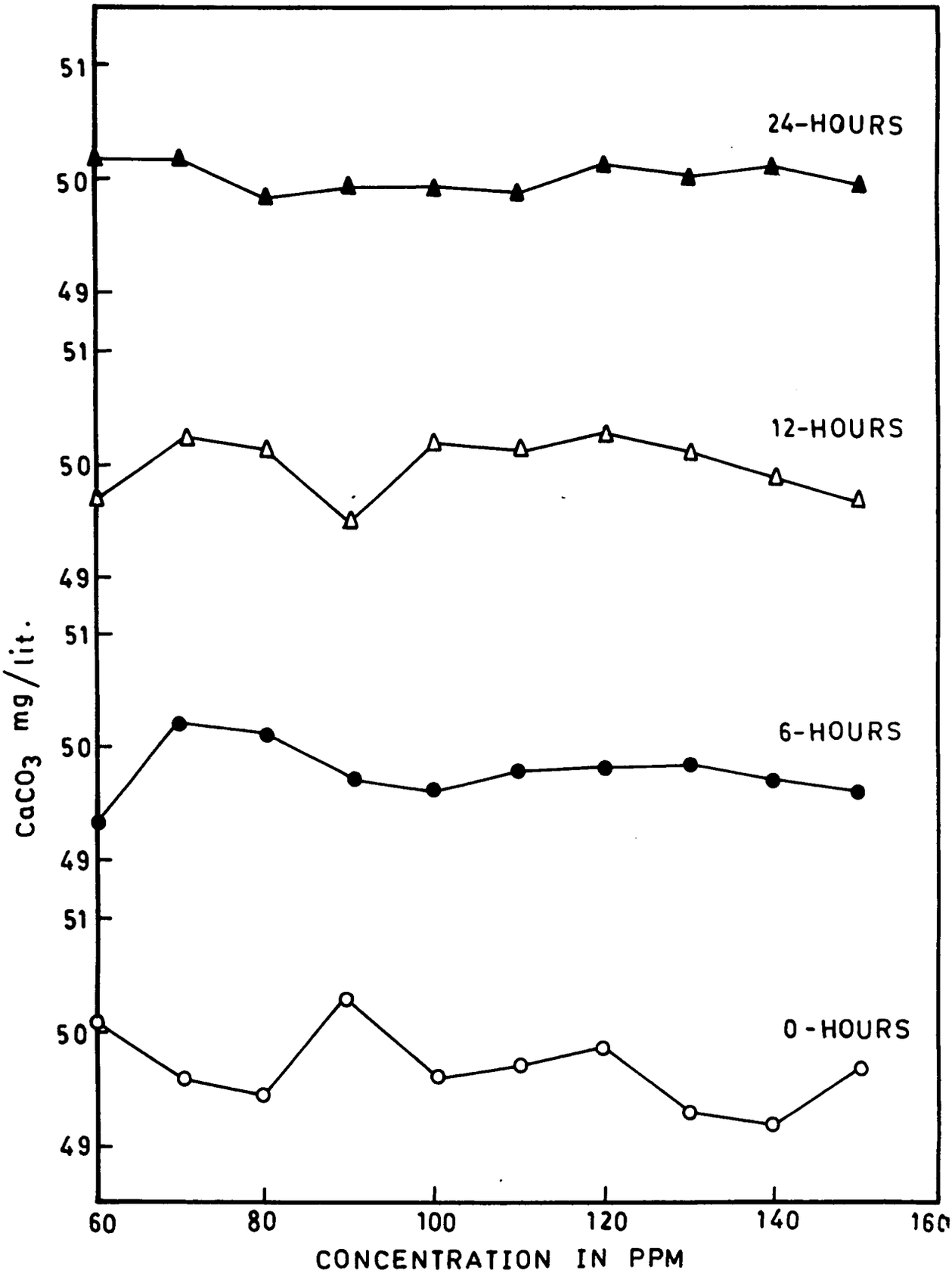

51
50
49
51
50
49
51
50
49
51
50
49
24-HOURS
12-HOURS
6-HOURS
0-HOURS
CaCO3 mg/lit.
60
80
100
120
140
160
CONCENTRATION IN PPM

Fig. No. 16

19 (12 Hrs.) and 20 (24 Hrs) give the LC_{50} values at different hours indicated in the parenthesis for the crude powder and for the ethanol extract. The Table No.5 also gives the statistical information containing the concentrations of the crude power and of the EE and the per cent mortality at different hours during the experimental procedure.

4) Behavioural Observations :

The behavioural responses of the fishes during experimental procedures using different extracts (BE, CE and EE) and crude powder were recorded as per visual observations. The responses to the extract BE and CE did not change the behaviour of Tilapia mossambica. The reactions to EE were remarkable. The reaction times was different with different concen trations selected for the experimental procedures. At lower concentrations (1, 3, 5 and 6 ppm) the behavioural changes were not noticeable but at higher concentrations (10, 12 and 15 ppm) behaviour of fish was remarkable. At these higher concentrations, the first visible effects occurred after 10-60 minutes. The fish at first showed a brief period of high excitability, followed by alternate periods of muscular spasms, and then a period of sluggishness. The fishes restricted to the corners of the test containers. The opercular movements were increased along with the activity of the fish. But towards the lethal phase its opercular movements were remarkably decreased. At this stage, it was also noticed that there was no response to different types of stimuli such as external movements, light, touch, etc. Then after some time slowly the fish lost its equilibrium, turning on one side along their long axis, and finally collapsed in upside down posture at the bottom of the aquarium.

TABLE No. 5

Showing the concentrations, percent mortality and period of exposure to the ethanolic extract and crude powder of Lasiosiphon Eriocephalus leaves.

Ethanolic Extract

No. of fishes	Concentration in PPM	Period of exposure to the toxin in hours			
		3	6	12	24
20	4	0	0	0	10
20	5	0	0	0	40
20	6	0	0	15	55
20	7	25	40	55	60
20	8	35	50	65	15
20	9	45	55	70	80
20	10	60	80	100	100
20	11	90	100	100	100
20	12	100	100	100	100
20	15	100	100	100	100

Crude Powder

No. of fishes	Concentration in PPM	Period of exposure to the powder in hours			
		3	6	12	24
20	80	0	0	0	30
20	90	0	0	25	50
20	100	0	25	50	75
20	110	50	50	75	100
20	120	63	75	100	100
20	130	75	100	100	100
20	140	87	100	100	100
20	150	100	100	100	100

FIG. NO. 17

(a) Showing Lc_{50} values at 3 hours for the crude powder and (b) for ethanol extract of L. eriocephalus leaves.

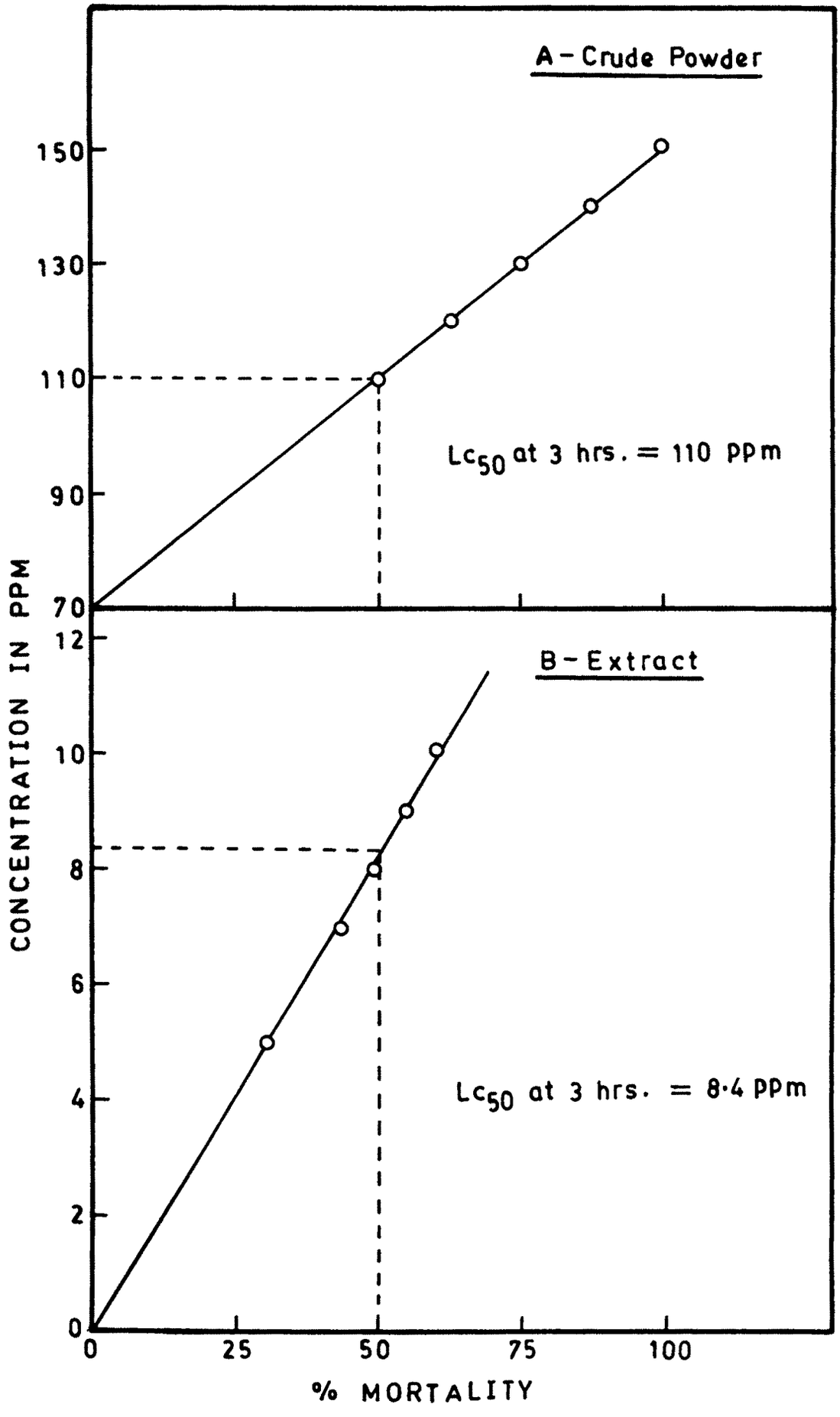

A - Crude Powder
150
130
110
90
70
Lc50 at 3 hrs. = 110 ppm
B - Extract
12
10
8
6
4
2
0
Lc50 at 3 hrs. = 8·4 ppm
CONCENTRATION IN PPM
0
25
50
75
100
% MORTALITY

Fig. No. 17

FIG. NO. 18

(a) Showing Lc_{50} values at 6 hours for the crude powder and (b) for ethanol extract of L. eriocephalus leaves.

