CHAPTER - III

Observations

C H A P T E R - I I I

OBSERVATIONS

A) OBSERVATIONS ON THE SOLVENT EXTRACTION OF FRUITS OF A. CONCINNA :

The shade dried fruits, after removal of seeds, were powdered and extraction was carried in five different solvents i.e. petroleum ether, benzene, ethyl acetate chloroform and ethanol. Percentage composition and weight in gms of each extract was obtained which is compiled in the following chart.

Percentage composition of five extracts of A. concinna

No.	Material	Solvent	Weight in gms.	Percentage
1.	Shade dried fruits	-	500.00	-
2.	Extract E_1	Petroleum ether	6.165	1.233
3.	Extract E_2	Benzene	4.760	0.954
4.	Extract E_3	Ethyl acetate	10.125	2.250
5.	Extract E_4	Chloroform	3.615	0.723
6.	Extract E_5	Ethanol	82-162	16.432

B) OBSERVATIONS ON THE PHYTOCHEMICAL ANALYSIS :

1) Melting points :

i) The melting point of Extract E_1 = 89°C

ii) The melting point of Extract E_2 = 92°C

iii) The melting point of Extract E_3 = 95°C

iv) The melting point of Extract E_4 = 99°C

v) The melting point of Extract E_5 = 97-98°C.

2) UV spectral observations :

UV spectra of the five extracts (E_1 - E_5) showed intense peaks at λ max in between 225 nm and 425 nm . Fig. Nos. 1 to 5 show the UV spectra for the petroleum ether, benzene, ethyl acetate, chloroform and ethanol soluble components, respectively.

The following chart shows the peak values of components extracted in the petroleum ether (E_1), Benzene (E_2), ethylacetate (E_3), chloroform (E_4) and ethanol (E_5).

Sr.No.	Extract	Peak values in nm	
		Peak I	Peak II
1.	Petroleum ether extract (E_1)	315	415
2.	Benzene extract (E_2)	315	420
3.	Ethyl acetate extract (E_3)	320	-
4.	Chloroform extract (E_4)	280	425
5.	Ethanol extract (E_5)	265	350

U V SPECTRUM OF PETROLEUM ETHER EXTRACT OF FRUITS OF

Acacia concinna (DC).

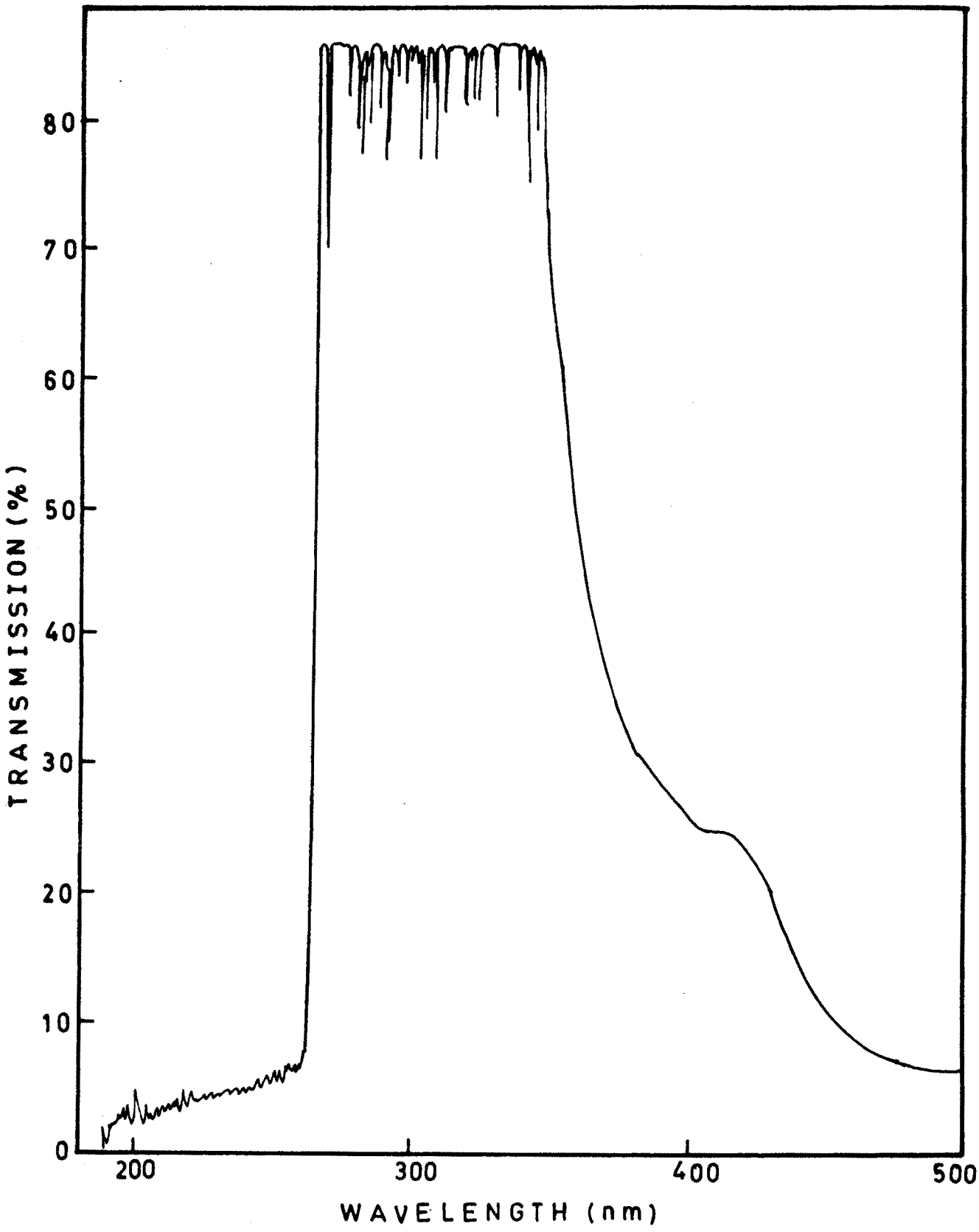

FIG. NO. 1

U V SPECTRUM OF BENZENE EXTRACT OF FRUITS OF

Acacia concinna (DC)

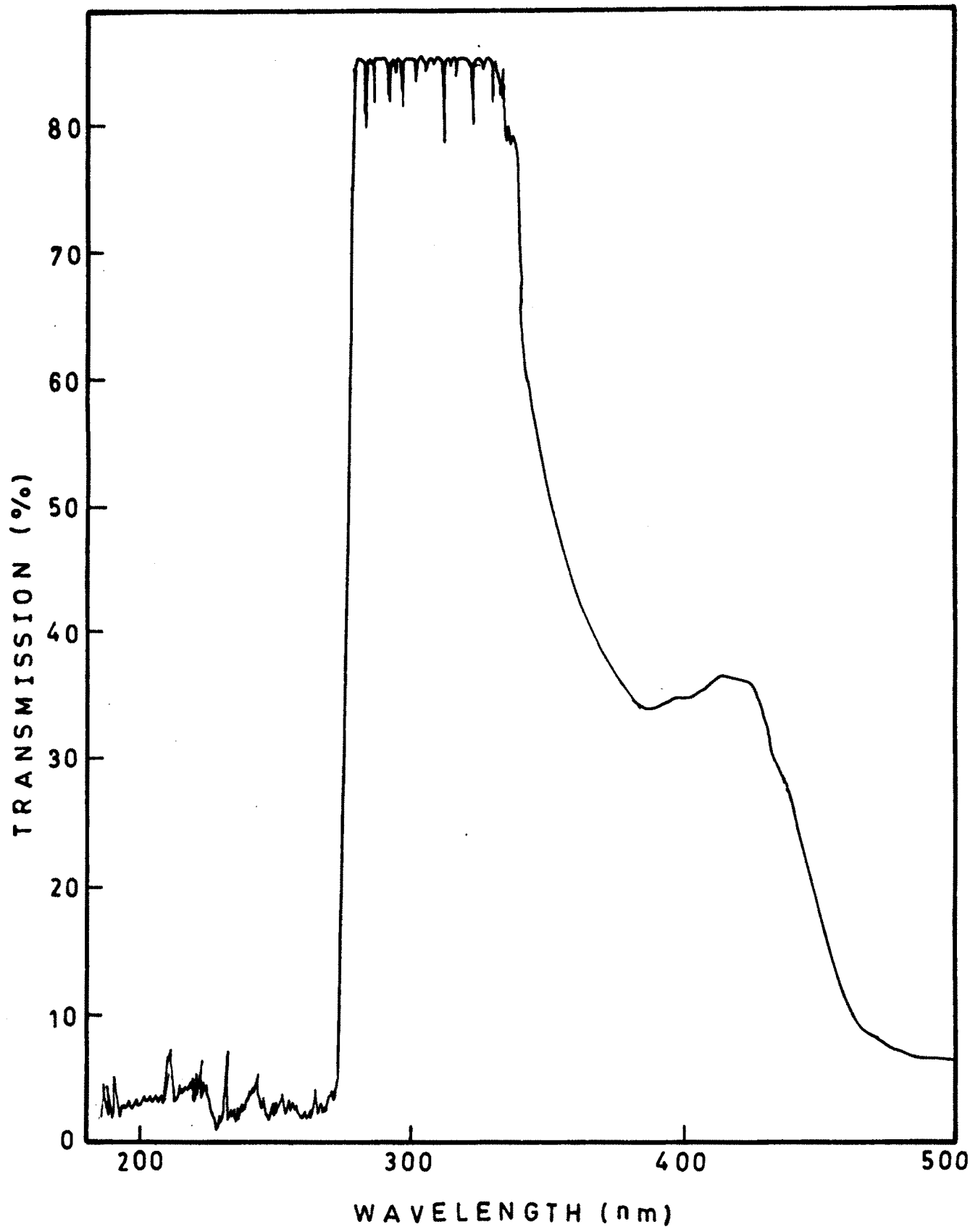

FIG. NO. 2

U V SPECTRUM OF ETHYL ACETATE EXTRACT OF FRUITS OF

Acacia concinna (DC)

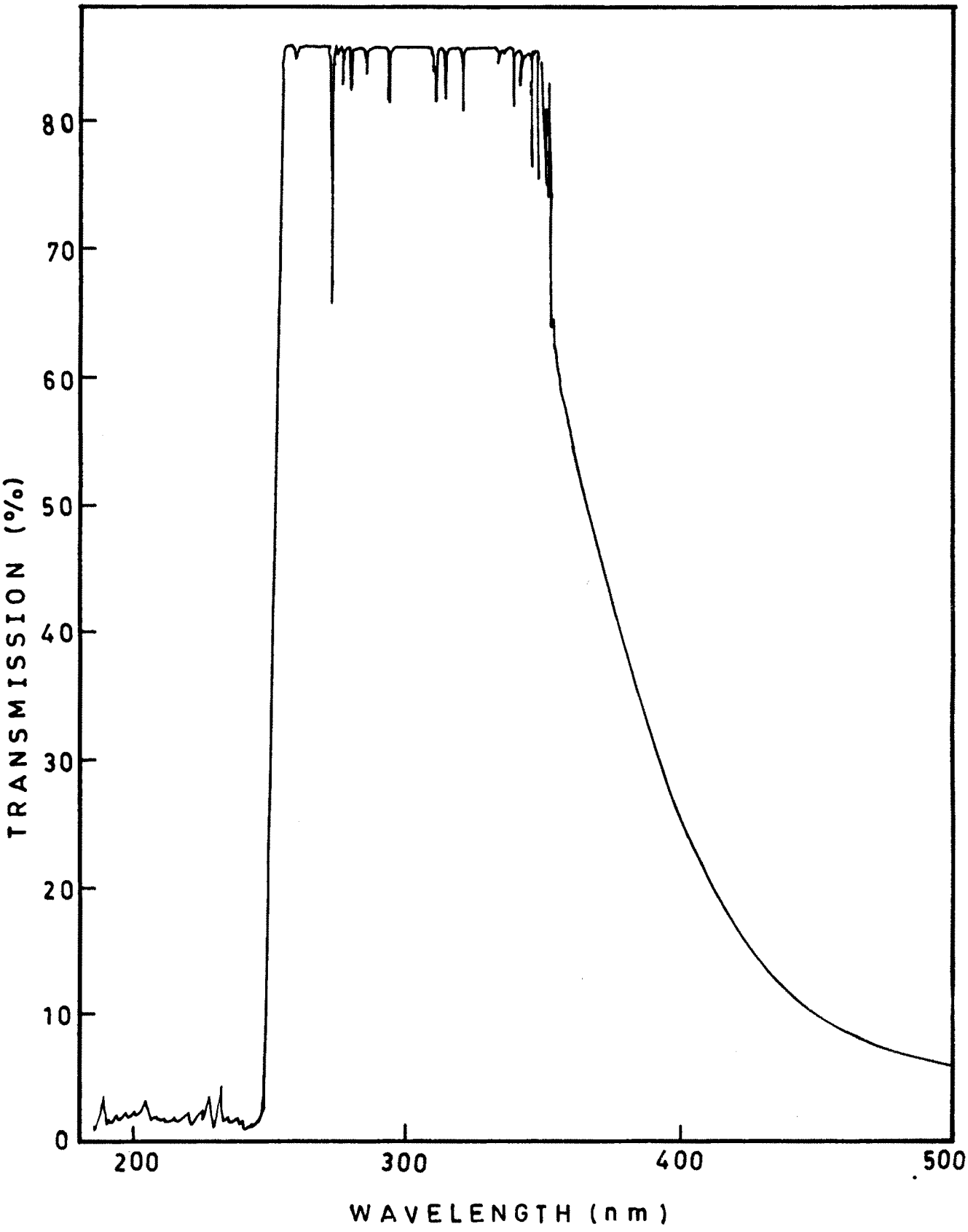

FIG. NO. 3

UV SPECTRUM OF CHLOROFORM EXTRACT OF FRUITS OF

Acacia concinna (DC).