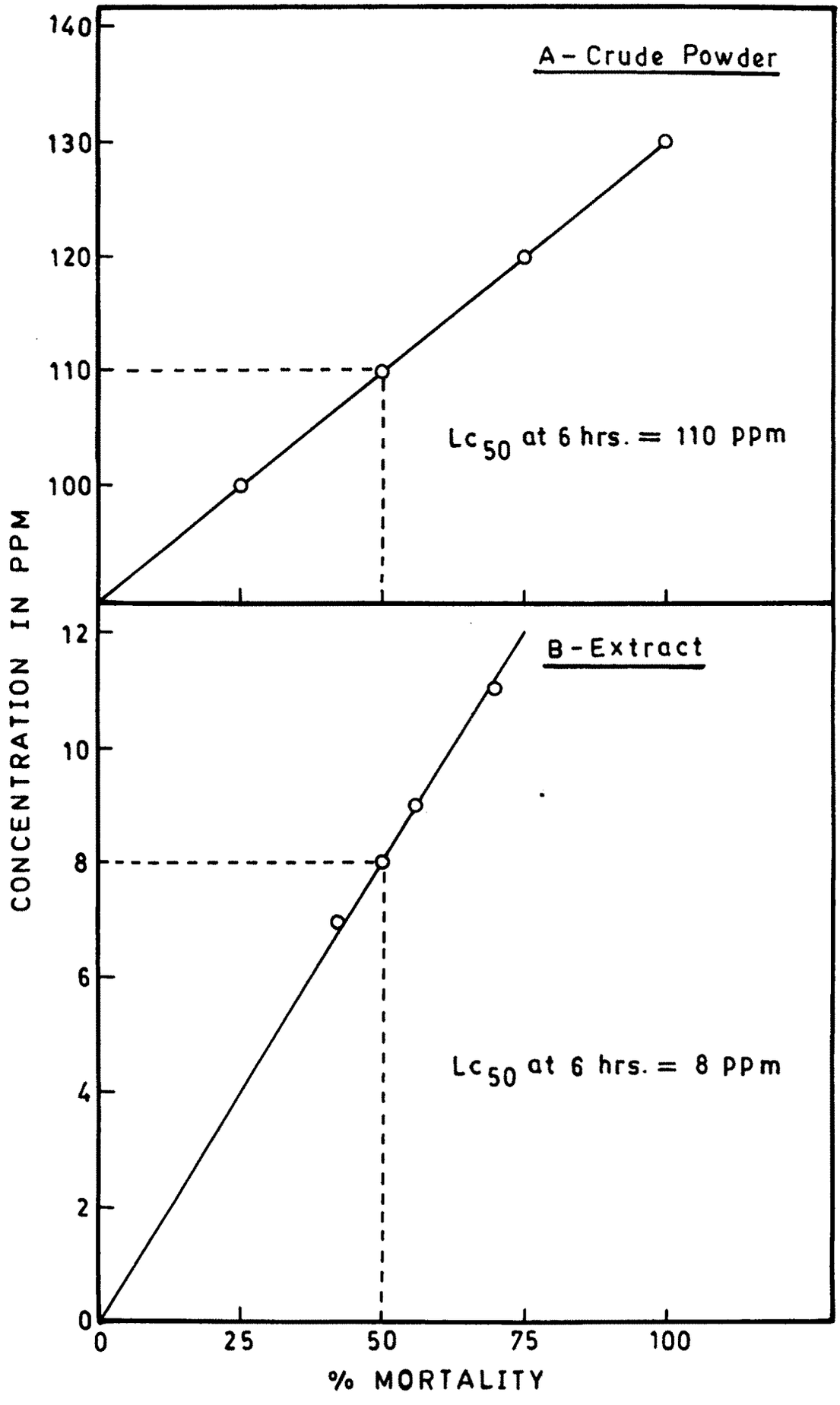

A - Crude Powder
B - Extract
Lc50 at 6 hrs. = 110 ppm
Lc50 at 6 hrs. = 8 ppm
CONCENTRATION IN PPM
% MORTALITY
140
130
120
110
100
12
10
8
6
4
2
0
0
25
50
75
100

Fig. No. 18

FIG. NO. 19

(a) Showing Lc_{50} values at 12 hours for the crude powder and (b) for ethanol extract of L. eriocephalus leaves.

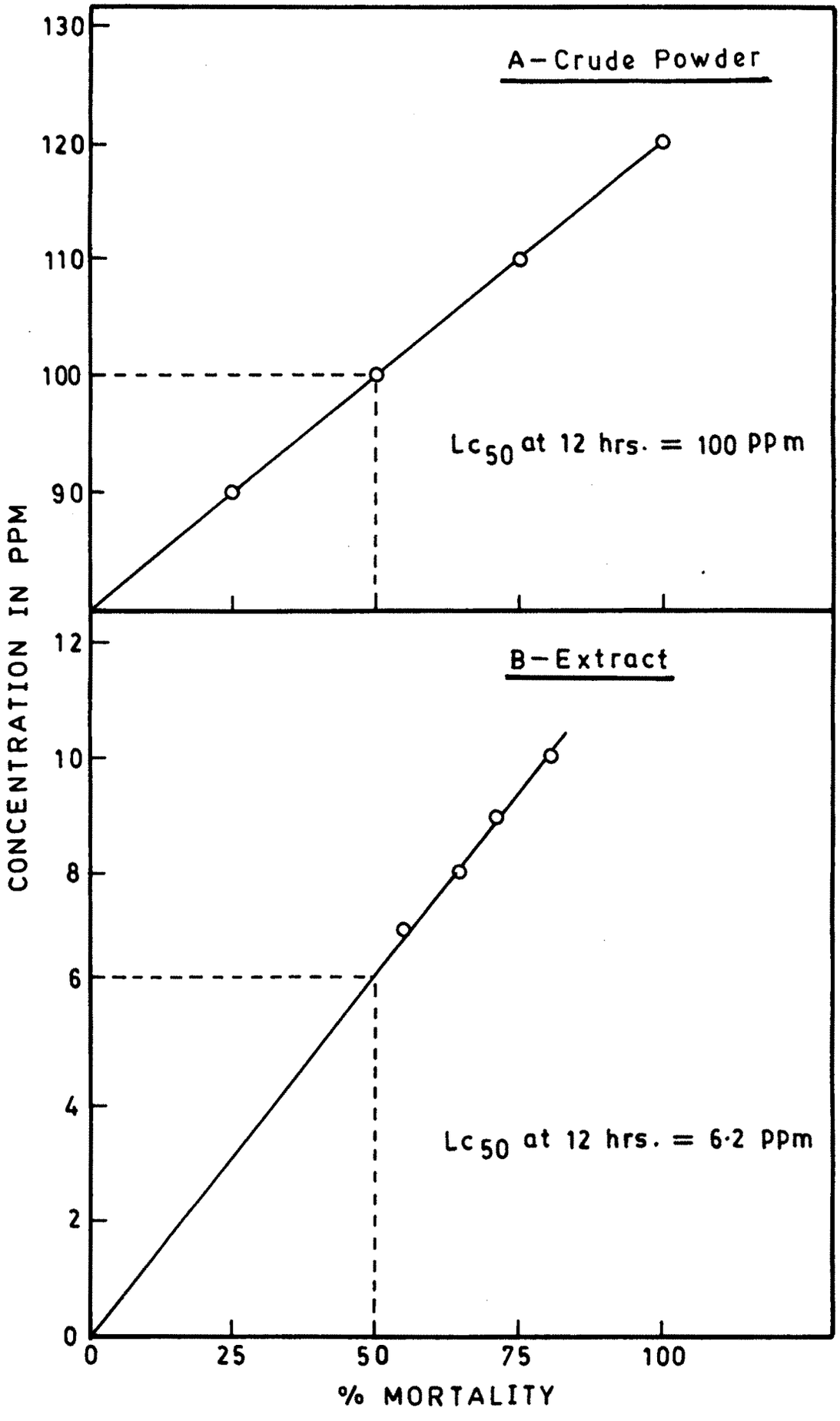

A–Crude Powder
130
120
110
100
90
Lc50 at 12 hrs. = 100 PPm
B–Extract
12
10
8
6
4
2
0
Lc50 at 12 hrs. = 6·2 PPm
CONCENTRATION IN PPM
0
25
50
75
100
% MORTALITY

Fig. No. 19

FIG. NO. 20

(a) showing Lc_{50} values at 24 hours for the crude powder and (b) for ethanol extract of L. eriocephalus leaves.

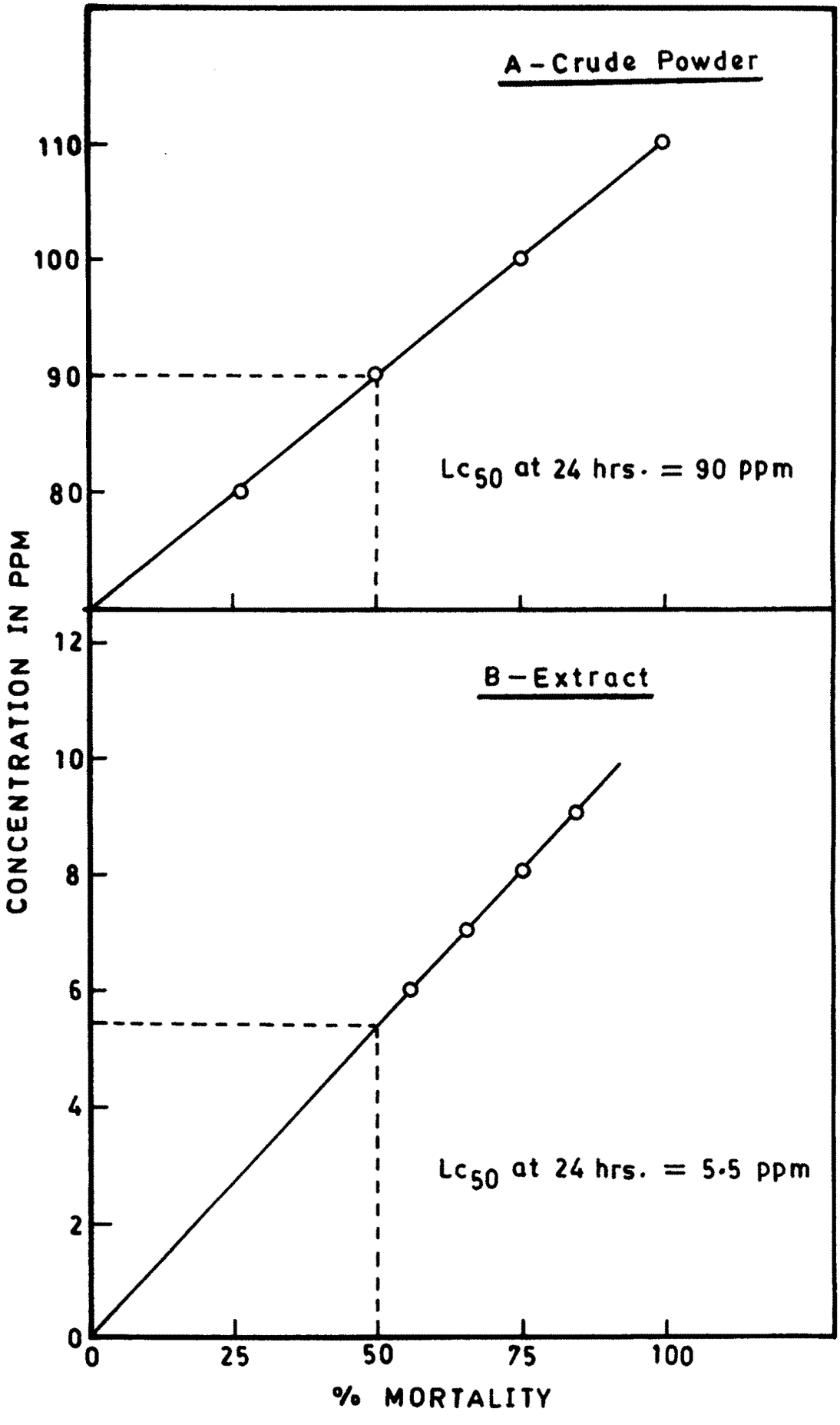

A - Crude Powder
110
100
90
80
Lc50 at 24 hrs. = 90 ppm
B - Extract
12
10
8
6
4
2
0
Lc50 at 24 hrs. = 5·5 ppm
CONCENTRATION IN PPM
0
25
50
75
100
% MORTALITY

Fig. No. 20

Interestingly, fishes treated with EE (ethanol extract) secreted thick slimy mucus with blood clots, oozing from their mouth before their collapsing and some times the whole surface of the water in the tank was covered with these secretions.