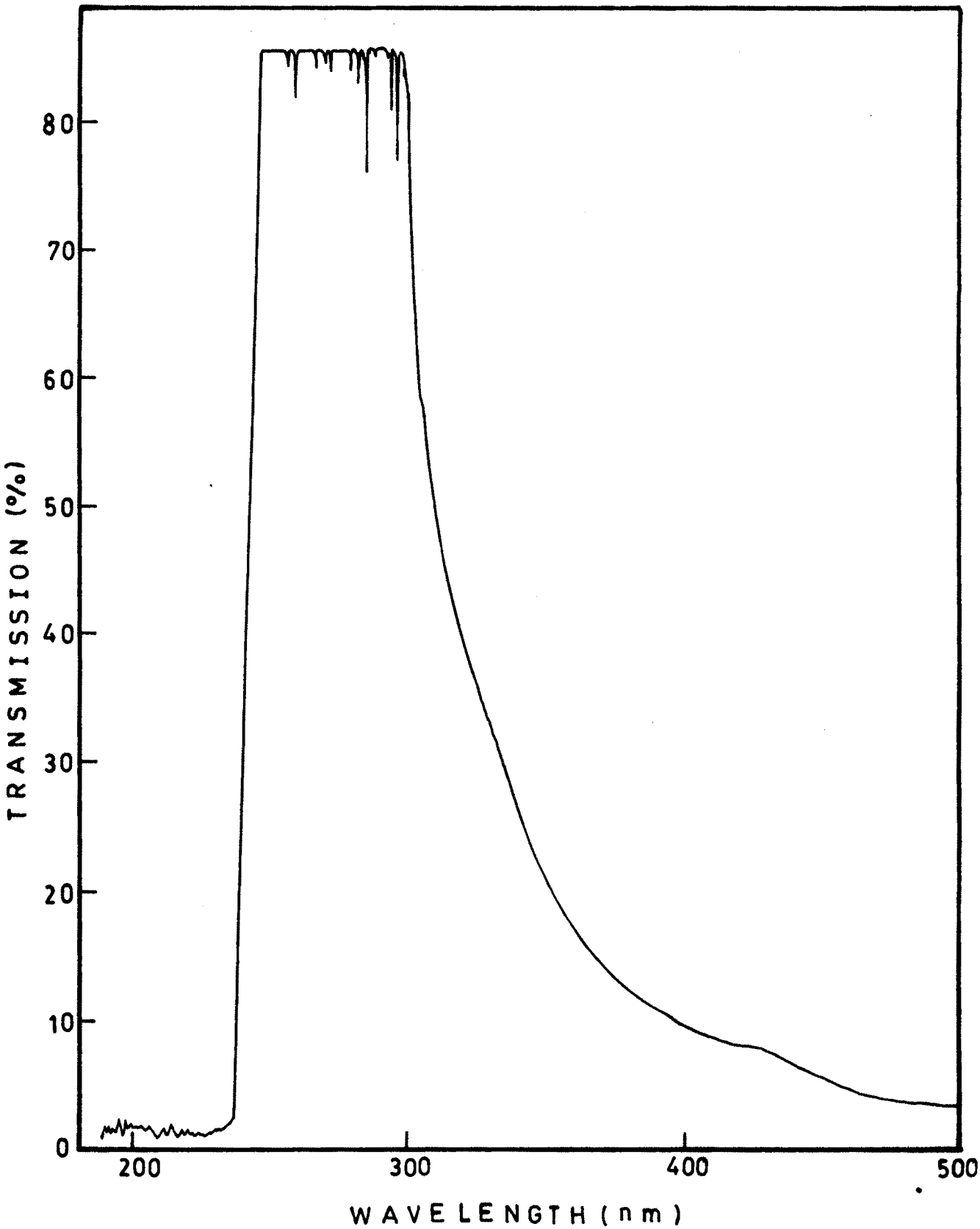

FIG. NO. 4

U V SPECTRUM OF ETHANOL EXTRACT OF FRUITS OF

Acacia concinna (DC).

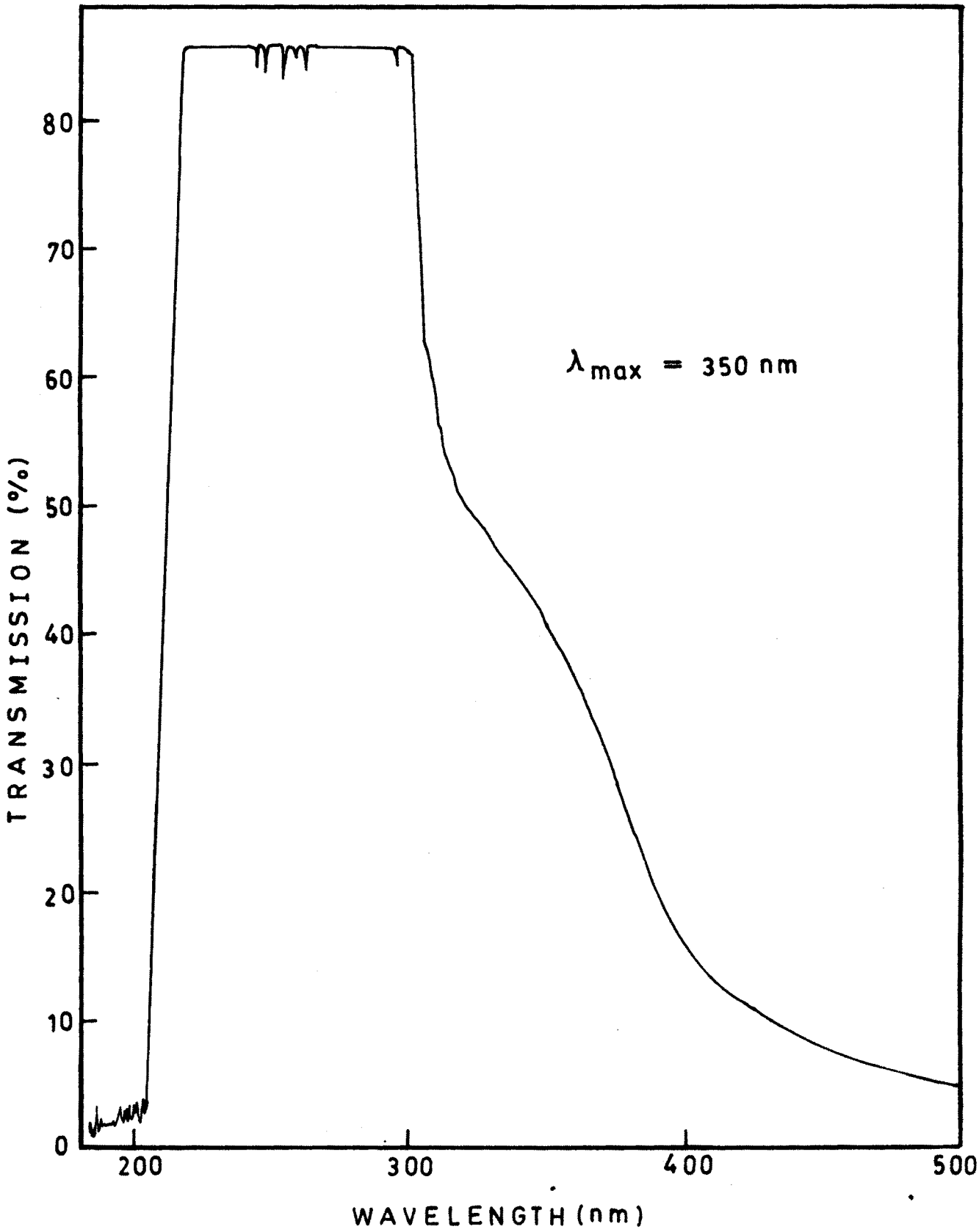

FIG. NO. 5

The UV spectra show that the extract E_3 has only one peak whereas extracts E_1, E_2, E_4 and E_5 have two peaks.

3) NMR spectral observations :

Fig. Nos. 6 to 10 show NMR spectra of five extracts E_1, E_2, E_3, E_4 and E_5, respectively.

After dissolving the five extracts in the CCl_4 and TFA and with TMS as an internal standard were scanned on Perkin-Elmer 90 MH_z, R-32, spectro photometer. The values of the chemical shift are expressed in δ ppm.

i) Fig. No.6 shows the NMR of the extract, E_1 of the fruits of A. concinna. The spectrum shows a singlet at δ 1.8, and unresolved multiplet at δ 0.6 and δ 1.5.

ii) Fig. No. 7 shows the NMR spectrum of the extract E_2 showing singlet at δ 1.8 and δ 7.4 and multiplets at δ 1.3 and δ 3.7.

iii) Fig. No. 8 shows the NMR spectrum of ethyl acetate soluble components in CCl_4. The values of chemical shift expressed in ppm, shows a singlet at δ 9.6 and two multiplets at δ 1.8 and δ 4.8

iv) Fig. No. 9 shows the NMR spectrum of the extract E_4 showing singlet at δ 9.5 and two multiplets at δ 0.8 and δ 3.8.

v) Fig. No. 10 shows the NMR spectrum of the extract E_5. The values of chemical shift are expressed in δ ppm. The spectrum shows peaks at δ 4.7, δ 5.1 and δ 7.1 and δ 9.5 and multiplet at δ 4.8.

N M R SPECTRUM OF PETROLEUM ETHER EXTRACT OF FRUITS OF *Acacia concinna* (D C).

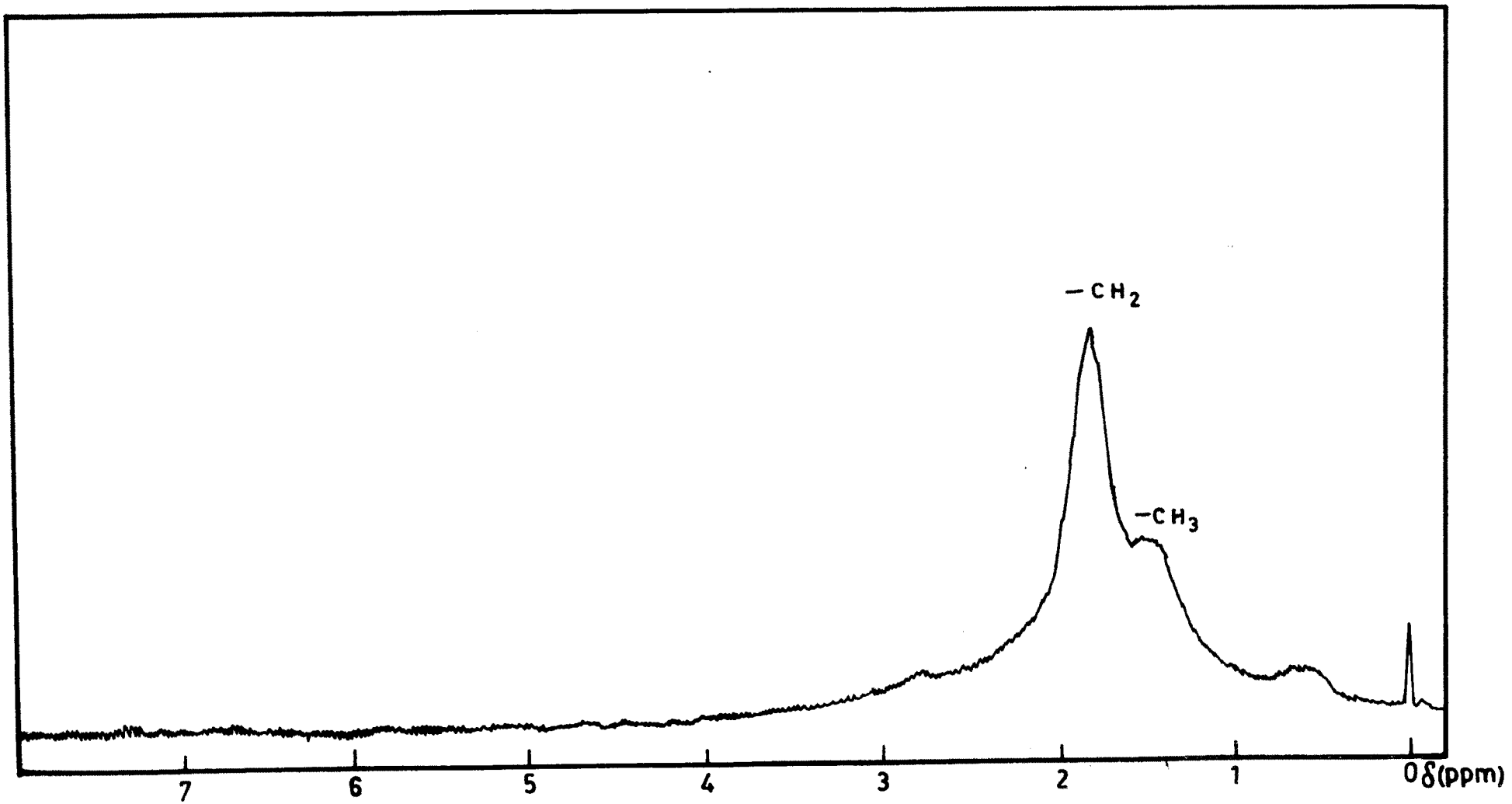

FIG. NO. 6

NMR SPECTRUM OF BENZENE EXTRACT OF FRUITS OF Acacia concinna (DC).

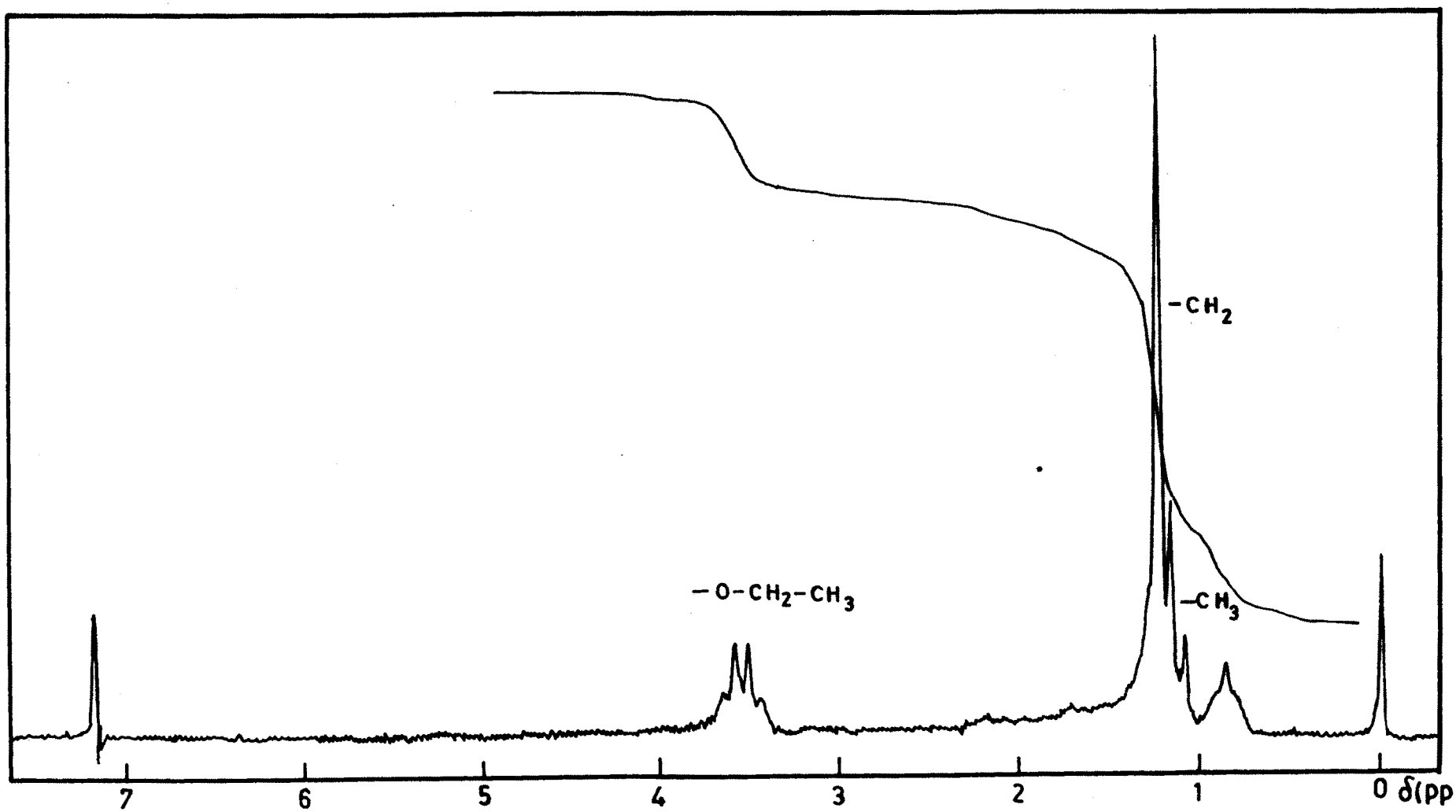

FIG. NO. 7

NMR SPECTRUM OF ETHYL ACETATE EXTRACT OF FRUITS OF Acacia concinna (DC).

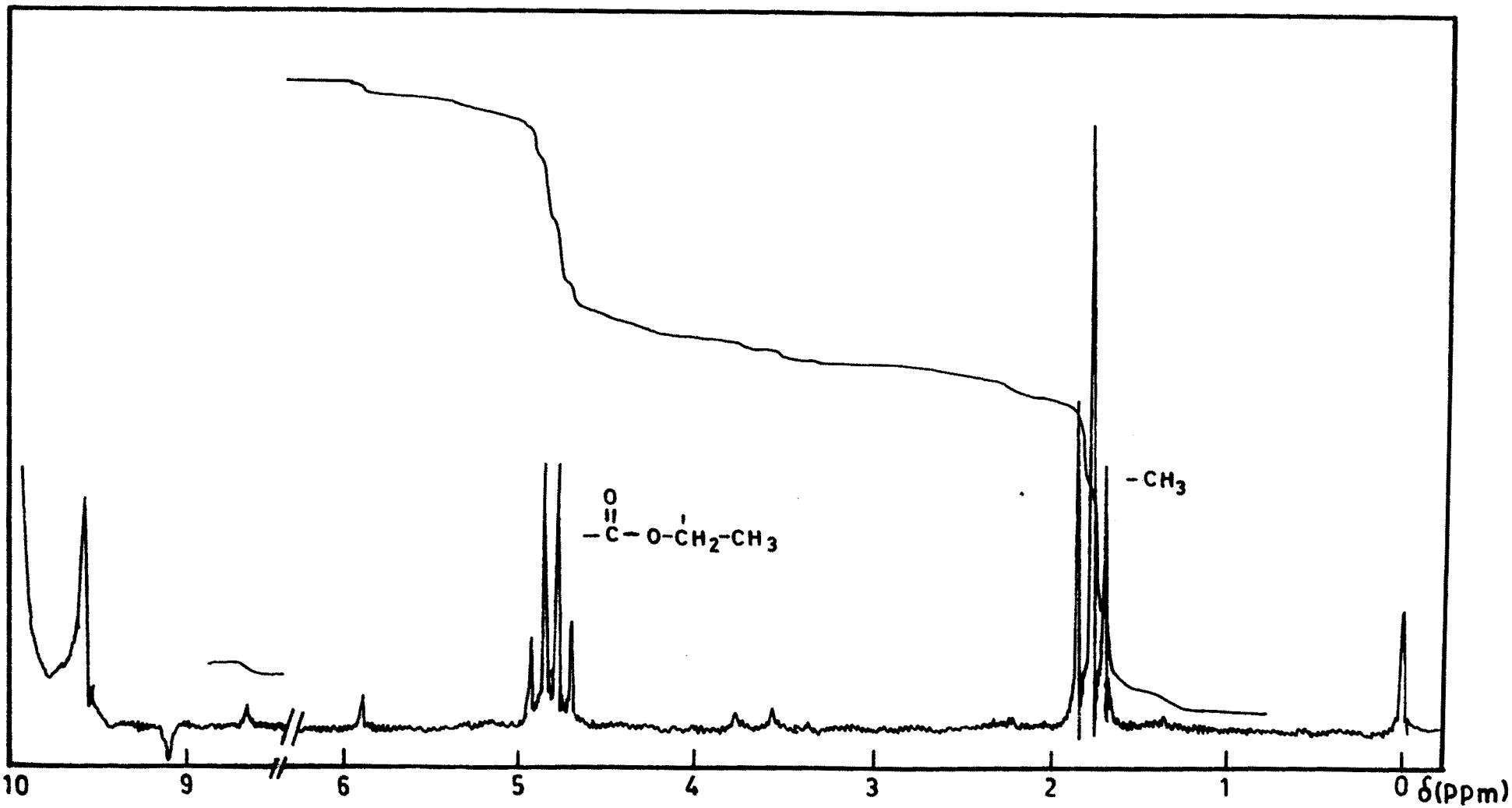

FIG. NO. 8

NMR SPECTRUM OF CHLOROFORM EXTRACT OF FRUITS OF Acacia concinna (DC).

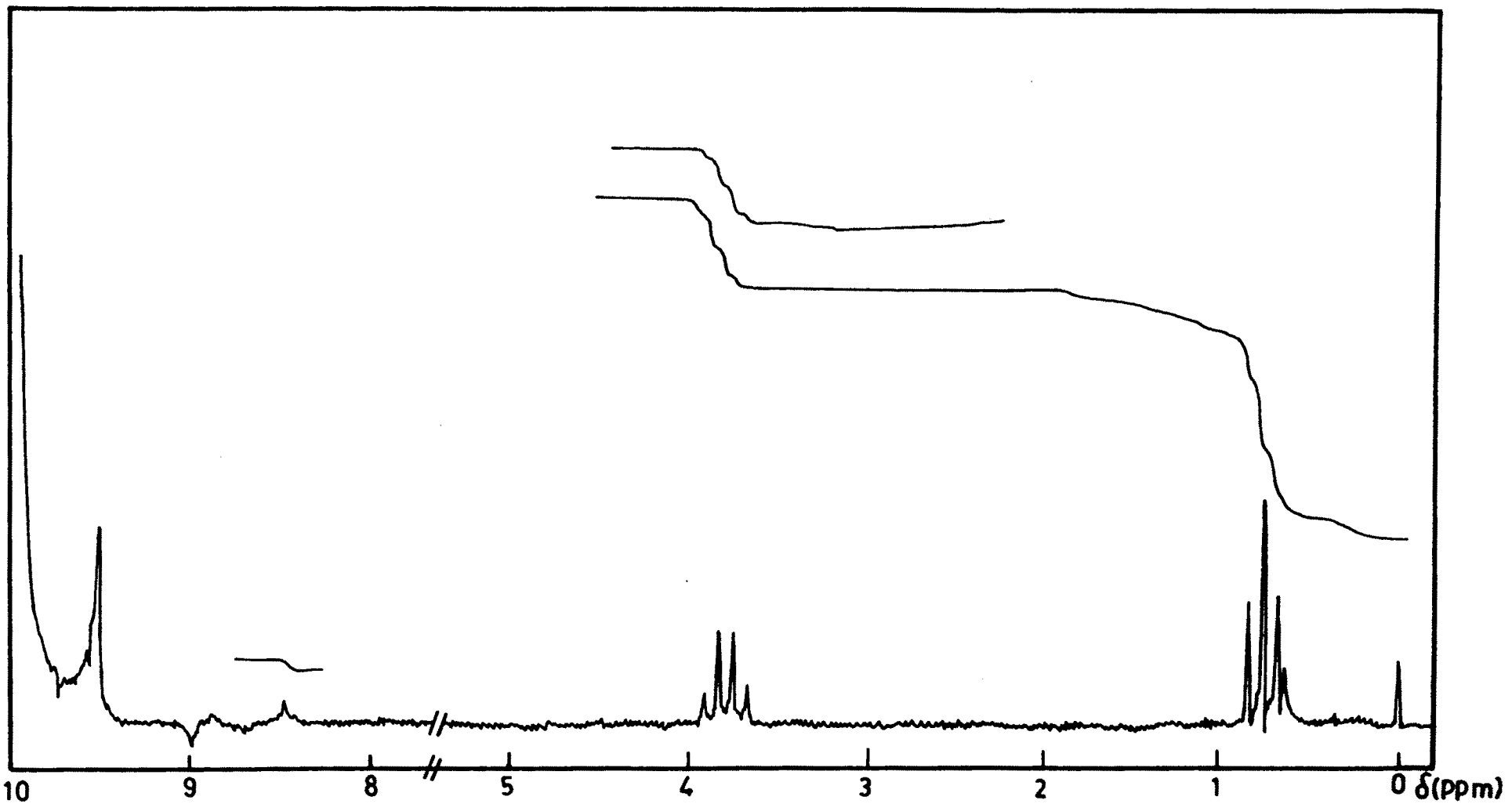

FIG. NO. 9

NMR SPECTRUM OF ETHANOL EXTRACT OF FRUITS OF *Acacia concinna* (DC).

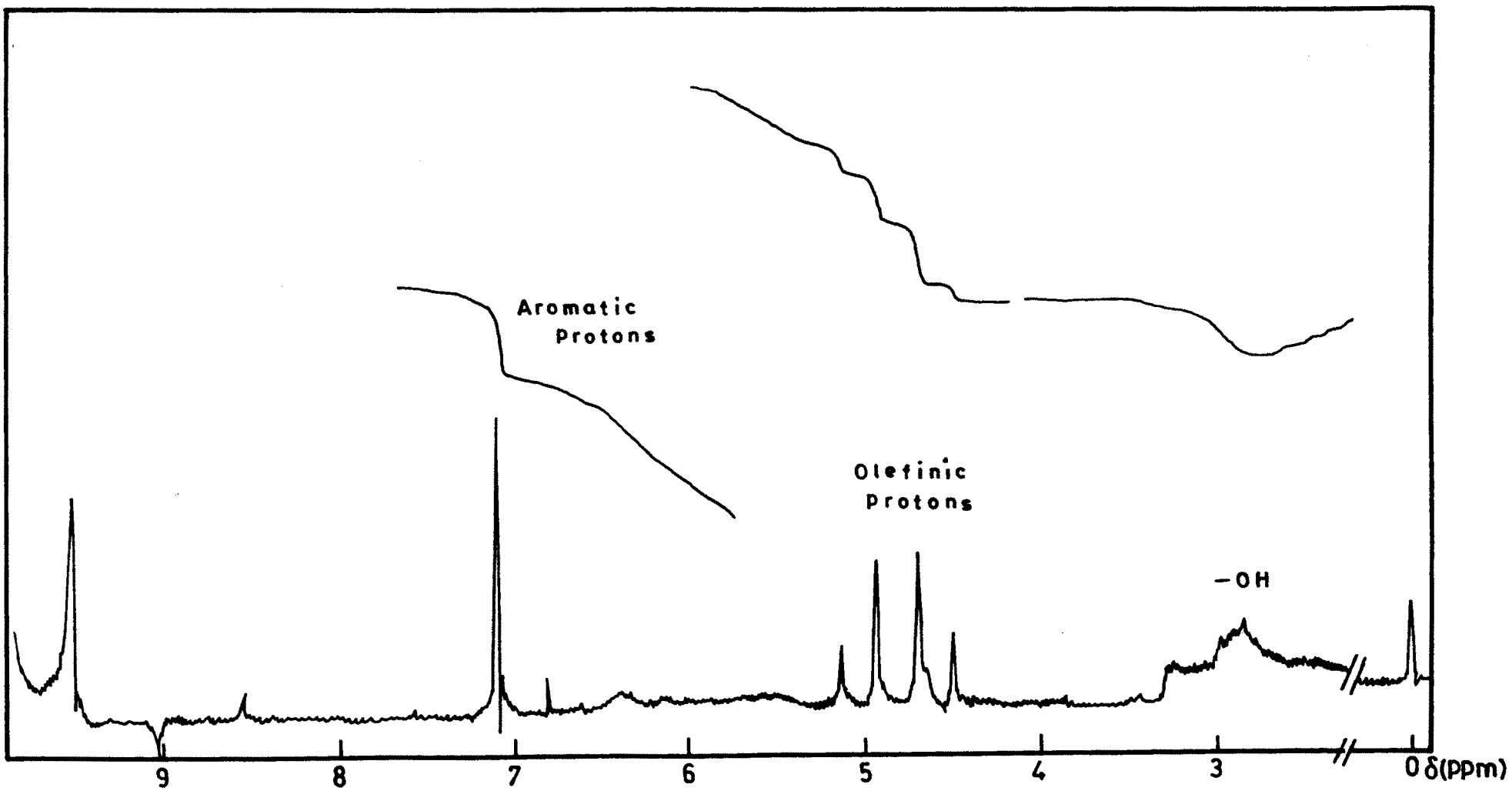

FIG. NO. 10

4) IR spectral observations :

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

i) The extract E_1 (Fig. No. 11) shows, streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1560 cm^{-1}, 1580 cm^{-1}, 1710 cm^{-1} and 2900 cm^{-1}.

ii) The extract E_2 (Fig. No. 12) shows five streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1710 cm^{-1} and 2900 cm^{-1}.

iii) The extract E_3 (Fig. No.13) shows five streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1780 cm^{-1} and 2900 cm^{-1} and broad band between 3500 cm^{-1} and 3350 cm^{-1}.

iv) The extract E_4 (Fig. No. 14) shows five streching bands at at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1710 cm^{-1} and 2900 cm^{-1}.

v) The extract E_5 (Fig. No. 15) shows streching broad band between 3500 - 3400 cm^{-1} and two bands between 2900 - 2800 cm^{-1} and 1730 - 1700 cm^{-1}.

5) Observations on atomic absorption spectrophotometry :

Table No. 3 shows the concentrations of various inorganic elements in the fruits of A. concinna.