5) Histological and Histochemical Observations :

i) Oral Cavity

A) Normal Histology of Oral Cavity :

The anterior chamber of the digestive tract of Tilapia mosambica is divided into an oral cavity and a pharynx. For the present study the earlier part is taken since the latter possesses the gill shifts. The oral (Buccal) cavity is lined with a thin stratified squamous epithelium which is supported by the connective tissue and muscular layer. The mucous membrane has several papillae, which are having lamina propria, a vascularized connective tissue core and epithelial covering. These papillae are more or less with flat-top indicating fungiform type. Some times a few conical shaped filiform papillae are also observed (Plate No.1, Figs. 5,6,7). The histological observations in normal and plant toxin treated tissues or oral cavity are photomicrographically illustrated in Plate No.1, Figs. 1 to 8.

B) Histological Alterations due to L.eriocephalus toxin :

The histological changes were clearly seen in the changes in the epithelium particularly in the thickness of the layer and in the diameter of the cells. The normal epithelium showed two types of cells in the epithelium small cuboidal cells (SC) in the outermost layer of the epithelium, scattered throughout the surface (Plate No.1,, Figs.1,3,4) and the Large columnar cells

Plate No.1, Fig. Nos. 1 to 9

(Histology of oral cavity of T.mossambica)

Fig. No. 1. Small portion of transverse section of the normal oral cavity stained with HE. Note thin stratified squamous epithelium (SEP) on small and large cells and with supporting connective tissue (CT). The mucous membrane shows fungiform papilla (P) with flat-top. Lumen (L) is inside the epithelium. X 150.

Fig. No. 2. Small portion of transverse section of oral cavity stained with HE of a fish intoxicated with lower dose (1 ppm) of L.eriocephalus toxin. Note the increased thickness of stratified epithelium (SEP), increase in the spaces of the connective tissue (CT). X 150.

Fig. No. 3. The oral cavity stained with HE of a fish intoxicated with dose (5 ppm) of L.eriocephalus toxin. Note the height of the large cells (LC) almost doubled and increased staining, connective tissue (CT) with randomly scattered cells. X 200

Fig. No. 4. The oral cavity stained with HE of a fish intoxicated with higher dose (10 ppm) of L. eriocephalus toxin. Note the thickening and staining intensity of stratified epithelium (SEP), number of small cells (SC) and height and size of large cells (LC) increased and proliferation reached maximum. X 200

Fig. No. 5. A single enlarged filiform papilla (P) of a fish stained with HE showing lateral large cells (LC), small cells (SC) and the top and lamina propria (LP) x 320.

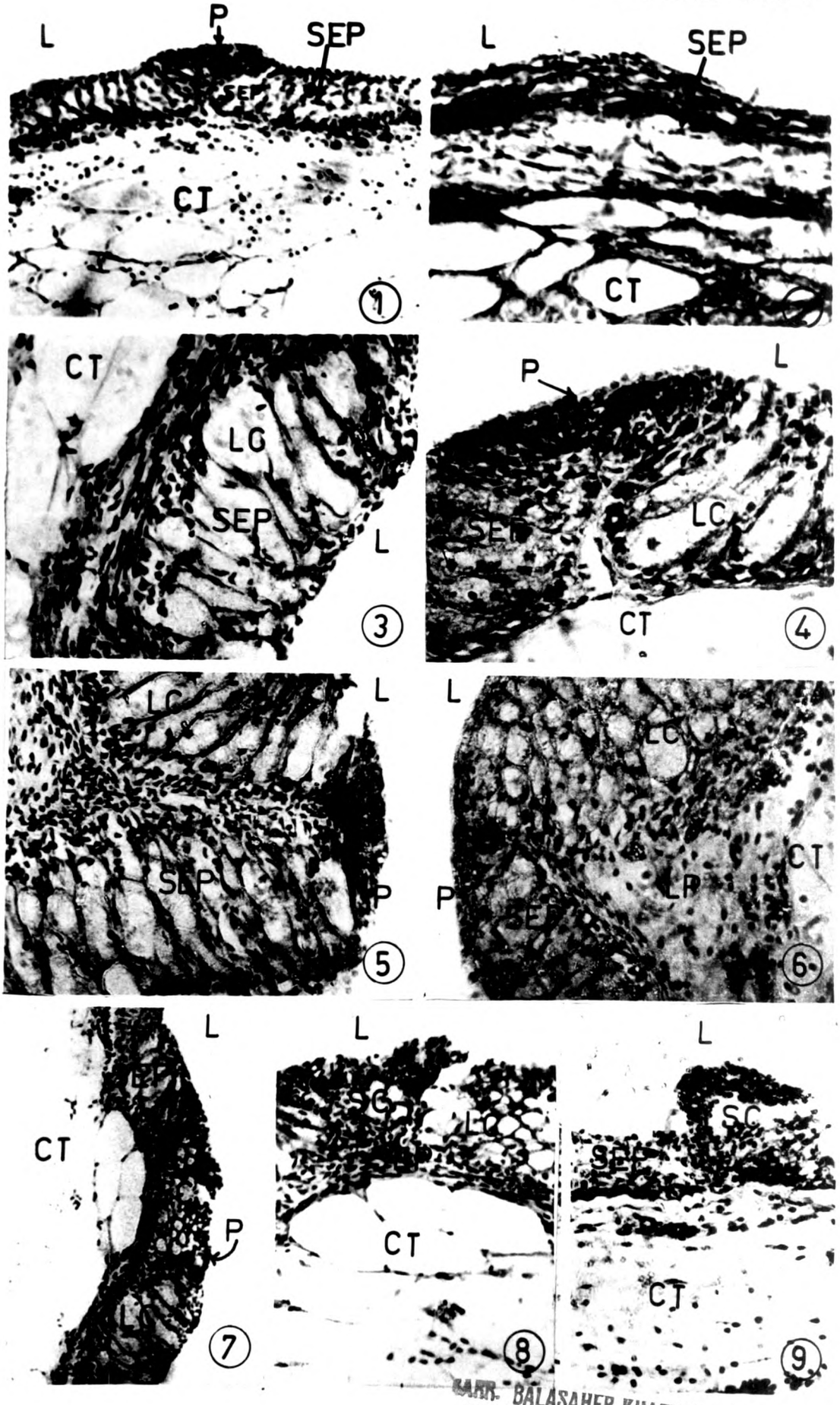
Fig. No. 6. A single enlarged oral papilla (P) of a fish intoxicated with 10 ppm of L.eriocephalus toxin stained with HE. Large cells (LC) become round, small cells (SC) number increased and they migrate towards lamina propria. Note enhanced staining, connective tissue (CT) thickened.X 320.

Fig. No. 7. A single oral papilla (P) of a fish intoxicated with (1 ppm) plant toxin and stained with HE. Note changes in the number of staining reactivities of large cells (LC) and small cells (SC) in stratified epithelium (SEP). Vacuolization in connective tissue (CT). X 150

Fig. No. 8. A single oral papilla of a fish intoxicated with 5 ppm toxin and stained with HE. Note proliferation of small cells (SC) and thickening of large cells (LC) and further vacuolization in the connective tissue (CT) whose cells are more towards epithelial side. X 200

Fig. No. 9. A disintegrated oral papilla of a fish intoxicated with higher dose (10 ppm) and stained with HE. Note the enhanced intensity of staining and increased number of small cells (SC) in stratified epithelium (SEP), connective tissue (CT) thickened. X 200 .

PLATE No 1



(LC) continuously forming many layers of the stratified epithelium. At a comparative level the number of large cells were more than the small cells The nuclei in the small cells were located at the centre whereas those of the large cells were at the base. During intoxication with low doses (1 ppm and 5 ppm) the number of small cells was found to be increased (Plate No.1 Figs.3,4). The number, the height and the size of the large cells were doubled. The staining capacity of these cells was also increased. In higher doses (10 ppm), the similar enhanced reactivity of the cells and the cell proliferation were distinctly observed (Plate No.1, Figs. 5,9). In the normal connective tissue a few cells were randomly scattered. Their number was more towards the stratified epithelium than the muscular side (Plate No.1, Fig.1). The cells and their sizes were increased in the toxin treatment (Plate No.1, Figs.3,4,5). There was no change in the muscle layer supporting the oral mucosa.

As pointed out earlier, there were two types of papillae (fungiform and filiform) in the normal oral cavity of Tilapia mosambica (Plate No.1, Figs.5,6). During toxin treatment cells bordering these papillae showed remarkable changes in their size, number and staining reactivities. The number of small cells lying at the top of these papillae was moderate in 1 ppm and 5 ppm toxin treatment but it was increased enormously in 10ppm toxin treatment. Sometimes they migrate in the lamina propria (Plate No.1, Fig.No.5). The large cells which were lying mainly on the sides of these papillae loose their columnar nature and became more or less round (Plate No.1, Figs.6,8). Their number was increased and staining reactivities were enhanced. The lamina propria of the papillae also showed increase in size and number of the cells. The connective tissue thickened and vacuolization

were prominent in this tissue. As in case of stratified epithelium, there was no change observed in the histological structure of the muscles.

C) Histochemical Observations on the Normal Oral Cavity :

The histochemical data on some important staining reactions employed in the present investigation of the oral cavity of a fish, Tilapia mossambica are recorded in Table No.6, according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The histochemical observations requiring further description and consideration, are presented hereafter along with the interpretations of the histochemical staining reactions. The distribution and alterations in the mucosubstances in various cellular elements in the oral cavity are photomicrographically illustrated in Plate No.2, Figs.1 to 8.