I R SPECTRUM OF PETROLEUM ETHER EXTRACT OF FRUITS OF Acacia concinna (DC).

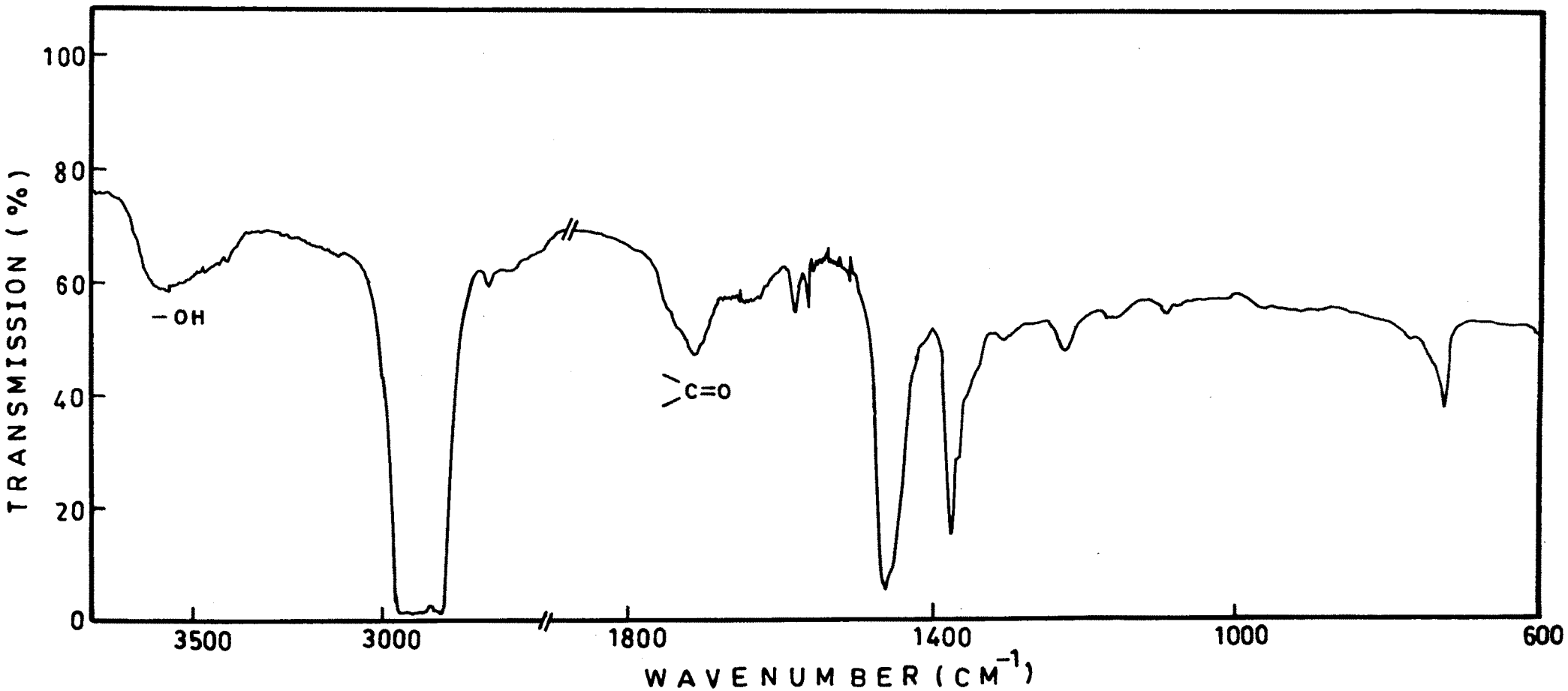

FIG. NO. 11

I R SPECTRUM OF BENZENE EXTRACT OF FRUITS OF Acacia concinna (DC).

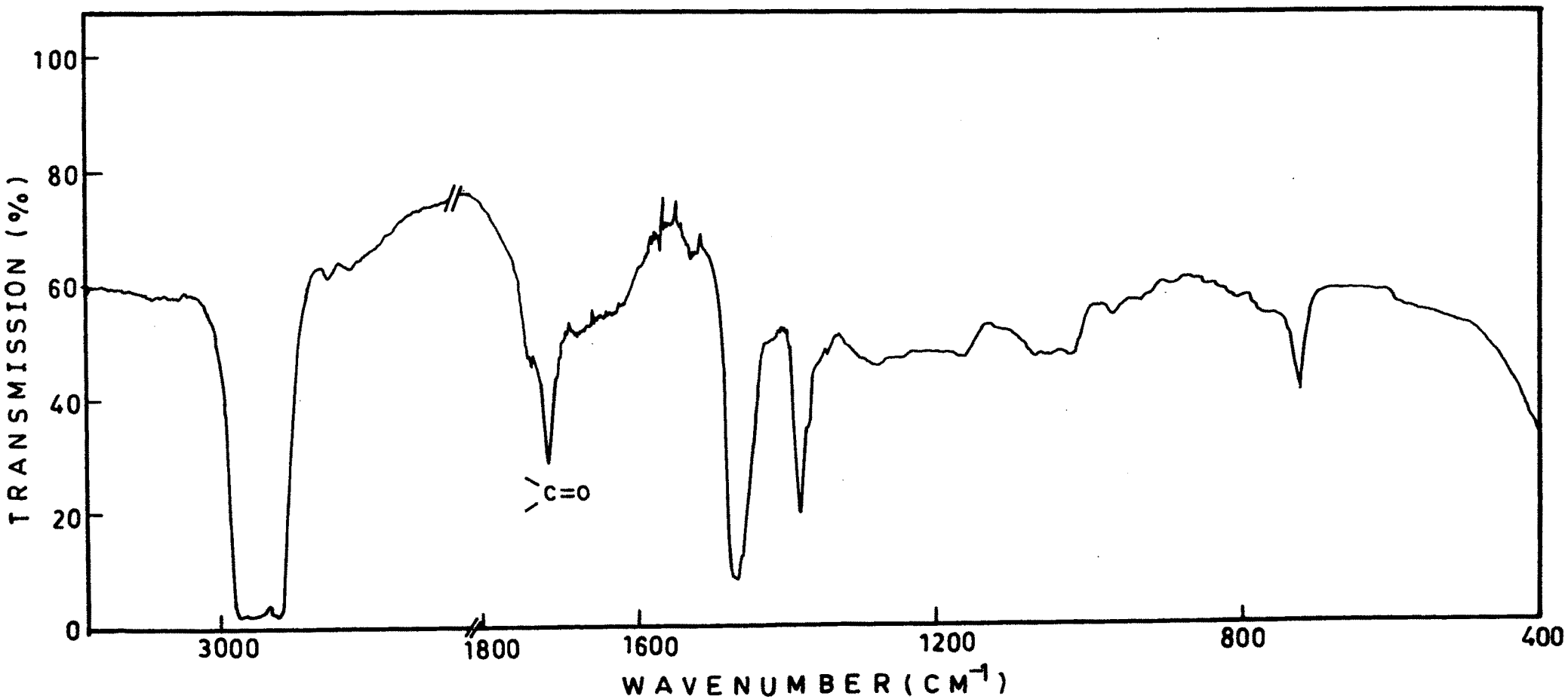

FIG. NO. 12

I R SPECTRUM OF ETHYL ACETATE EXTRACT OF FRUITS OF Acacia concinna (DC).

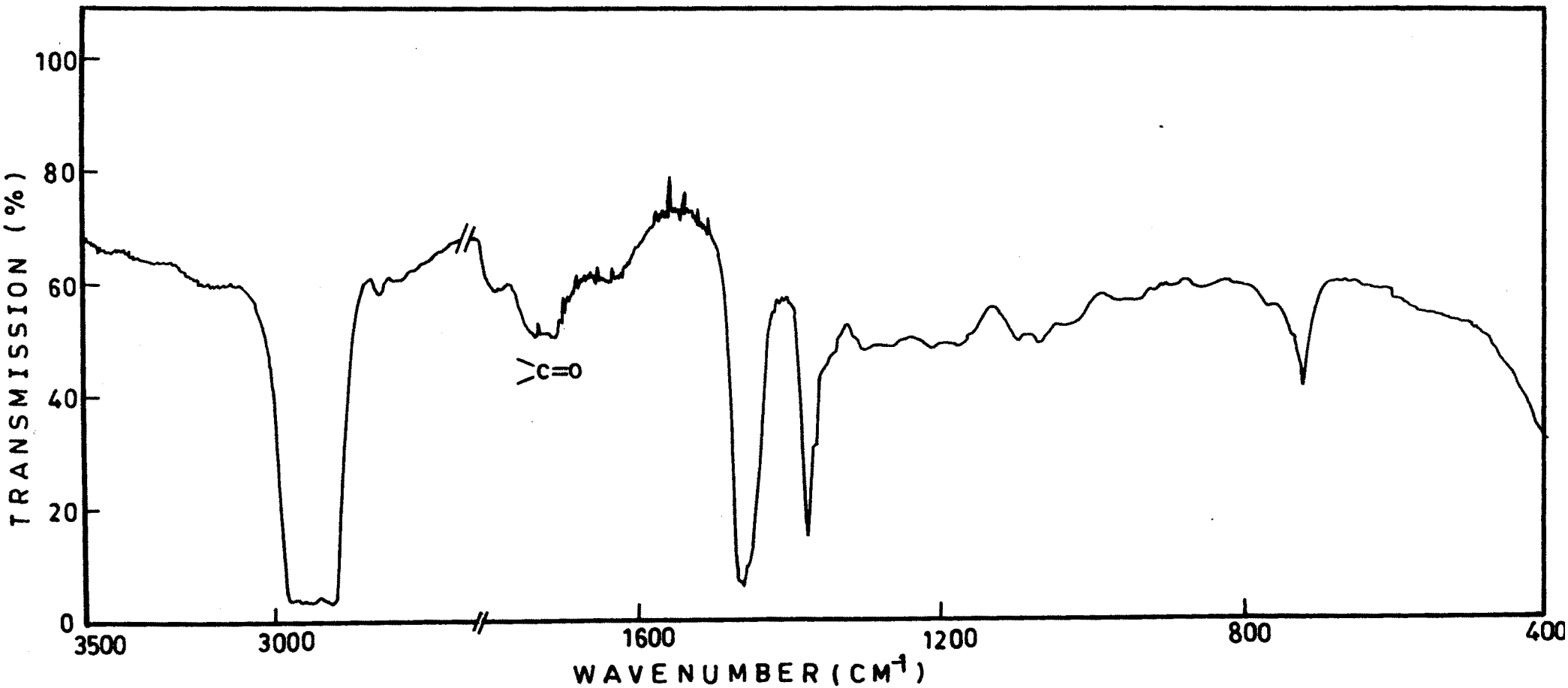

FIG. NO. 13

I R SPECTRUM OF CHLOROFORM EXTRACT OF FRUITS OF Acacia concinna (DC).

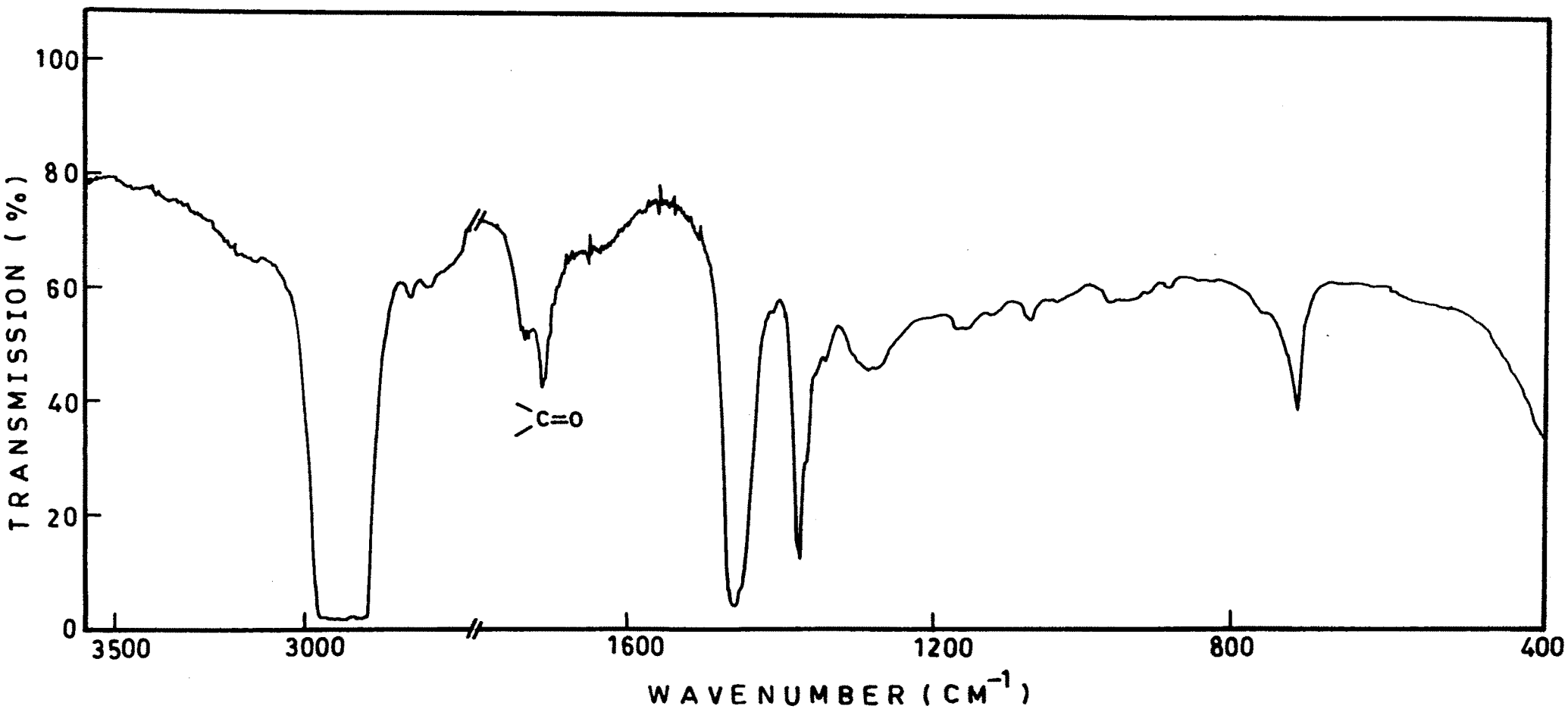

FIG. NO. 14

I R SPECTRUM OF ETHANOL EXTRACT OF FRUITS OF Acacia concinna (DC).

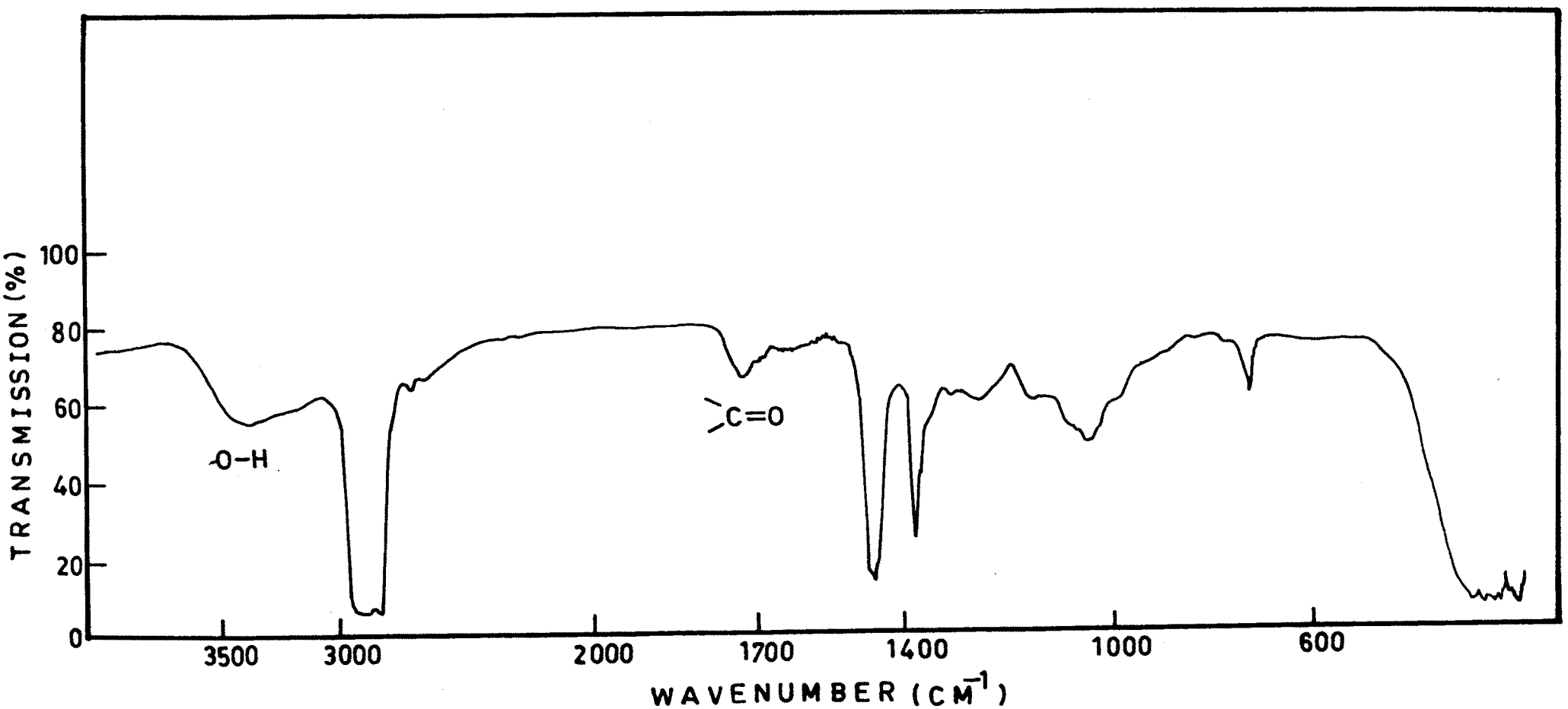

FIG. NO. 15

TABLE NO. 3

Inorganic ions in the petroleum ether, benzene, ethylaccetate, chloroform and ethanol extracts of the fruits of Acacia concinna.

No.	Extracts	Inorganic ions*				
		Cu^{++}	Fe^{++}	Mg^{++}	Ca^{++}	Zn^{++}
1.	Petroleum ether extract	0.010	Not	0.12	0.38	0.077
	Readings	0.01	found	0.12	0.37	0.077
		0.01		0.13	0.38	0.076
	Mean	0.01		0.12	0.38	0.077
2.	Benzne extract					
	Readings	0.07	Not	1.05	2.04	0.281
		0.07	found	1.05	2.03	0.282
		0.06		1.06	2.04	0.281
	Mean	0.07		1.05	2.04	0.281
3.	Ethyl acetate extract					
	Readings	0.01	Not	0.12	0.41	0.071
		0.01	found	0.11	0.41	0.072
		0.01		0.12	0.42	0.071
	Mean	0.01		0.12	0.41	0.071
4.	Chloroform extract					
	Readings	0.34	not	0.17	0.26	0.094
		0.34	found	0.17	0.27	0.094
		0.33		0.18	0.26	0.093
	Mean	0.34		0.17	0.26	0.094
5.	Ethanol extract					
	Readings	0.32	0.14	0.21	0.33	0.109
		0.33	0.15	0.22	0.33	0.109
		0.32	0.14	0.22	0.33	0.110
	Mean	0.32	0.14	0.22	0.33	0.109

* N.B. : All figures of inorganic ions are expressed in ppm.

C) OBSERVATIONS ON THE IMPACT OF PLANT TOXIN FROM A. CONCINNA ON WATER QUALITY :

The fishes were collected in rectangular glass acquaria (25 liter capacity) and were acclimatized to the laboratory conditions similar to their natural habitat. These acclimatized fishes were used for the test of extracts E_1, E_2, E_3, E_4 and E_5.

The feeding was stopped before 24 hrs of the commencement of the test. The fishes were also not fed during the test period. For each experiment twenty fishes were transferred into a well cleaned test container. The DO, pH, temperature and hardness of water were measured before and sometime after addition of fruit extracts. All the five extracts were tested against the fish T. mossambica. It was found from the observations that there was no effect on the fish mortality on addition of the fruit extracts E_1, E_2, E_3 and E_4 of A. concinna. But the fruit extract E_5 of A. concinna had lethal effect on the fish T. mossambica. Hence only extract E_5 was used for different experimental procedures to study the toxicological effect on water quality as well as on mortality, behaviour and mucosubstances in the target organs like gills, buccal mass, intestine, liver and the kidney of the fish T. mossambica.

The ethanol extract (E_5) is water soluble. Hence various concentrations were prepared by using chlorine free tap water and desired concentrations (200, 225, 250, 275, 300, 350 and 400 ppm) were made. The temperature, pH, DO and hardness of the normal experimental water was measured before the addition of the fruit extract. Then extract E_5 of different

concentrations such as 200, 225, 250, 275, 300, 350 and 400 ppm was added to the water in each acquarium and the temperature, pH, DO and hardness of this extract added water was measured and the same procedure was continued at regular intervals such as 1, 3, 6, 9, 12, 24, 48, 72 and 96 hrs.

Table No. 4 provides the temperature range and Table No. 5 gives the pH values of the experimental water. DO values have been tabulated in Table No. 6 whereas Table No. 7 gives the values of the hardness of experimental water.

The effects of different concentrations of the plant toxin from A. concinna on temperature, pH, DO and hardness of experimental water at different time intervals have also been graphically represented in Fig. Nos. 16, 17, 18 and 19, respectively.

D) OBSERVATIONS ON THE PHYSIOLOGICAL RESPONSES TO PHYTOTOXIN OF A. CONCINNA :

1) Mortality effect : LC_0, LC_{50} and LC_{100}

The ethanol extract (E_5) of the fruits of A. concinna showed lethal effects at various concentrations such as 200, 225, 250, 275, 300, 350 and 400 ppm, at different time intervals such as 1, 3, 6, 9, 22, 24, 48, 72 and 96 hrs.

Table No. 8 gives the LC_0, LC_{50} and LC_{100} values at different hrs for the E_5 extract, which shows per cent mortality and per cent survival at different hrs during the toxicological experiments.

TABLE NO. 4

The temperature of the experimental water during Acacia concinna fruit toxin intoxication to the fish T. mossambica.

No.	Concentration of fruit toxin (ppm)*	Duration of intoxication to fish in hrs.								
		1	3	6	9	12	24	48	72	96
1	Control water	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	26 ± 2	26 ± 2
2	200	26 ± 2	26 ± 2	26 ± 2	26 ± 2	26 ± 2	26 ± 2	25 ± 2	25 ± 2	26 ± 2
3	225	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	26 ± 2	27 ± 2	27 ± 2	26 ± 2
4	250	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	26 ± 2	27 ± 2	27 ± 2	26 ± 2
5	275	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	26 ± 2	26 ± 2
6	300	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	25 ± 2	26 ± 2	25 ± 2
7	350	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	27 ± 2	26 ± 2
8	400	26 ± 2	26 ± 2	26 ± 2	26 ± 2	26 ± 2	26 ± 2	26 ± 2	27 ± 2	27 ± 2

*N.B. : 1) The ethanol extracted fruit powder was used as fruit toxin during the experiment.

2) The temperature values are expressed in °C.

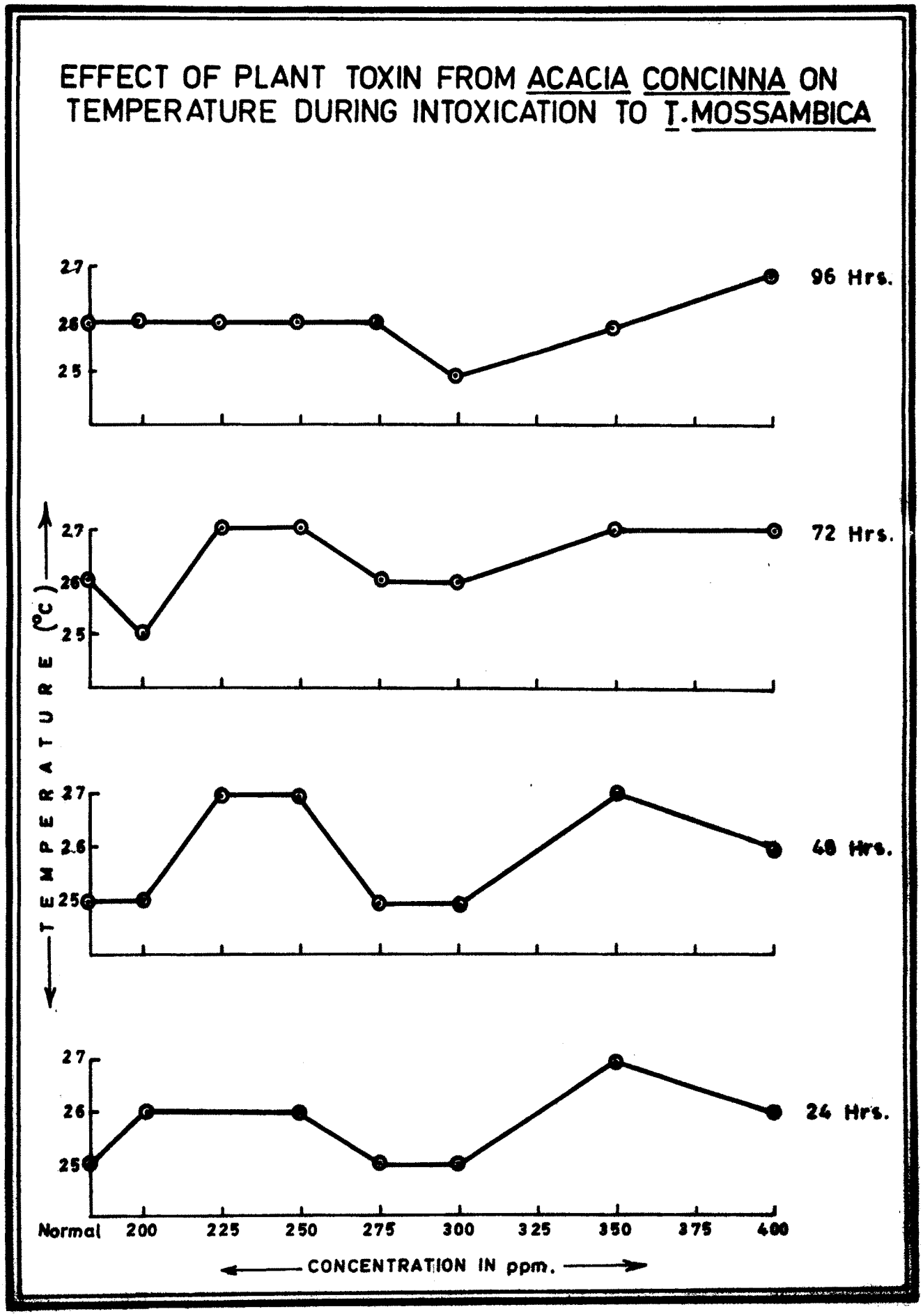
EFFECT OF PLANT TOXIN FROM ACACIA CONCINNA ON TEMPERATURE DURING INTOXICATION TO T.MOSSAMBICA
96 Hrs.
72 Hrs.
48 Hrs.
24 Hrs.
TEMPERATURE (°C)
27
26
25
27
26
25
27
2.6
25
27
26
25
Normal
200
225
250
275
300
325
350
375
400
CONCENTRATION IN ppm.

Fig. No. 16

TABLE NO. 5

The pH values of the experimental water during Acacia concinna fruit toxin intoxication to the fish T. mossambica.

No.	Concentration of fruit toxin (ppm)*	Duration of intoxication to fish in hrs.								
		1	3	6	9	12	24	48	72	96
1	Control water	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.03	7.0 ± 0.3
2	200	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04	7.0 ± 0.04
3	225	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	6.9 ± 0.02	7.0 ± 0.02
4	250	7.1 ± 0.03	7.1 ± 0.03	7.1 ± 0.03	7.1 ± 0.03	7.1 ± 0.03	7.1 ± 0.03	7.0 ± 0.03	7.2 ± 0.03	7.2 ± 0.03
5	275	7.1 ± 0.01	7.1 ± 0.01	7.1 ± 0.01	7.1 ± 0.01	7.1 ± 0.01	7.1 ± 0.01	7.1 ± 0.01	7.0 ±0.01	7.1 ± 0.01
6	300	7.2 ± 0.02	7.2 ± 0.02	7.2 ± 0.02	7.2 ± 0.02	7.2 ± 0.02	7.2 ± 0.02	7.2 ± 0.02	7.1 ± 0.02	7.2 ± 0.02
7	350	7.1 ± 0.08	7.1 ± 0.08	7.1 ± 0.08	7.1 ± 0.08	7.1 ± 0.08	7.1 ± 0.08	7.0 ± 0.08	7.1 ± 0.08	7.1 ± 0.08
8	400	7.3 ± 0.04	7.3 ± 0.04	7.3 ± 0.04	7.3 ± 0.04	7.3 ± 0.04	7.1 ± 0.04	7.1 ± 0.04	7.1 ± 0.04	7.2 ± 0.04

*N.B. : 1) The ethanol extracted fruit powder was used as fruit toxin during the experiment.