The histochemical study on the oral cavity revealed the following significant facts about the elaboration of the mucosubstances - (1) on the basis of elaboration of the mucosubstances, the stratified epithelial cells showed two different histochemical reactivities in large cells and in the small cells. (2) These two types of cells showed distinct responses in elaboration of their mucosubstances in plant toxin treated tissues. (3) The connective tissue showed very lower mucin secretion. (4) The muscles contained only glycogen in them.

i) Elaboration of mucosubstances by the large cells :

The histochemical reactions of these cells showed a moderate PAS reactivity, which was partially lost by diastase digestion indicating the presence of some glycogen in these cells. These cells showed alcianophilia with AB both at pH 1 and 2.5, the degree of intensity of staining at the

Plate No.2, Fig. Nos.1 to 8

(Histology and Mucosubstances of oral cavity of T.mossambica)

Fig.No. 1. A small portion of transverse section of normal oral cavity of a fish stained with AB pH 2.5 - PAS. Note large cells in epithelium (SEP) with intense PAS and alcianophilia towards luminal (L) side. Connective tissue (CT) with PAS reaction. X 200.

Fig.No. 2. The oral cavity stained with AF of a fish intoxicated with 1 ppm L.eriocephalus toxin. Note minimum mucin concentration in large cells (LC) and in small cells (SC); connective tissue (CT) reactivities diminished. X 200.

Fig.No. 3. The oral cavity section intoxicated with 5 ppm toxin containing papilla (P) with lamina propria (LP) and negatively stained connective tissue (CT) in AB pH 1 method. Large cells in epithelium (SEP) and papilla showed moderate alcianophilia. X 150.

Fig.No. 4. The enlarged section of oral cavity of a fish intoxicated with 10 ppm dose of L.eriocephalus stained with AB 2.5-PAS. Note maximum staining intensity and concentration of mucosubstances in large cells (LC) of epithelium (SEP), in small cells and in scattered cells of connective tissue (CT). X 150.

Fig.No. 5. A single oral papilla (P) intoxicated in 1 ppm plant toxin and stained with PAS. Note increase in PAS positivity in large cells (LC) in epithelium (SEP), moderate staining in lamina propria (LP) and connective tissue(CT) without any staining. X 320.

Fig.No. 6. A single normal oral papilla (P) stained with AB pH 2.5-PAS. Note very few alcianophilia in large cells (LC) and PAS staining in small cells, moderate PAS staining in lamina propria (LP) and slight PAS staining in connective tissue (CT). X 370.

Fig.No. 7. A Single oral papilla (P) intoxicated in 5 ppm plant toxin and stained with AB pH 2.5. Note intense alcianophilia in large cells (LC) of stratified epithelium (SEP). Lamina propria was without any staining. X 320.

Fig.No. 8. A single oral papilla (P) intoxicated with 10 ppm plant toxin and stained with AB pH 1 - PAS. Note enhanced staining in large cells (LC), in small cells (SC), lamina propria (LP) and in connective tissue (CT). X 320.

PLATE No.2

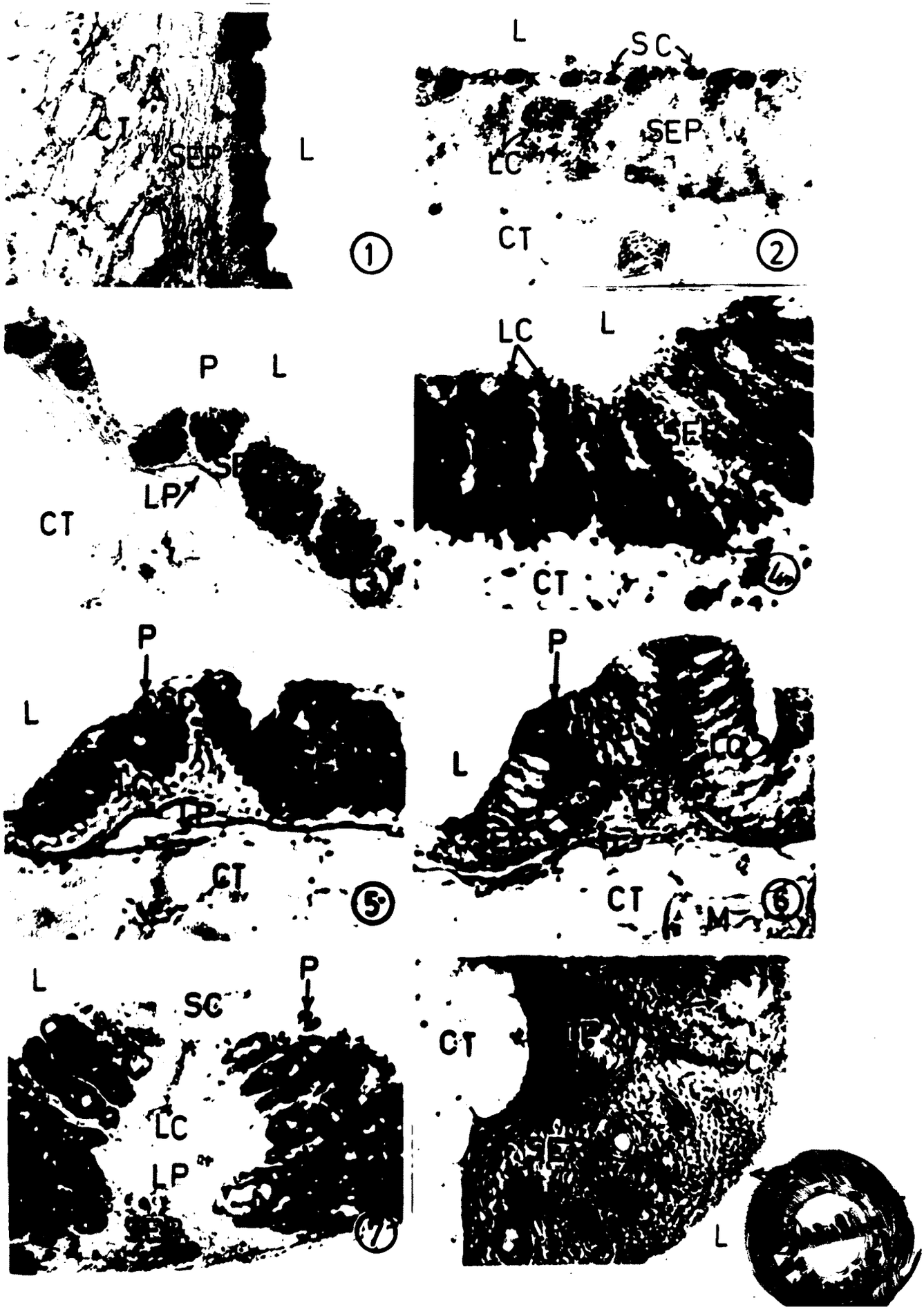

CT
SEP
L
①
L
SC
SEP
LC
CT
②
P
L
SE
LP
CT
③
LC
L
SEP
CT
④
P
L
LP
CT
⑤
P
L
LC
CT
M
⑥
L
SC
P
LC
LP
⑦
CT
LC
L
⑧

Table No.6

Histochemical observations on mucosubstances in the various tissues of the oral cavity in normal and plant toxin (L.eriocephalus) treated Tilapia mossambica (Peter's)

No.	Histochemical Methods	TISSUES											
		Epithelium						Connective tissues			Muscles		
		Large cells			Small cells								
		Normal	Low conc.	High conc.	Normal	Low conc.	High conc.	Normal	Low conc.	High conc.	Normal	Low conc.	High conc.
1	HE	N-blue E-ve	Staining moderate	Staining increased	N-blue E-+ve	Staining moderate	Staining increased	++	+++	++++	++	+++	++++
2	PAS	+++	++++	++++	+++	++++	++++	++	+++	++++	++	+++	++++
3	M Diastase-PAS	++	++	++	-	±	+	++	+++	++++	-	-	-
4	AB pH 1	++	+++	++++	-	++	++	-	-	-	-	-	-
5	AB pH 2.5	+++	+++	++++	-	++	++	-	-	-	-	-	-
6	AB pH 1 - PAS	++B ++P	+++B ++P	++++B +++P	++P -	+++P +B	+++P +B	++P	+++P	++++P	++P	+++P	++++P
7	AB pH 2.5-PAS	+++B ++P	++++B ++P	++++B +++P	++P	+++P +B	+++P +B	++P	+++P	++++P	++P	+++P	++++P
8	AF	++	+++	+++	-	-	-	-	-	-	-	-	-
9	AF-ABpH 2.5	++P ++B	+++P +++B	+++P +++B	-	-	-	-	-	-	-	-	-

N.B. :++++ = Very intense reaction; +++ = Intense reaction; ++ = Moderate reaction; + = Poor reaction; - = No reaction.
Abbreviations : HE = Haematoxyline-Eosine; PAS = Periodic Acid Schiff; M.Diastase = Malt diastase;
AB = Alcian blue 8GX-300; AF = Aldehyde fuschin; P = pink; B = blue.

latter pH level being somewhat higher than that at the former. The sequential AB (pH 1, 2.5) - PAS staining techniques indicated simultaneous presence of both the acidic and glycogen in these cells. AF and AF-AB pH 2.5 staining procedures also evidenced such simultaneous occurrence of these two types of mucosubstances in the cells.

Thus, the large cells of the general stratified epithelium as well as in the oral papillae, are endowed with a capacity to elaborate the glycogen and acidic mucosubstance (especially sulfomucins as evidenced by positive reaction towards AB pH 1 and AF).

ii) Elaboration of mucosubstances by the small cells :

These cells exhibited intense PAS reactivity. The PAS reactivity was completely lost after prior malt diastase digestion. Towards other histochemical techniques, these cells showed negative reactivities indicating the presence of only glycogen in these cells.

iii) Elaboration of mucosubstances by the connective tissue :

The connective tissue showed negative reactivities towards the many of the histochemical staining techniques except trace PAS reaction in small cells scattered in between the connective tissue fibers. The PAS reactivity was resistant to prior diastase digestion indicating the presence of only neutral mucosubstances in it.

iv) Elaboration of mucosubstances by the muscular layer :

The PAS reactivity in the muscle cells was completely lost after diastase digestion. These cells showed negative reactivities towards other histochemical techniques indicating the presence of glycogen in this layer.

D) Histochemical Alterations due to L.eriocephalus toxin:

The plant toxin studies revealed interesting alterations in the staining intensity and concentration of the mucosubstances in the large cells, small cells, connective tissue and muscle layer of the oral cavity of T.mossambica.

The large cells reflected variations in the intensity of staining and concentration of the mucosubstances synthesized by these cells. The cells synthesizing glycogen and acidic mucins showed minimum staining in the normal tissues. The staining intensity started increasing in the low doses of the plant toxin showing moderate reactivities and reached its maximum in the higher doses of the toxin.

The small cells which contained only glycogen, showed interesting alterations in them after the toxin treatment. Their PAS reactivity was not completely abolished after prior diastase digestion indicating the transformation of glycogen into the neutral mucosubstances. Their intensity being moderate in lower doses of toxin treatment whereas reached maximum in higher doses.

The connective tissue showed the same staining reactivities even after the toxin treatment but in some PAS positive cells, which contained neutral mucosubstances in them the intensity of staining and concentration of the mucosubstances were increased in the tissues, treated with the plant toxin.

The muscle layer, which contained only glycogen in it also showed similar alterations to that of the connective tissue in the oral cavity.

ii) Gills

A) Normal Histology of Gills :

Each gill of Tilapia mossambica has two rows of primary gill lamellae internally supported by a bony structure and the spaces are filled with many blood cells (Plate No.3, Figs.9, 11). The primary gill filaments give rise to several secondary gill lamellae placed at regular intervals. Externally, the primary gill lamellae are covered with epithelial layers from the both sides. The epithelium is followed by thin connective tissue and vascular layer. The mucous secreting cells and acidophilic cells are distributed in the epithelium of the primary gill lamellae (Plate No.3,Figs.3,5,6,8).

The secondary gill lamellae are separated from each other by inter branchial septum and are free at their distal ends. These gill filaments are supported by cartilagenous tissue. The secondary lamellae are also supported internally by pillar cells (Plate No.3,, Fig.5,6). These cells are stained in HE and are arranged in the lamellae in such a fashion that they lie just adjacent to the capillaries. The epithelial cells lining the secondary gill lamellae also consists of numerous mucous cells and the acidophils are seen at the bases of these lamellae.

B) Histological Alterations due to L.eriocephalus toxin :

The effects of the crude powder and of the ethanol extracted EE showed very similar effects in the alterations in histology of fish gills. These changes observed after the treatment of lower and higher concentrations were totally different.

a) Effects with lower concentrations of crude powder (80,90 ppm) and ethanol extract (1,3,5 and 6 ppm) :

At lower doses, the effects were not prominent, however, slight changes in the morphology and in the histology were observed. The staining reactivities were altered. These changes are summarized : the increase in interlamellar space, reduction in the primary gill lamellae, displacement of the epithelium from the basement membrane, initiation of the histolysis and increase in number of mucous secreting cells and acidophil cells in the primary gill lamellae.

The secondary gill lamellae were unevenly curved. Some had became thin and slightly shortened. The apical ends were affected but basal regions of these lamellae were intact. The blood spaces were reduced. The pillar cells were enlarged and their staining reactivities were enhanced. The number of mucous secreting cells in the epithelium and of the acidophil cells were very much increased.

b) Effects with higher concentrations of crude powder (130, 140 and 150 ppm) and EE (10,12 and 15 ppm) :

The remarkable histological changes in the primary and secondary gill lamellae were observed in the treatment of higher concentrations of toxins of L.eriocephalus. The primary gill lamellae were affected and showed the alterations including reduction in the supporting bony element indicating degenerative changes, enlargement of distal gill lamellar blood spaces, increased subepithelial spaces, ruptured lamellar capillaries and detachment of epithelium.

The secondary gill lamellae also showed noticeable changes due to this plant toxin. The main alterations include the reduction and shortening of secondary gill lamellae, accumulation of blood cells in the intercellular spaces, loss of pillar cells, formation of haematomas, overlapping on one another of the filaments, uneven curling and discontinuation of the epithelium at the basal regions, increasing mucous secreting cells in the epithelium, increase in number and size of the acidophil cells. In secondary gill lamellae there were many major degenerative changes occurred suggesting the histolysis in this tissue.

C) Histochemical observations on the normal Gills :

The mucosubstance elaborating cells showed a regular distribution along the free margins of primary gill filaments, bases of the gill lamellae, interlamellar spaces and on the secondary gill filaments.

The histochemical data on some important staining reactions employed in the present investigation of the gills of T.mossambica are recorded in Table No.7, according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The distribution and alterations in the various cellular elements and their mucosubstances in the gills are photomicrographically illustrated in Plate No.3, Figs. 1 to 12.

The histochemical study on the gills revealed the following significant facts about the elaboration of the mucosubstances - (1) The mucosubstance elaborating cells showed a regular distribution along the free margins of primary gill filaments, secondary gill filaments, bases of the gill lamellae and interlamellar spaces. (2) The mucosubstance elaborating cells responded to the plant toxin treatment.

Plate No.3, Fig.Nos. 1 to 12

(Histology and mucosubstances of Gills of T.mossambica)

Fig. No.1 Small portion of transverse section of normal gills stained with HE, showing primary filament (PF), secondary filament (SF) with pillar (PC). X 150.

Fig. No.2 Small portion of transverse section of toxin treated (5 ppm) gills stained with HE. Note enhanced staining at tips (GT) of secondary gill filaments (SF) and acidophil cells (AC). X 200.

Fig. No.3 Small portion of transverse section of toxin treated (5 ppm) gills stained with AB pH1-PAS. Note curled and shortened secondary gill filaments (SF), decreased numbers of pillar cells (PC). X 200.

Fig. No.4 Small portion of transverse section of toxin treated (10 ppm) gills stained with HE. Note increase in number of acidophil cells (AC) and histolysis was evident in primary filaments (PF) and in secondary filaments (SF). X 150.

Fig.No. 5 Small portion of transverse section of toxin treated (.1 ppm) gills stained with PAS. Note intense staining in pillar cells (PC), Acidophils (AC) and at the tips of secondary filaments (SF). X 320.

Fig.No. 6 Gills treated with toxin (5 ppm) and stained with AB pH 2.5-PAS. Note the altered staining reactivities in the gill epithelium, in primary filament (PF), secondary filament (SF) and in pillar cells (PC). X 320.

Fig.No. 7 Gills treated with toxin (10 ppm). Epithelial, primary filament (PF) mucins. Stained intensely but pillar cells (PC) staining was decreased. X 320.

Fig.No. 8 Gills treated with toxin (10 ppm) stained with AB pH 2.5. Maximum staining in other gill structures except pillar cells. Note curled secondary gill filaments. X 320.

Fig.No. 9 Gills treated with plant toxin (10 ppm), stained with AB-pH 1-PAS showing shortened and histolysed secondary filaments (SF) and primary filaments (PP). Gill rachis showed strong positivity. X 150.

Fig.No.10 Enlarged gill rachis stained with AB pH 1. Note intense alcianophilla. X 320.

Fig.No.11 Enlarged gill rachis stained with AB pH 2.5 - PAS. Note intense alcianophilia. X 320.

Fig.No.12 Small portion of transverse section of toxin (5 ppm) treated gills, stained with AB pH 1. Note intensely stained pillar cells (PC) in the secondary filaments (SF), acidophils (AC) are moderately stained. X 200.

PLATE No. 3

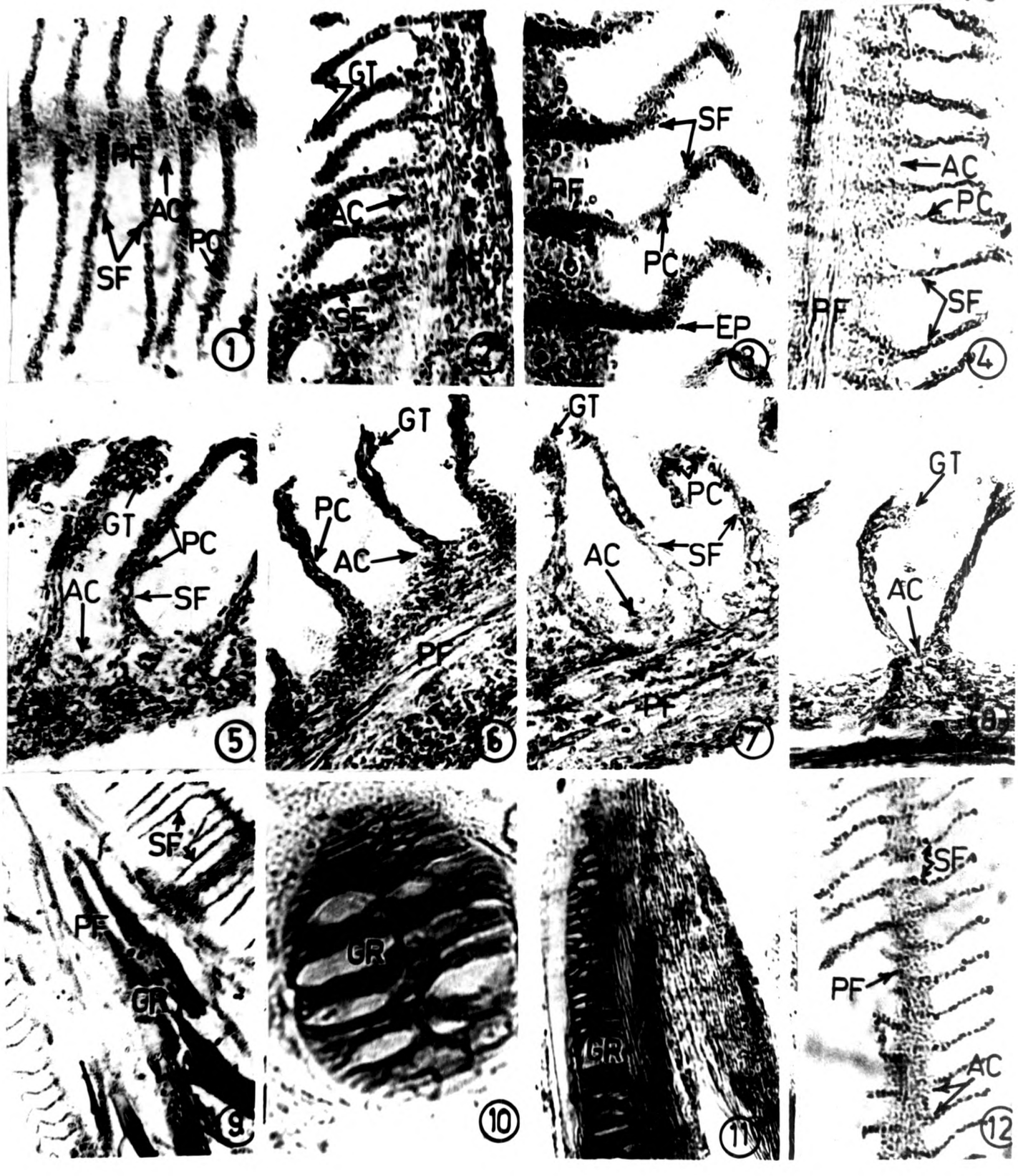

PF
AC
PC
SF
1
GT
AC
SE
2
SF
PF
PC
EP
3
AC
PC
PF
SF
4
GT
PC
AC
SF
5
GT
PC
AC
PF
6
GT
PC
AC
SF
PF
7
GT
AC
8
SF
PF
GR
9
GR
10
GR
11
SF
PF
AC
12

Table No.7

Histochemical observations on mucosubstances in the various tissues of the Gills in normal and plant toxin (L.eriocephalus) treated fish, Tilapia mossambica (Peter's)

No,	Histochemical Methods	TISSUES							
		Epithelial cells		Mucous cells				Basement Lamina	Gill rachis
		Neutral MPS cells	Mixed N+ Acidic cells	Neutral MPS cells	Neutral + Acidic cells	Sulfated	-CaOH containing cells		
1	HE	++++H ++E	++++H +++E	++++H	++++H +++E	++++H +++E	++++H ++E	++++H +++E	++++H +E
2	PAS	++++P	++++P	++++P	++++P	++++P	++++P	++++P	++++P
3	M.Diastase-PAS	++++P	++++P	++++P	++++P	++++P	++++P	++++P	++++P
4	AB pH 1	-	+++B	-	++++B	++++B	-	++B	++++B
5	AB pH 2.5	-	+++B	-	++++B	++++B	++++B	+++B	++++B
6	AB pH 1-PAS	++++P	++++P +++B	++++P	++++P ++++B	++++B	++++P	++P ++B	++++B
7	Ab pH 2.5-PAS	++++P	++++P +++B	++++P	++++P ++++B	++++B	++++B	++P ++B	++++B
8	AF	-	+++P	-	++P	++++P	-	±	++++P
9	AF-AB-pH 2.5	-	+++P	-	++P	++++P	-	±	++++P

N.B. :++++ = Very intense reaction; +++ = Intense reaction; ++ = Moderate reaction; + = Poor reaction; - = No reaction.