2) The figures under duration of intoxication are pH values.

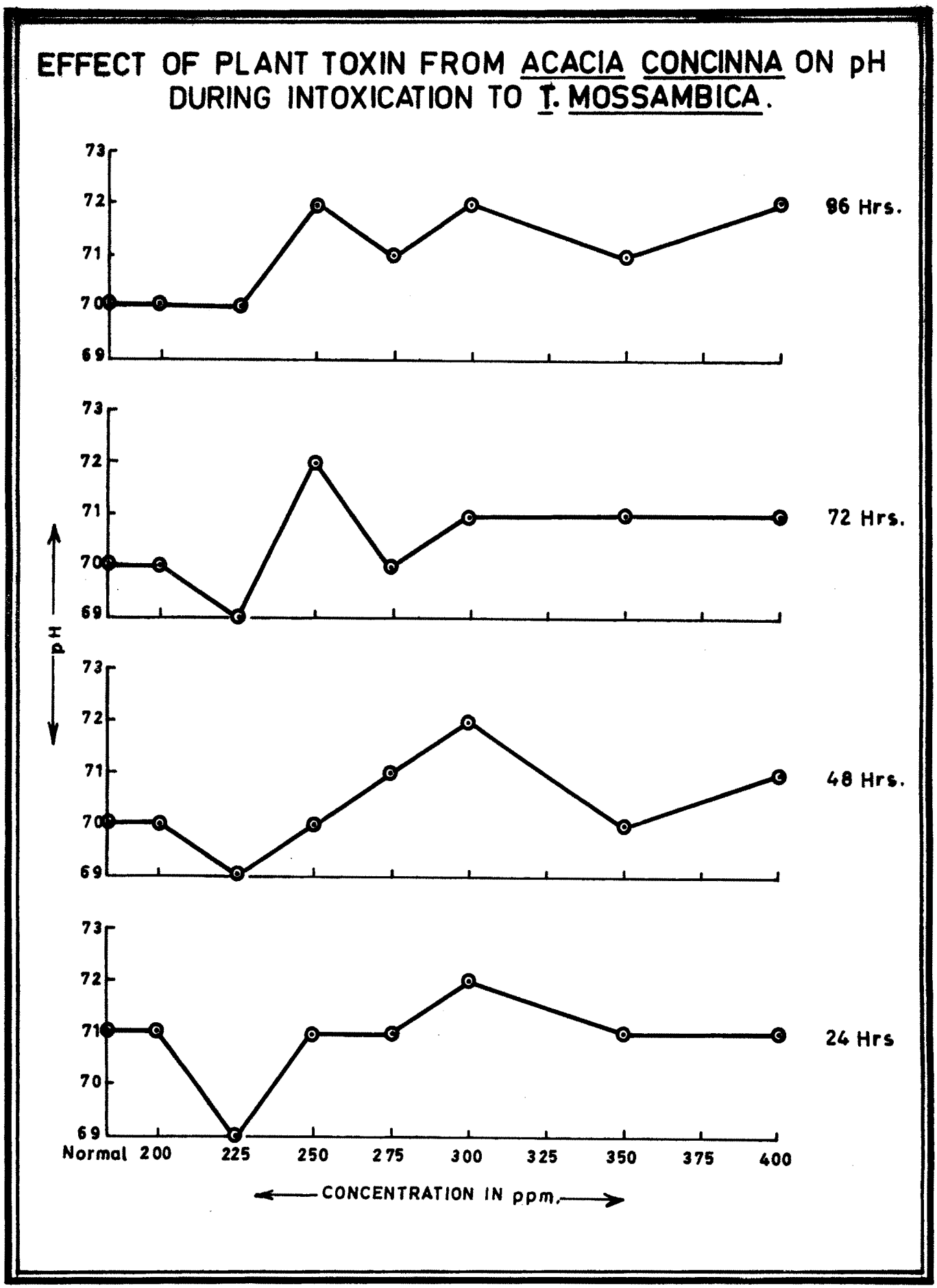
EFFECT OF PLANT TOXIN FROM ACACIA CONCINNA ON pH DURING INTOXICATION TO T. MOSSAMBICA.
96 Hrs.
72 Hrs.
48 Hrs.
24 Hrs
pH
73
72
71
70
69
Normal 200 225 250 275 300 325 350 375 400
CONCENTRATION IN ppm.

Fig. No. 17

TABLE NO. 6

The dissolved oxygen values of the experimental water during Acacia concinna fruit toxin intoxication to the fish T. mossambica

No.	Concentration of fruit toxin (ppm)*	Duration of intoxication to fish in hrs.								
		1	3	6	9	12	24	48	72	96
1	Control water	6.2 ± 0.4	6.2 ± 0.4	6.2 ± 0.4	6.2 ± 0.4	6.1 ± 0.1	6.2 ± 0.4	6.2 ± 0.4	6.2 ± 0.4	6.2 ± 0.4
2	200	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
3	225	5.8 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	5.8 ± 0.3	5.8 ± 0.3
4	250	6.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.6	5.9 ± 0.6	6.0 ± 0.6	6.1 ± 0.6	6.0 ± 0.6
5	275	5.7 ± 0.4	5.7 ± 0.4	5.7 ± 0.4	5.7 ± 0.4	5.7 ± 0.4	5.6 ± 0.4	5.6 ± 0.4	5.7 ± 0.4	5.6 ± 0.4
6	300	6.1 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.0 ± 0.2	6.1 ± 0.2	6.0 ± 0.2	6.1 ± 0.2
7	350	6.1 ± 0.8	6.1 ± 0.8	6.1 ± 0.8	6.1 ± 0.8	6.1 ± 0.8	6.1 ± 0.8	6.0 ± 0.8	6.0 ± 0.8	6.1 ± 0.8
8	400	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	6.0 ± 0.2	5.9 ± 0.2

*N.B. : 1) The ethanol extracted fruit powder was used as fruit toxin during the experiment.

2) The D.O. values are expressed in mg/lit.

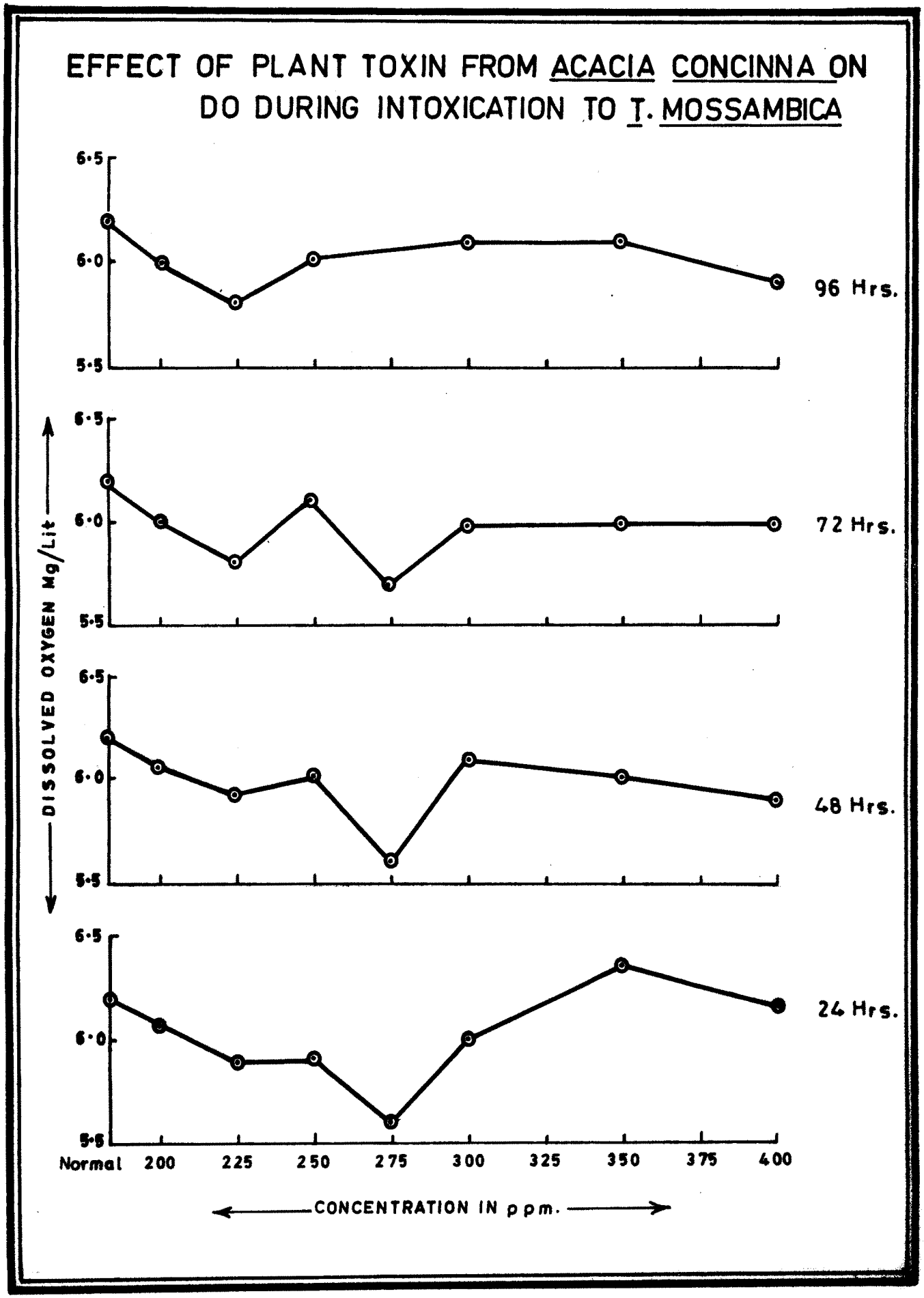
EFFECT OF PLANT TOXIN FROM ACACIA CONCINNA ON DO DURING INTOXICATION TO T. MOSSAMBICA
DISSOLVED OXYGEN Mg/Lit
6·5
6·0
5·5
96 Hrs.
72 Hrs.
48 Hrs.
24 Hrs.
Normal
200
225
250
275
300
325
350
375
400
CONCENTRATION IN ppm.

Fig. No. 18

TABLE NO. 7

The values of hardness of the experimental water during Acacia concinna fruit toxin intoxication to the fish T. mossambika.

No.	Concentration of fruit toxin (ppm)*	Duration of intoxication to fish in hrs.								
		1	3	6	9	12	24	48	72	96
1	Control water	31.67±1.4	31.67± 1.4	31.67±1.4	31.67±1.4	31.40±1.6	31.60±1.4	31.70±1.4	31.55±1.4	31.35±1.8
2	200	35.40±2.2	35.45±2.2	35.40±2.2	35.60±2.1	31.80±2.2	31.80±2.8	31.30±2.4	31.90±2.4	31.25±3.2
3	225	36.40±1.8	36.40±2.0	36.40±2.0	36.80±2.6	35.80±3.2	35.60±2.2	35.60±2.8	36.25±3.4	36.80±3.4
4	250	38.30±2.1	38.30±2.1	38.30±2.4	37.75±3.0	37.60±2.4	38.25±2.1	38.90±2.1	40.80±2.1	39.25±2.4
5	275	40.56±1.6	40.56±1.6	40.86±1.4	41.80±2.4	41.76±2.2	42.0±2.4	40.25±2.2	41.60±3.3	41.75±3.2
6	300	43.65±1.9	44.19±2.1	43.54±1.3	43.26±1.4	44.25±1.4	43.45±1.9	43.45±1.9	44.90±3.2	45.15±2.6
7	350	49.36±1.6	49.50±1.6	49.50±1.6	49.10±1.4	48.90±1.9	48.35±1.1	50.20±2.1	51.20±1.7	50.72±2.6
8	400	48.10±2.3	48.15±2.4	48.15±2.3	48.10±2.3	48.90±2.1	47.80±1.9	52.00±3.2	50.25±1.9	49.00±2.4

*N.B. : 1) The ethanol extracted fruit powder was used as fruit toxin during the experiment.

2) The hardness values are expressed in Hg $CaCO_3$/lit.

EFFECT OF PLANT TOXIN FROM ACACIA CONCINNA ON HARDNESS OF WATER DURING INTOXICATION TO T. MOSSAMBICA

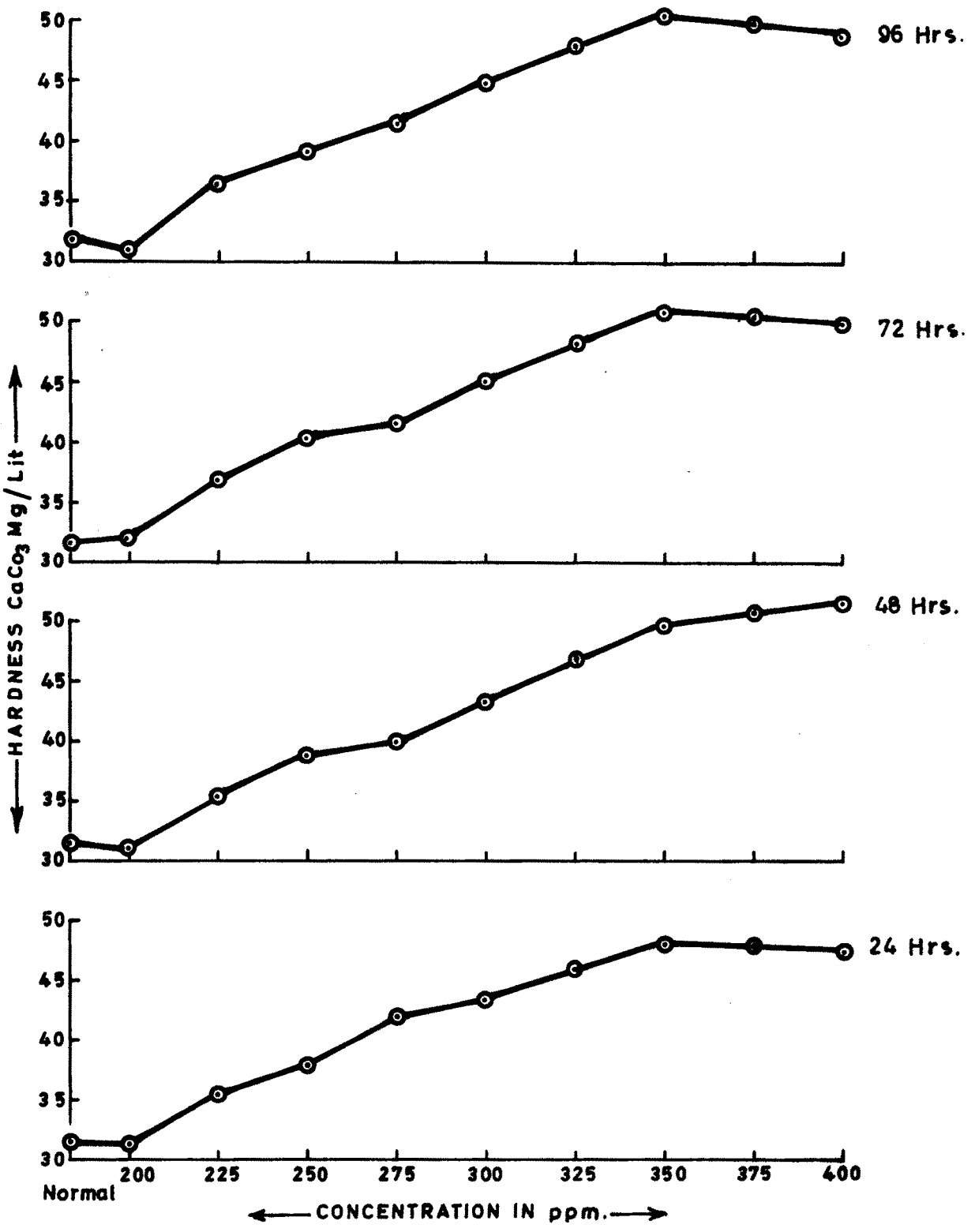

Fig. No. 19

TABLE NO. 8

Summary of mortality test results with ethanol extract of Acacia concinna.