Abbreviations : HE = Haematoxyline-Eosine; PAS = Periodic Acid Schiff; M.Diastase = Malt diastase; AB = Alcian blue 8GX-300; AF = Aldehyde fuschin; P = pink; B = blue.

a) Mucosubstances elaboration by epithelial cell :

The epithelial cells exhibited PAS reactivities which was resistant to prolonged diastase digestion indicating absence of glycogen in them. The other histochemical techniques for demonstration of acid mucosubstances showed negative reactivities, indicating their absence from these sites. Thus the gill epithelial cells of T.mossambica elaborated only neutral mucosubstances in them.

But some few cells in the epithelium showed PAS reactivity and slight alcianophilia at AB pH 2.5 and in the combined (AB 2.5-PAS) staining methods, indicating probable simultaneous occurrence of acidic mucosubstances along with the neutral mucins in them.

b) Mucosubstances elaboration by Mucous cells :

There are different cells on the gill lamellae which stained deeply with various mucin demonstrating techniques employed in the present investigation.

All the mucous secretory cells were PAS positive. Their PAS positivity was resistant to prolonged diastase digestion, indicating absence of glycogen in them. In combined sequential staining techniques like AB pH 2.5 - PAS and AB pH 1-PAS, some of these cells stained only purple (Plate No.3, Figs. 3,6 and 9) thus showing elaboration of neutral mucosubstances in them.

Some cells contained a mixture of neutral and sulfated mucosubstances. These cells showed PAS reactivity resistant to prior diastase digestion. In AB pH 1-PAS and AB pH 2.5-PAS sequential staining techniques, these cells showed mixed staining. The probable occurrence of sulfated

mucosubstances were deduced from their alcianophilia both at pH 2.5 and pH 1.0 (Plate No.3, Figs. 8 and 12). AF and AB pH 2.5-AF also showed mixed staining in these cells. Thus, these cells have capacity to synthesize and elaborate mixed, neutral and acidic (sulfated) mucosubstances.

There were some acidic mucosubstances secreting cells. These cells showed PAS reactivity resistant to diastase digestion. These cells showed intense alcianophilia both at pH 1.0 and pH 2.5. They stained blue with sequential staining techniques (AB pH 1-PAS and AB pH 2.5-PAS). Some of the acidic mucosubstances secreting cells were AF positive and in the combined sequential staining procedure. AF-AB pH 2.5 also showed pink staining in them. Thus some of the acidic mucosubstance secreting cells including the pillar cells have capacity to elaborate sulfomucins whereas other contained carboxyl containing acid mucosubstances in them (Plate No. 3, Figs. 1,3,4,6 and 8).

c) Mucosubstances elaboration by the Basement lamina :

The epithelial cells rest on the basement lamina which showed moderate PAS reactivities. The PAS staining was not reduced by prior diastase digestion indicating absence of glycogen but the neutral mucins. The basement lamina, simultaneously, showed moderate alcianophilia both at pH 1 and pH 2.5. In the combined sequential staining procedures like AB (pH 1, 2.5)-PAS, this tissue stained bluish-pink indicating acid moieties along with the neutral mucins in it (Plate No.3, Figs. 3,5,6,7 and 8).

d) Mucosubstances elaboration by the Gill rachis :

The cartilagenous tissue present in the gill rachis showed strong PAS reactivity, deep alcianophilia both at pH 1 and pH 2.5, stained only

blue in the combined sequential staining techniques like AB pH 1-PAS and AB pH 2.5-PAS, AF staining was prominent indicating sulfated acic muco-substances in them (Plate No.3, Figs.9, 10 and 11).

D) Histochemical Alterations in the Mucins Elaboration due to L.eriocephalus Toxin :

The application of plant toxin L.eriocephalus revealed several interesting alterations in the staining intensity and concentration of the mucosubstance in the epithelial cells, mucin elaborating cells, pillar cells, basement lamina and in the gill rachis of T.mossambica.

The epithelial cells and mucous elaborating cells reflected variation in the intensity of staining and in the concentration of the mucosubstances elaborated by these cells. During lower doses of the plant toxins their mucin secretion was increased (Plate No.3, Fig.7) and during the treatment of higher doses their elaboration reached maximum, covering the whole tissue (Plate No.3, Figs.6 and 8).

The pillar cells of the secondary filaments showed reverse effects to that of the epithelial cells during the plant toxin treatment. In the low concentration the pillar cells showed maximum concentration of muco-substances (Plate No. 3, Fig.3) while in the higher concentration due to the reduction in the number of pillar cells, their concentration and staining intensities were minimum (Plate No. 3, Fig. 7).

The basement lamina of primary and secondary gill filaments showed moderate staining intensities and concentration of mucosubstances in the lower dose of plant toxin (Plate No.3, Figs. 4,12) and peak maxima in the high doses (Plate No. 3, Figs. 6 and 8).

The mucosubstance elaboration by gill rachis was moderate in the normal environment (Plate 3, Fig. 10), which was increased considerably in the 1 ppm and 5 ppm of plant toxin concentrations (Plate 3, Fig. 11) but reached a maximum and their staining intensities were also highest in the higher doses (10 ppm and 15 ppm) of the plant toxin treatment (Plate No.3, Fig. 9).

III - Liver

A) Normal Histology of Liver:

The Liver of Tilapia mossambica is composed of many lobules richly supplied with blood by many vessels. Each lobule consists of a central lumen surrounded by many cords of polygonal hepatic cells. The hepatic cells or hepatocytes contain granules of different sizes in the cytoplasm and a centrally placed nucleus. A number of blood spaces - sinusoids are scattered in the hepatic tissue. In between the hepatic cords of the hepatocytes, small bile canaliculi originate and many of them unite to form bile duct, which is seen in the hepatic lobules (Plate No.4, Figs. 1,2,7). In some sections the pancreatic tissue contains big and differently staining Islet-cells (Plate No.4, Fig.4).

B) Histological Alterations due to L.eriocephalus toxin :

The effects of the crude powder and of the ethanol extract showed very similar effects on the histology of the fish liver. The histological changes observed in low and high doses of the toxin did not differ very much. The histologically distinct observable changes in the liver are summarized below :

The displacement of hepatocytes was the foremost effect of the plant toxin. The cells with the increasing doses started enlarging. There was aggregation of cytoplasmic contents. Due to this precipitation black patches could clearly be seen in this tissue (Plate No.4, Figs.6 and 8). Some hepatocytes were swollen with large vacuoles. With higher concentrations (15 ppm) due to the swelling and vacuolization it was difficult to mark the cell boundaries. Disruption of sinusoids was also evident (Plate No.4, Fig.11).

The other detectable histological change was observed in hepatocyte nuclei. Some cells were without nuclei, whereas in some the size of the nuclei was increased. The nuclei picnosis was observed. There were some binucleated hepatocytes. In some cells nuclei with mitotic divisions were observed.

The net result of the plant toxin showed the changes in the cordal arrangement, leading to deformation of liver histology (Plate No.4, Fig. 12) showing large gaps in the hepatic tissues.

C) Histochemical Observations on the Normal Liver :

The histochemical data on some important staining reactions employed in the present investigation of the liver of T.mossambica are recorded in Table No.8, according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The histochemical observations requiring further description and consideration, are presented hereafter along with the interpretations of the histochemical staining reactions. The distribution and alterations in the mucosubstances in the liver are photomicrographically illustrated in Plate No.4, Figs.1 to 12.

Plate No.4, Fig. Nos. 1 to 12

(Histology and mucosubstances of Liver of T.mossambica)

Fig.No.1 Small portion of transverse section of normal liver stained with HE. Note lobules (L) with bile canaliculi and strands of hepatocytes (HC). X 200.

Fig.No.2 Small enlarged portion of transverse section of normal liver stained with HE. Note lobules (L) and bile canaliculi stained darkly. Faint staining in hepatocytes (HC). x 200

Fig.No.3 Small enlarged portion of transverse section of normal liver stained with PAS. Note moderate PAS staining in Lobules(L) showing hepatocytes (HC). X 200

Fig.No.4 Small enlarged portion of transverse section of normal liver stained with AB pH 2.5 - PAS. Note intensely stained Islet cells (BC) with AB pH 2.5 and moderate PAS reactivity in the hepatocytes (HC). X 200

Fig.No.5 Small enlarged portion of transverse section of toxin treated (.1 ppm) liver stained with HE. Note some nuclei showing mitotitic divisions (M) in hepatocytes (HC) of liver lobule (L). X 200

Fig.No.6 Toxin treated (1 ppm) enlarged liver section stained with HE, Nuclei in mitosis (M) are clearly seen in hepatocytes (HC). Note the black patches due to aggregation of cytoplasmic contents. X 320.

Fig.No.7 Toxin treated (5 ppm) liver section stained with PAS. Note moderately stained hepatocytes (HC) and intensely stained contents in the Canaliculi. X 320.

Fig.No.8 Toxin treated (5 ppm) liver section stained with AB pH 2.5 - PAS. Note increased PAS reactivity in the hepatocytes bordering blood sinusoids. Glycogen patches intensely stained (SP) with PAS. X 370.

Fig.No.9 Toxin treated (10 ppm) liver section stained with PAS showing maximum PAS reactivity in the hepatocytes (HC). Number of nuclei showing mitotic divisions reached maximum (MN). X 320.

Fig.No.10 Toxin treated (10 ppm) liver section stained with AB pH 2.5 - PAS. Note reduced staining intensity of hepatocytes (HC) and initiation of vacuolization. X 320

Fig.No.11 Toxin treated (10 ppm) liver section stained with HE. Note ruptured blood sinusoid, binucleated hepatocytes and loss of cell borders. X 320

Fig.No.12 Toxin treated (10 ppm) enlarged liver section stained with PAS. Large vacuoles (V) were observed and intense PAS reactivity surrounding vacuoles. X 320

PLATE No. 4

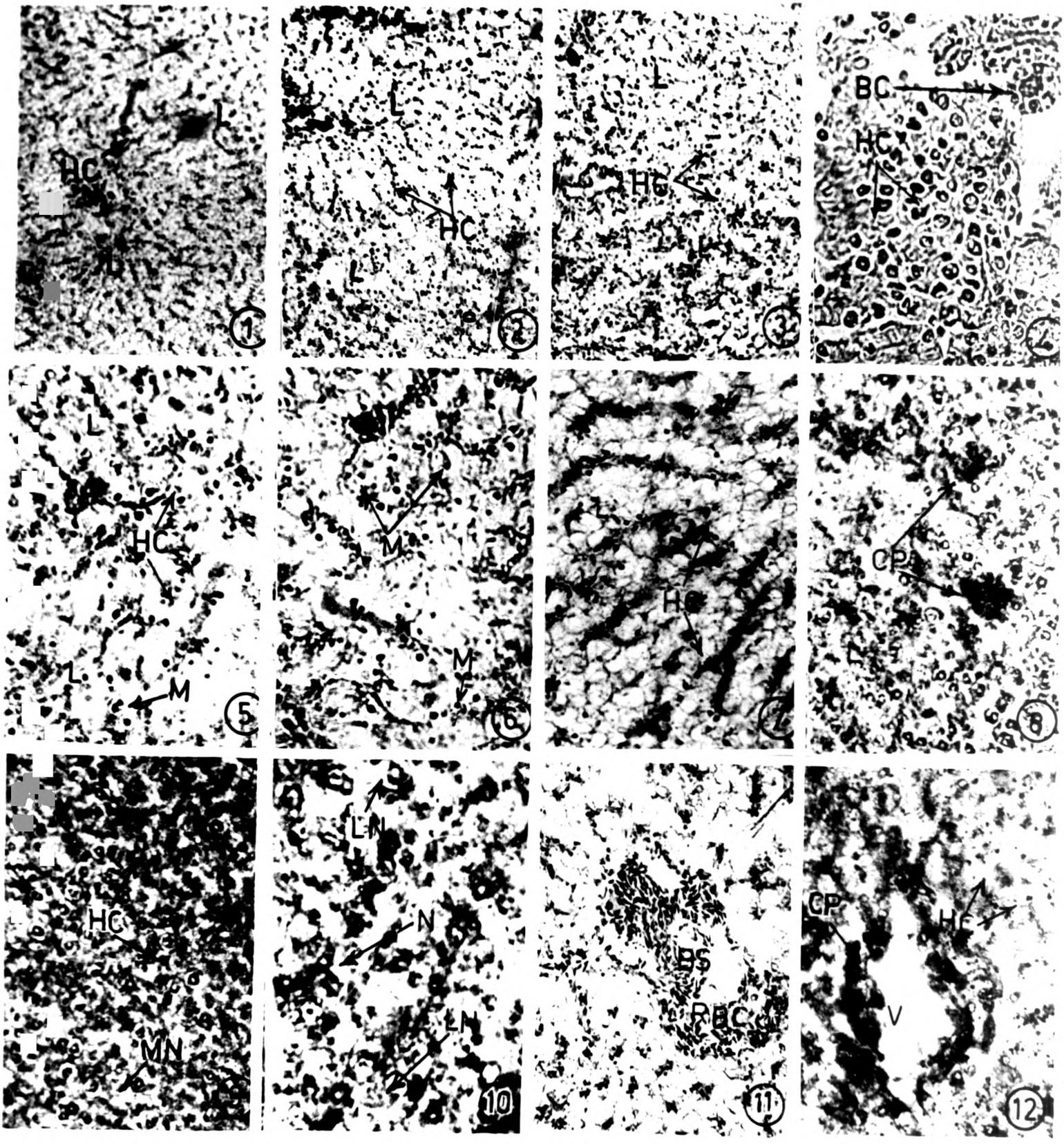

HC
L
1
L
HC
2
L
HC
3
BC
HC
4
L
HC
L
M
5
M
M
6
H
7
CP
8
HC
MN
9
LN
N
LN
10
BS
RBC
11
CP
HC
V
12

Table No.8

Histochemical observations on mucosubstances in the various tissues of the Liver in normal and plant toxin (L.eriocephalus) treated fish, Tilapia mossambica (Peter's)

No.	Histochemical Methods	Hepatocytes		
		Normal	Low concentration of plant ploxin	High concentration of plant toxin
1	HE	++++H +++E	++++H +++E	++++H ++++E
2	PAS	++P	++++P	+++P
3	M.Diastase-PAS	-	-	-
4	AB pH 1	+B	++B	+++B
5	AB pH 2.5	+B	++B	+++B
6	AB pH 1-PAS	+B ++P	++B ++++P	+++B +++P
7	AB pH 2.5-PAS	+B ++P	++B ++++P	+++B ++P
8	AF	-	-	-
9	AF-AB pH 2.5	-	-	-

N.B. : ++++ = Very intense reaction; +++ = Intense reaction; ++ = Moderate reaction; + = Poor reaction; - = No reaction.

Abbreviations : HE = Haematoxyline-Eosine; PAS = Periodic Acid Schiff; M.Diastase = Malt diastase;

AB = Alcian blue 8GX-300; AF = Aldehyde fuschin; P = pink; B = blue.

Elaboration of mucosubstances by Hepatocytes:

The liver cells exhibited intense PAS reactivities (Plate No.4, Figs. 9 & 12). To test the nature of the PAS staining some of the sections were treated for enzymatic digestion by malt diastase. Such digestion indicated the glycogen nature of the PAS staining. Acid mucosubstances were very low in concentration as compared to Glycogen. There was very faint alcianophilia both at pH 1 and pH 2.5 (Plate No.4, Fig.4). In the combined AB (pH 1, 2.5)-PAS staining techniques also they stained only pink with very faint blue staining. Thus, the hepatic tissue contained glycogen and very few acidic mucosubstances.

The few patches of pancreatic cells showed PAS positivity resistant to diastase digestion and moderate alcianophilia at pH 2.5 (Plate No.4, Fig.No.4), and in the combined sequential staining methods, such as AB (pH 1 and 2.5)-PAS. Alcianophilia was not visible at pH 1 and AF reactivity was also negative. Such reactivities indicated the presence of carboxyl group containing acidic mucosubstances in these pancreatic cells.

Interestingly the cells bordering the blood sinusoids were highly PAS positive and contained high concentration of glycogen in them (Plate No.4, Figs. 10 and 12).

D) Histochemical Alterations in the Mucosubstance Elaboration due to L.eriocephalus Toxin :

The application of plant toxin, L. eriocephalus to the T.mossambica revealed many striking changes in the intensity of staining and concentrations of the mucosubstances of the hepatic cells only.

The liver sections in the lower concentrations (1 ppm, 3 ppm and 5 ppm) of plant toxin produced very few increase in the staining intensity (Plate No.4, Fig. 4) whereas in higher succeeding concentration (10 ppm) it was found to intensify gradually reaching maximum at 12 ppm (Plate No.4, Fig. 9). But at still higher concentration (15 ppm) the staining was reduced considerably but at places it was concentrated surrounding certain vacuolar spaces (Plate No.4, Fig.12). Thus, it indicated that with the high doses of toxin treatment glycogen content of the tissue was considerably depleted. The accumulation or aggregation of cells consequently formed, number of glycogen patches throughout the hepatic tissue (Plate No.4, Fig. 8).

IV - Kidney

A) Normal Histology of Kidney :

The kidney of Tilapia mossambica composed of many functional units the nephrons. The Malpighian bodies are clearly seen (Plate No.5, Fig.4) with Bowman's capsules in which glomeruli are enclosed (Plate No.5, Fig. 1,3,4,5,6,7 and 8). The Bowman's capsule has basement membrane. The Bowman's capsule leads to a narrow neck, with ciliated epithelium, continues into proximal and distal tubules. The proximal tubules are characterised by the tall columnar epithelium which shows brush border towards the luminal side, whereas the distal tubules are lined with epithelial cells only. The distal tubules ends into collecting duct. There are various patches of the haemopoietic tissue interpreted in the kidney tissue (Plate No. 5, Fig. 2,9).

B) Histological Alterations due to L.eriocephalus Toxin :

The histology of the kidney was totally changed due to the toxin treatment even at lower doses also. The initial toxin dose affected the malpighian body. In the low concentration (1 ppm) the glomeruli of the kidney immediately increased in size. Subsequently at higher concentrations increase in capsular size, shrinkage in glomeruli, damage to the capillaries, degeneration of basement membrane and endothelial cells development of intertubular space etc. took place particularly in the higher doses, the glomeruli became diffused (Plate No.5, Fig. 9).

The lumen of the proximal tubules was reduced and the brush border showed disintegration. There was loss of cytoplasmic material in few proximal tubule cells. Some tubules showed distortion leading to change in the kidney structure. In some of the cells, nuclear material was lost. The edema in these tubules was prominent. The cytoplasm of the cells in these tubules showed increased eosinophilic activity.

The swelling and the damage of epithelial cells in the distal tubules were evidenced. The concentrated substances were observed towards the luminal side showing honey-comb like structure. The hyportrophy and edema led to disorientation in the distal tubules, thus causing distortion in the kidney structure. There were formation of spaces in between the intertubules, whereas lumen size was very much reduced. The severe necrotic changes were prominent in these tubules.

The cells of the collecting tubules were also enlarged. The vacuoles were observed in these tubules cells. The lumen size was very much reduced due to the enormous increase of the cells.

C) Histochemical Observations on the Normal Kidney :

The histochemical data on some important staining reactions employed in the present investigation of the kidney of T. mossambica are recorded in Table No.9, according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The histochemical observations requiring further description and consideration, are presented here after along with the interpretations of the histochemical staining reactions. The distribution and alterations in the mucosubstances in various parts of the kidney are photomicrographically illustrated in Plate No.5, Figs.1 to 9.

The histochemical study on the kidney revealed the following significant facts about the elaboration of the mucosubstances - (1) The mucosubstance elaboration exhibited by the malpighian bodies, proximal tubules and distal tubules. (2) There were very significant alterations in the mucosubstance elaborating tissues in response to the plant toxin (L.eriocephalus) treatment.

(a) Elaboration of mucosubstances by Malpighian bodies :

Both glomeruli and Bowman's capsule showed intense PAS reactivities (Plate No.5, Fig.2) which were partly sussceptible to prior malt diastase digestion, indicating partial presence of some glycogen at both these sites. The non-susceptible chemical moiety was confirmed to be acidic mucosubstances by various alcian blue staining procedures. The basophilia (alcianophilia) with AB pH 2.5 gave a moderate staining reaction (Plate No.5, Fig.3). In AB pH 1-PAS and AB 2.5 - PAS sequential staining procedures these parts showed mixed staining (Bluish-purple). Thus, Malpighian bodies indicated the presence of glycogen and acidic mucopolysaccharides in their cells.