Temperature of water - 26°C. Hardness of water - 32.67
pH of water - 7.02 DO of water - 6.4
Average wt. of fish - 95 gms. Average length of fish - 9.0 cms.

No.	Concentration of fruit toxin (ppm)*	% survival and mortality for different time intervals in hrs.																	
		1		3		6		9		12		24		48		72		96	
		S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M
1	Control water	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0
2	200	100	0	95	5	90	10	75	25	65	35	70	30	55	45	20	80	20	80
3	225	100	0	95	5	90	10	70	30	60	40	60	40	40	60	15	85	15	85
4	250	100	0	95	5	85	15	80	20	60	40	50	50	30	70	10	90	5	95
5	275	100	0	95	5	85	15	70	30	50	50	40	60	25	75	5	95	0	100
6	300	90	10	80	20	50	50	20	80	20	80	20	80	10	90	0	100	0	100
7	350	90	10	50	50	20	80	5	95	10	90	10	90	0	100	0	100	0	100
8	400	80	20	40	60	5	95	0	100	0	100	0	100	0	100	0	100	0	100

There was no mortality of T. mossambica in concentrations 200, 225, 250 and 275 ppm upto one hour, mortality began to occur in concentrations 200, 225, 250 and 275 ppm from three hours onwards. While mortality began to occur from first hour onwards in concentrations 300, 350 and 400 ppm. 10% mortality was observed in concentrations 300 and 350 ppm at one hour and 20% mortality was observed in concentration 400 ppm at one hour.

The 50% mortality was observed at more than 24 hours in conc. 250 ppm, at 12 hours in conc. 275 ppm, at 6 hours in conc. 300 ppm, at 3 hours in conc. 350 ppm and in between 1 and 3 hours in conc. 400 ppm.

There was no 100% mortality in concentrations 200, 225 and 250 ppm at 96 hours. 100% mortality was observed at 96 hours in conc. 275 ppm; at 72 hours in conc. 300 ppm; at 48 hours in conc. 350 ppm and at 9 hours in concentration 400 ppm.

Table Nos. 9 to 14 show values of X and Y for estimation of LC_{50} value of ethanol extract for fish T. mossambica for 6 hrs, 12 hrs, 24 hrs, 72 hrs and 96 hrs, respectively.

By using values of X and Y in the aforementioned tables values of 'b' and 'a' for respective time intervals were calculated. These values of 'b' and 'a' for respective time interval are given in Table No. 15.

By using the values of 'b' and 'a' the regression equation and LC_{50} values for each time interval were calculated statistically, which are given in Table No. 16.

TABLE NO. 9

Values of Y and X for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 6 hours.

No.	No.of intoxicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	10	3.72	200	5.298	28.0688	19.7085
2	10	3.72	225	5.416	29.3330	20.1475
3	15	3.96	250	5.521	30.4814	21.8631
4	15	3.96	275	5.616	31.5574	22.2313
5	50	5.00	300	5.703	32.5242	28.515
6	80	5.84	350	5.857	34.3044	34.2048
7	95	6.65	400	5.991	35.8920	39.8401
		$\Sigma Y = 32.85$ $\bar{Y} = 4.6928$	Σ No.of concentrations (n) = 7	$\Sigma Ln\bar{X} = 39.4036$ $Ln\bar{X} = 5.6290$	$\Sigma LnX^2 = 222.1615$	$LnXY = 222.1615$

TABLE NO. 10

Values of Y and X for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 12 hours.

No.	No.of intoxicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	35	4.61	200	5.298	28.0688	24.4237
2	40	4.75	225	5.416	29.3330	25.726
3	40	4.75	250	5.521	30.4814	26.2247
4	50	5.00	275	5.616	31.5394	28.08
5	80	5.84	300	5.703	32.5242	33.3055
6	90	6.28	350	5.857	34.3044	36.7819
		$\Sigma Y = 31.23$ $\bar{Y} = 5.205$	No.of concen.=n=6	$\Sigma LnX = 33.414$ $Ln\bar{X} = 5.5690$	$\Sigma LnX^2 = 186.2894$	$\Sigma LnXY = 174.542$

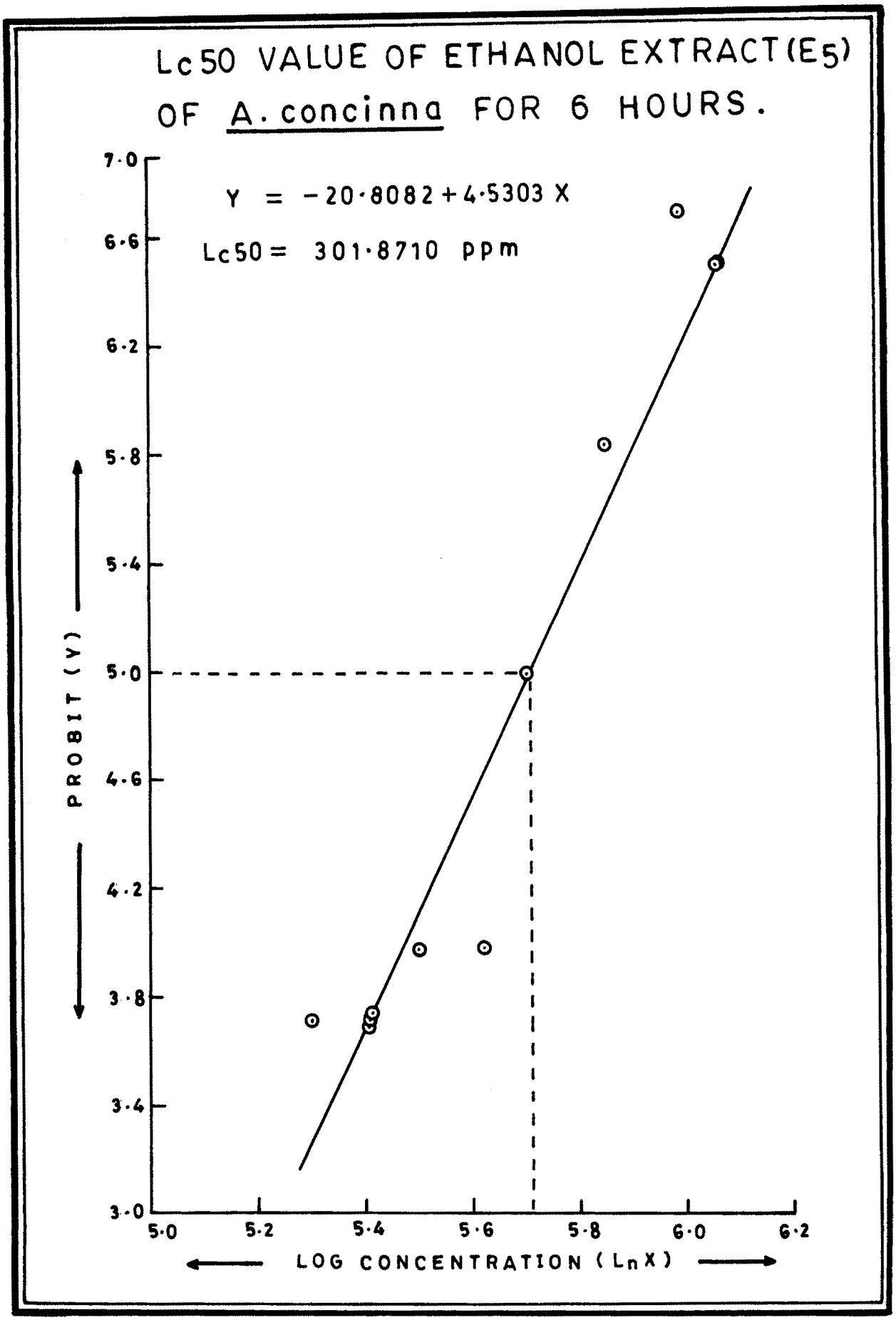
Lc50 VALUE OF ETHANOL EXTRACT(E5)
OF A. concinna FOR 6 HOURS.
Y = -20·8082 + 4·5303 X
Lc50 = 301·8710 ppm
PROBIT (Y)
7·0
6·6
6·2
5·8
5·4
5·0
4·6
4·2
3·8
3·4
3·0
5·0
5·2
5·4
5·6
5·8
6·0
6·2
LOG CONCENTRATION (LnX)

FIG. NO. 20

Lc 50 VALUE OF ETHANOL EXTRACT (E5) OF A. concinna FOR 12 HOURS.

$Y = -11.88 + 3.0682\ X$

Lc 50 = 243.4725 ppm

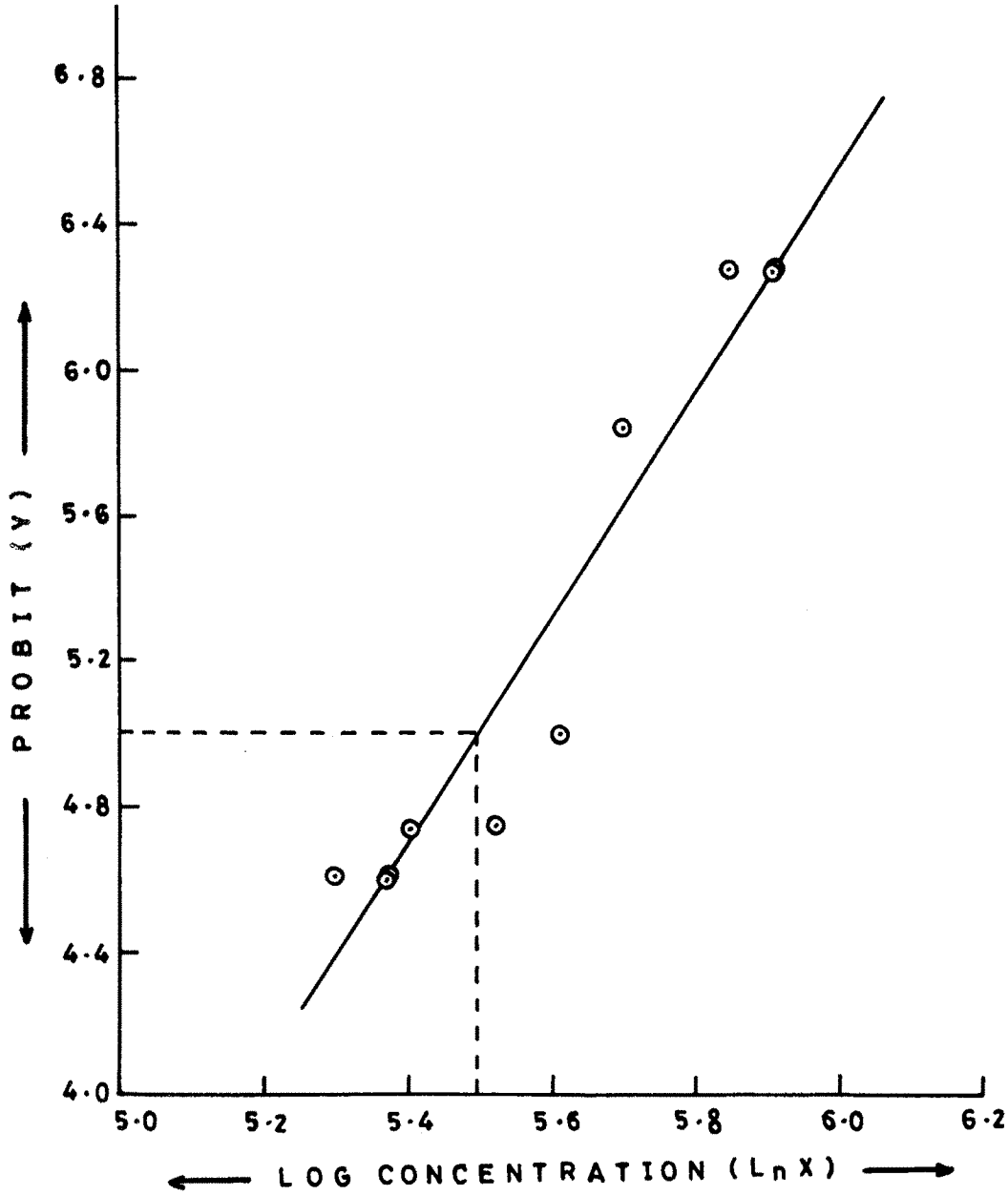

FIG. NO. 21

TABLE NO. 11

Values of X and Y for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 24 hours.

No.	No.of into-xicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	30	4.48	200	5.298	28.0688	23.7350
2	40	4.75	225	5.416	29.3330	25.726
3	50	5.00	250	5.521	30.4814	27.605
4	60	5.25	275	5.616	31.5574	29.484
5	80	5.84	300	5.703	32.5242	33.3055
6	90	6.28	350	5.857	34.3044	36.7819
		$\Sigma Y=31.6$	No.of conc.	$\Sigma LnX=33.414$	$\Sigma LnX^2=$	$\Sigma LnXY =$
		$\bar{Y}=5.2667$	$=n=6$	$\bar{LnX}=5.5690$	186.2894	176.65

TABLE NO. 12

Values of X and Y for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 48 hours.