Plate No.5, Fig. Nos. 1 to 9

(Histology and mucosubstances of Kidney of T.mossambica)

Fig.No.1 Small portion of transverse section of the normal kidney stained with HE, Glomeruli (GL), proximal tubules (PT), Distal tubules were clearly seen. X 200

Fig.No.2 Small portion of normal kidney stained with PAS. Note intense PAS staining in Glomeruli (GL) and Bowman's capsule (BC) X 200

Fig.No.3 Small portion of normal kidney stained with AB pH 2.5. Glomeruli (GL), Proximal convoluted tubules (PT) and Distal tubules showed alcianophilia. X 320

Fig.No.4 Small portion of toxin treated (5 ppm) kidney section stained with AB pH 2.5. Note enlargement of glomeruli (GL) and Bowman's capsule (BC) and alcianophilia at brush border of proximal tubules (PT). X 320

Fig.No.5 Portion of toxin treated (5 ppm) kidney section stained with AB pH 2.5 - PAS. Note increased staining reactivities of Glomeruli (GL), Bowman's capsules and proximal tubules (PT). X 320

Fig.No.6 Small portion of toxin treated (5 ppm) kidney section stained with HE. Note the enlarged glomerular (GL) size and cells bordering the Bowman's capsule (BC) and proximal tubules. X 320.

Fig.No.7 Small portion of toxin treated (10 ppm) kidney section stained with AB pH 2.5 - PAS. Note vacuolization and shrinkage in the glomeruli (GL). Bowman's capsule with intense PAS staining. X 320

Fig.No.8 Section of kidney treated with toxin (10 ppm), stained with AB ph 2.5-PAS. Glomeruli (GL) forms thick zone of mucosubstances forming ring. Other structures showed decreased staining. X 320

Fig.No.9 Small portion of toxin treated (10 ppm) kidney section stained with HE. Note diffused glomeruli (GL), enlarged cells in the proximal (PT) and distal tubules and Bowman's capsule (BC). Necrotic changes were evident X 320 .

PLATE No. 5

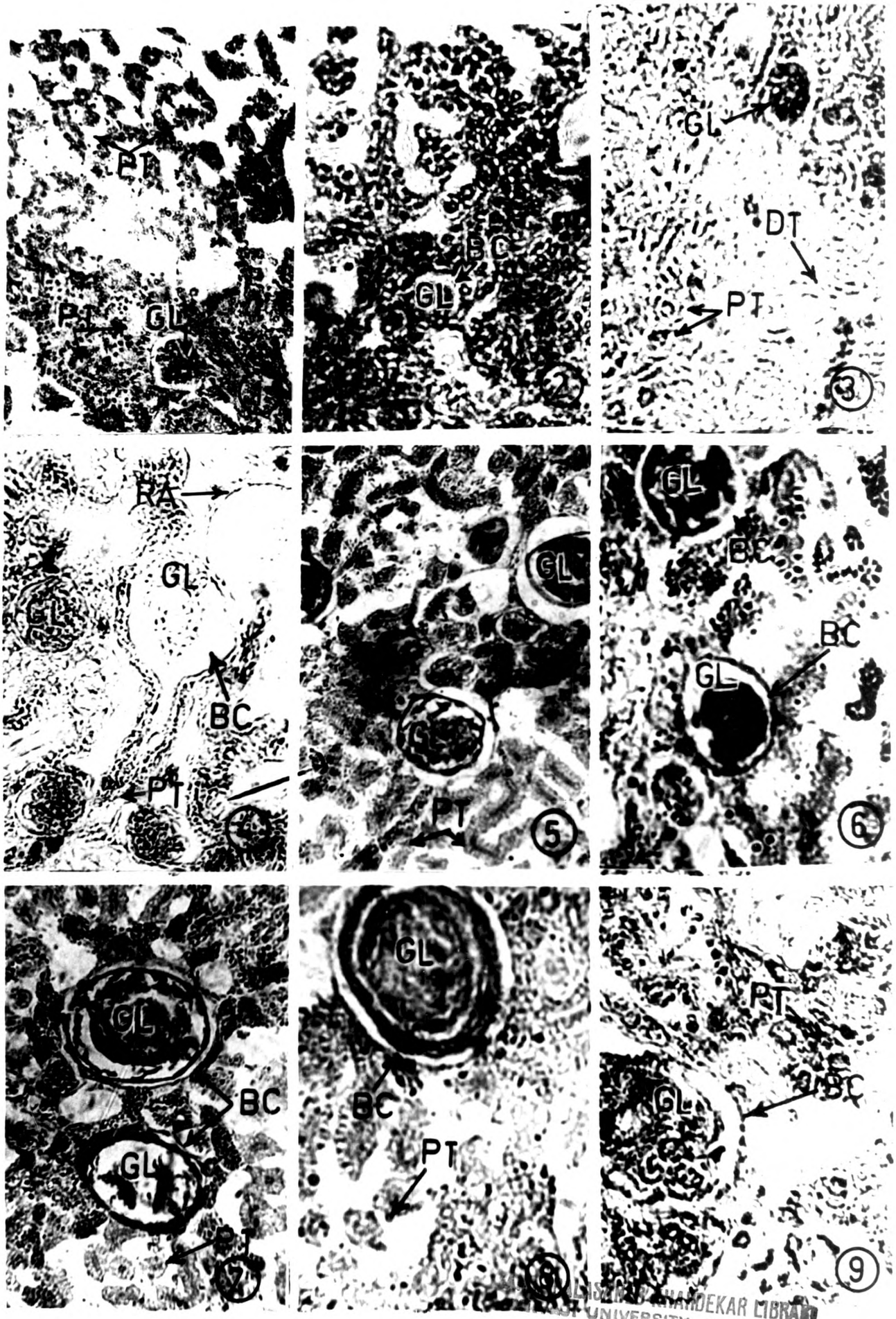


Table No. 9

Histochemical observations on mucosubstances in the various tissues of the kidney in normal and plant toxin (L.eriocephalus) treated fish, Tilapia mossambica (Peter's)

No.	Histochemical Methods	TISSUES								
		Malphigian Body			Proximal Tubules			Distal tubules		
		Normal	Low dose of plant toxin	High doses of plant toxin	Normal	Low doses of plant toxin	High doses of plnat toxin	Normal	Low doses of plant toxin	High doses of plant toxin
1.	HE	++++H +++ E	++++H ++++E	++++H ++++E	++++H +++E	++++H +++E	++++H ++++E	+++H +++E	++++H ++++E	++++H ++++E
2.	PAS	+++P	++++P	++P	++P	+++P	++++P	++P	+++P	++++P
3.	M.Diastase-PAS	++P	+++P	+P	++P	+++P	++++P	-	-	-
4.	AB-pH 1	-	-	-	++B	+++B	++++B	++B	+++B	++++B
5.	AB pH 2.5	+++B	++++B	+B	++B	+++B	++++B	++B	+++B	++++B
6.	AB pH 1 - PAS	+++P	++++P	++P	++P ++B	+++P +++B	++++P ++++B	++P ++B	++P +++B	++++P ++++B
7.	AB pH 2.5-PAS	+++P +++B	++++P ++++B	++P +B	++P +++B	+++P +++B	++++ ++++B	++P ++B	+++P +++B	+++P ++++B
8.	AF	-	-	-	++P	+++P	++++P	++P	+++P	++++P
9.	AF-AB pH 2.5	++B	++++B	+B	++P ++B	++++P ++++B	++++P ++++B	++P	+++P	++++P

N.B. : +++ = Very intense reaction; +++ = Intense reaction; ++ = Moderate reaction; + = Poor reaction; - = No reaction.
Abbreviations : HE = Haematoxyline-Eosine; PAS = Periodic Acid Schiff; M.Diastase = Malt diastase; AB = Alcian blue 8GX-300; AF = Aldehyde fuschin; P = pink; B = blue.

(b) Elaboration of mucosubstances by Proximal tubules :

The PAS reaction was selectively intense in the luminal brush border of proximal tubules and cellular staining was slightly faint (Plate No.5, Fig.3). PAS positivity with prior diastase digestion had no effect indicating absence of glycogen in these tubules.

The luminal brush border, as in case of PAS showed intense alcianophilia at pH 2.5, indicating the presence of acidic mucins in these sites. AB 2.5-PAS combination produced a magenta colour only at the sites of luminal lining which showed intense reactivity for PAS whereas in the remaining cytoplasm AB staining was evident implying the presence of either neutral mucopolysaccharide or acidic mucins in these tubules (Plate No.5, Fig.3).

(c) Elaboration of mucosubstances by the distal tubules :

The distal tubules showed uniform PAS reactivity which had a slight suppressive effect with prior diastase digestion indicating the presence of glycogen in these tubules. Alcianophilia with AB pH 1 and AB pH 2.5- was also uniform but with very low intensity. AB (pH 1, 2.5)-PAS combined staining procedures showed mixed staining. AF staining was very faint and in the combined AF-AB pH 2.5 also AF reactivity was observed. Thus, the distal tubules were synthesising and elaborating glycogen and slightly sulfated acid mucosubstances.

D) Histochemical Alterations in the Mucosubstances due to *L. eriocephalus* Toxin :

The application of plant toxin, *L.eriocephalus* to fish, *T.mossambica* revealed interesting changes in the staining intensity and concentration of the mucosubstances elaborated by the kidney tissue.

The glomeruli and Bowman's capsule showed increasing and intensifying staining of the mucosubstances. These structures contained glycogen and acid mucopolysaccharide, immediately showed their concentration reaching maximum even in low doses of plant toxin (Plate No.5, Figs.4 to 6) and started decreasing as the concentrations of the plant toxin in the treatment increased (Plate No.5, Figs. 7 to 9). Interestingly the accumulation of these mucosubstances in the glomeruli were so much that their sizes were at least 10 to 15 time greater than those of the normal ones (Plate No.5, Figs.7 and 8). The mucosubstance form a ring surrounding the glomeruli near the Bowman's capsule (Plate No.5, Figs.7 & 8). In the higher treatment accumulated mucosubstances slowed and steadily diminished (Plate No.5, Fig. 9).

The proximal tubule mucosubstances were also increased immediately after the low doses treatment of the plant toxin (Plate No.5, Fig.5) which were distinctly observed towards luminal brush borders. As against the Malpighian bodies the increasing trend of mucosubstances accumulation or concentration and intensifying in the sites of these tubules continued in the higher doses of the plant toxin treatment (Plate No.5, Figs. 7 and 8).

The distal tubule mucosubstances showed similar alteration pattern to that of the proximal tubule mucosubstances. Their concentration was moderate the normal sections which slightly increased in the lower doses and continued in the higher concentration of plant toxin treatment. Thus, in distal tubules glycogen and sulfated acid mucosubstance varied with the concentration of plant toxin treatment, showed linear relationship as regards to their concentration and intensity of the mucosubstances.

****