No.	No.of into-xicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	45	4.87	200	5.298	28.0688	25.8012
2	60	5.25	225	5.416	29.3330	28.434
3	70	5.52	250	5.521	30.4814	30.4759
4	75	5.67	275	5.616	31.5574	31.8427
5	90	6.28	300	5.703	32.5242	35.8148
		$\Sigma Y=27.59$	No.of conc.	$\Sigma LnX=27.554$	$\Sigma LnX^2=$	$\Sigma LnXY =$
		$\bar{Y}=5.518$	$= n = 5$	$\bar{LnX}=5.5108$	151.9461	152.3687

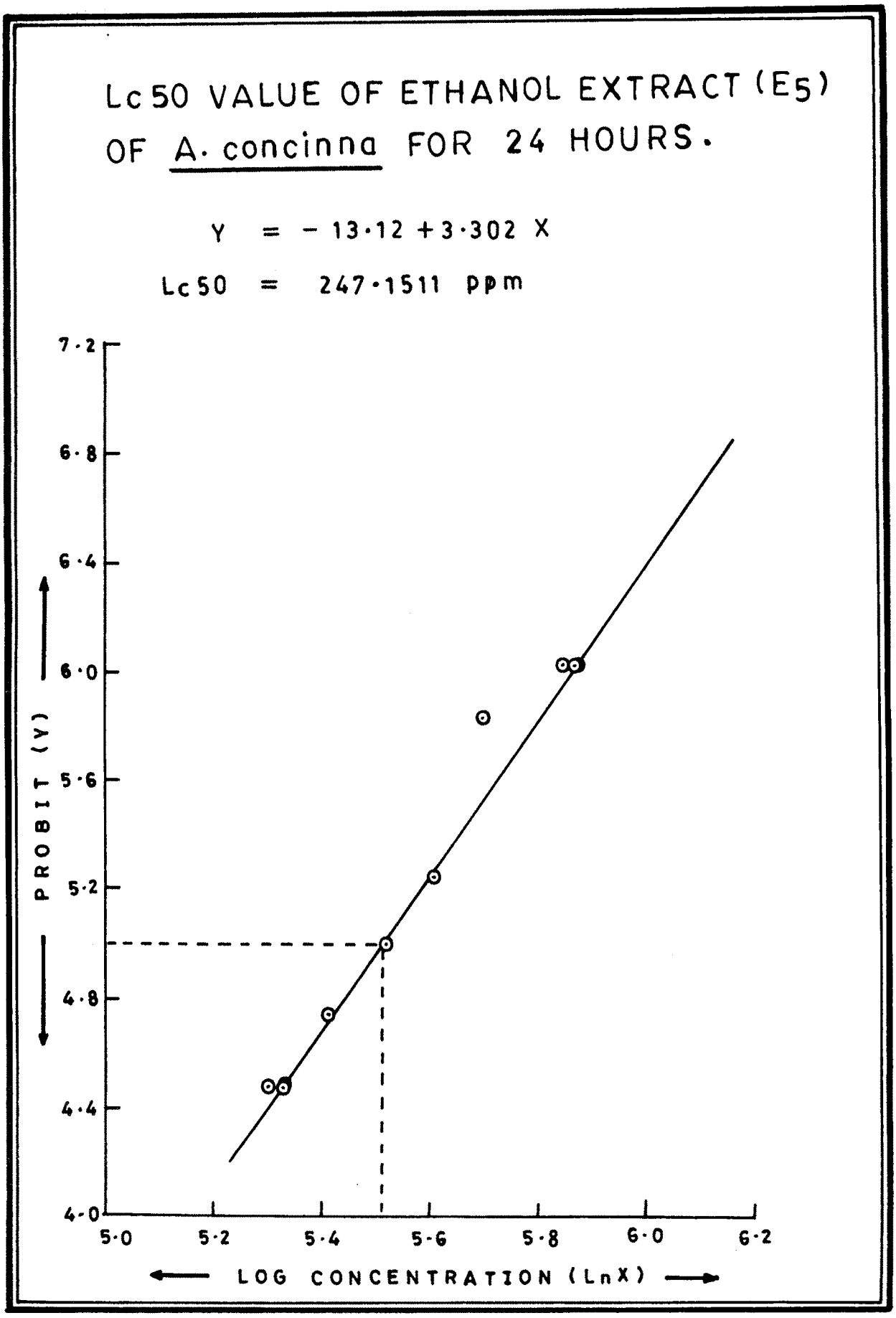
Lc50 VALUE OF ETHANOL EXTRACT (E5) OF A. concinna FOR 24 HOURS.
Y = - 13·12 + 3·302 X
Lc50 = 247·1511 ppm
7·2
6·8
6·4
6·0
5·6
5·2
4·8
4·4
4·0
PROBIT (Y)
5·0
5·2
5·4
5·6
5·8
6·0
6·2
LOG CONCENTRATION (LnX)

FIG. NO. 22

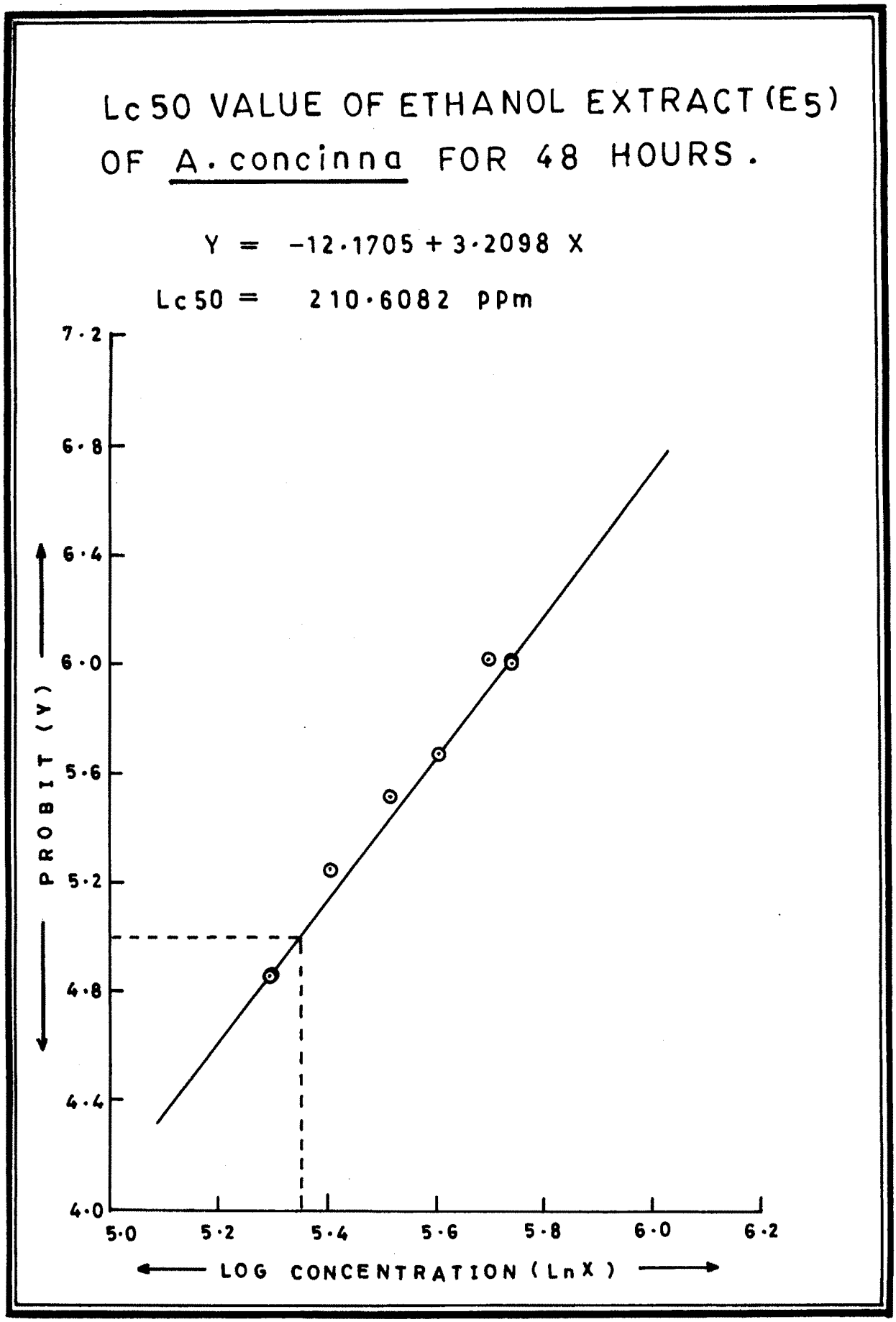
Lc50 VALUE OF ETHANOL EXTRACT (E5)
OF A. concinna FOR 48 HOURS.
Y = -12·1705 + 3·2098 X
Lc50 = 210·6082 PPm
7·2
6·8
6·4
6·0
5·6
5·2
4·8
4·4
4·0
PROBIT (Y)
5·0
5·2
5·4
5·6
5·8
6·0
6·2
LOG CONCENTRATION (LnX)

FIG. NO. 23

TABLE NO. 13

Values of X and Y for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 72 hours.

No.	No.of intoxicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	80	5.84	200	5.298	28.0688	30.9403
2	85	6.04	225	5.416	29.3330	32.7126
3	90	6.28	250	5.521	30.4814	34.6718
4	95	6.65	275	5.616	31.5574	37.3464
		$\Sigma Y=24.81$	No.of conc.	$\Sigma LnX=21.851$	$\Sigma LnX^2=$	$\Sigma LnXY =$
		$\bar{Y}=6.2025$	$= n = 4$	$Ln\bar{X}=5.4627$	119.4227	135.67

TABLE NO. 14

Values of X and Y for estimation of Lc50 value of ethanol extract of A. concinna for T. mossambica for 96 hours.

No.	No.of intoxicant fishes	Probit (Y)	Conc. in ppm(X)	LnX	LnX^2	LnXY
1	80	5.84	200	5.298	28.0688	30.9403
2	85	6.04	225	5.416	29.3330	32.7126
3	95	6.28	250	5.521	30.4814	32.7188
		$\Sigma Y=18.16$	No.of conc.	$\Sigma LnX=16.235$	$\Sigma LnX^2=$	$\Sigma LnXY =$
		$\bar{Y}=6.0533$	$= n = 3$	$Ln\bar{X}=5.4116$	87.88	98.3248

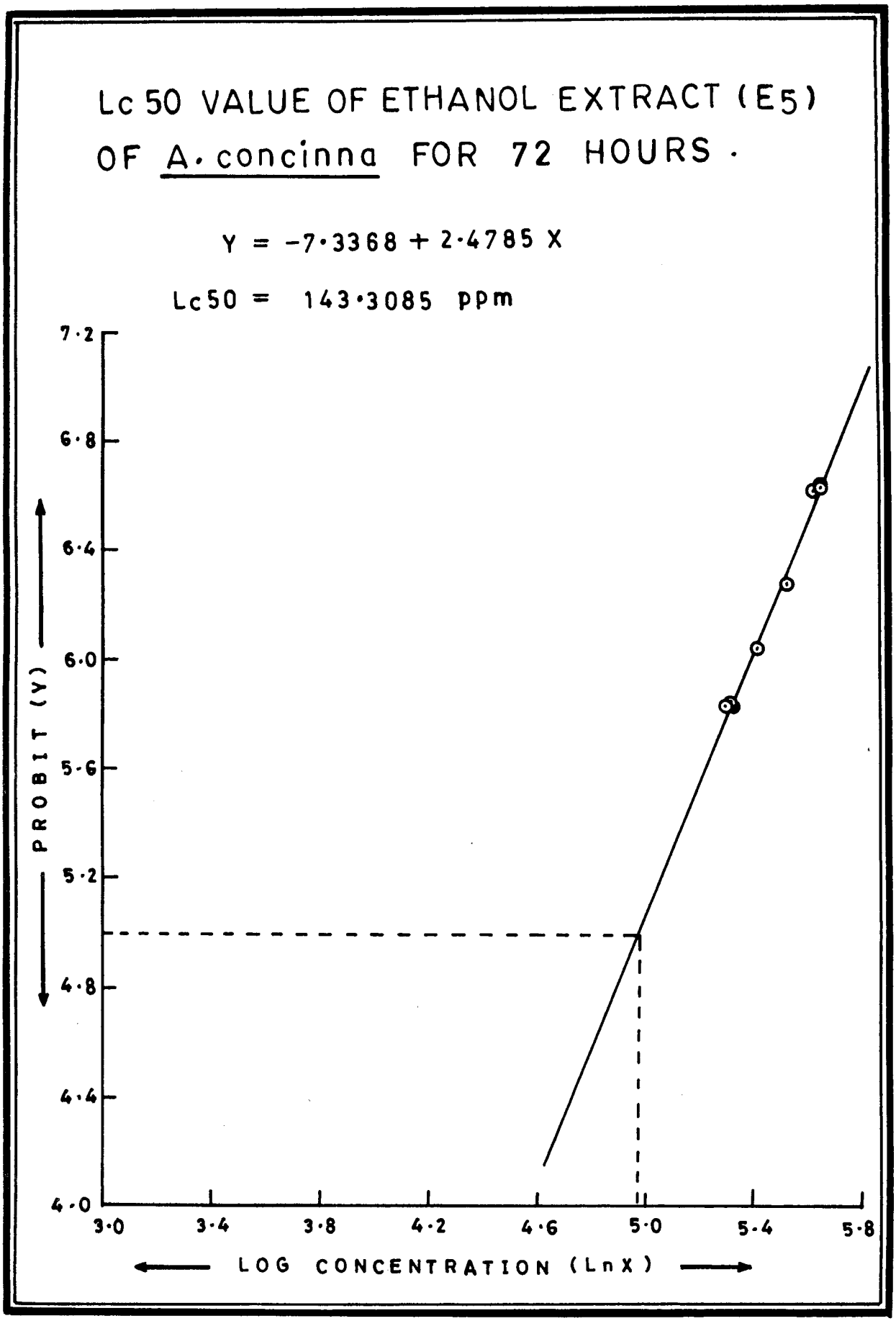
Lc 50 VALUE OF ETHANOL EXTRACT (E5)
OF A. concinna FOR 72 HOURS.
Y = -7·3368 + 2·4785 X
Lc50 = 143·3085 ppm
7·2
6·8
6·4
6·0
5·6
5·2
4·8
4·4
4·0
PROBIT (Y)
3·0
3·4
3·8
4·2
4·6
5·0
5·4
5·8
LOG CONCENTRATION (LnX)

FIG. NO. 24

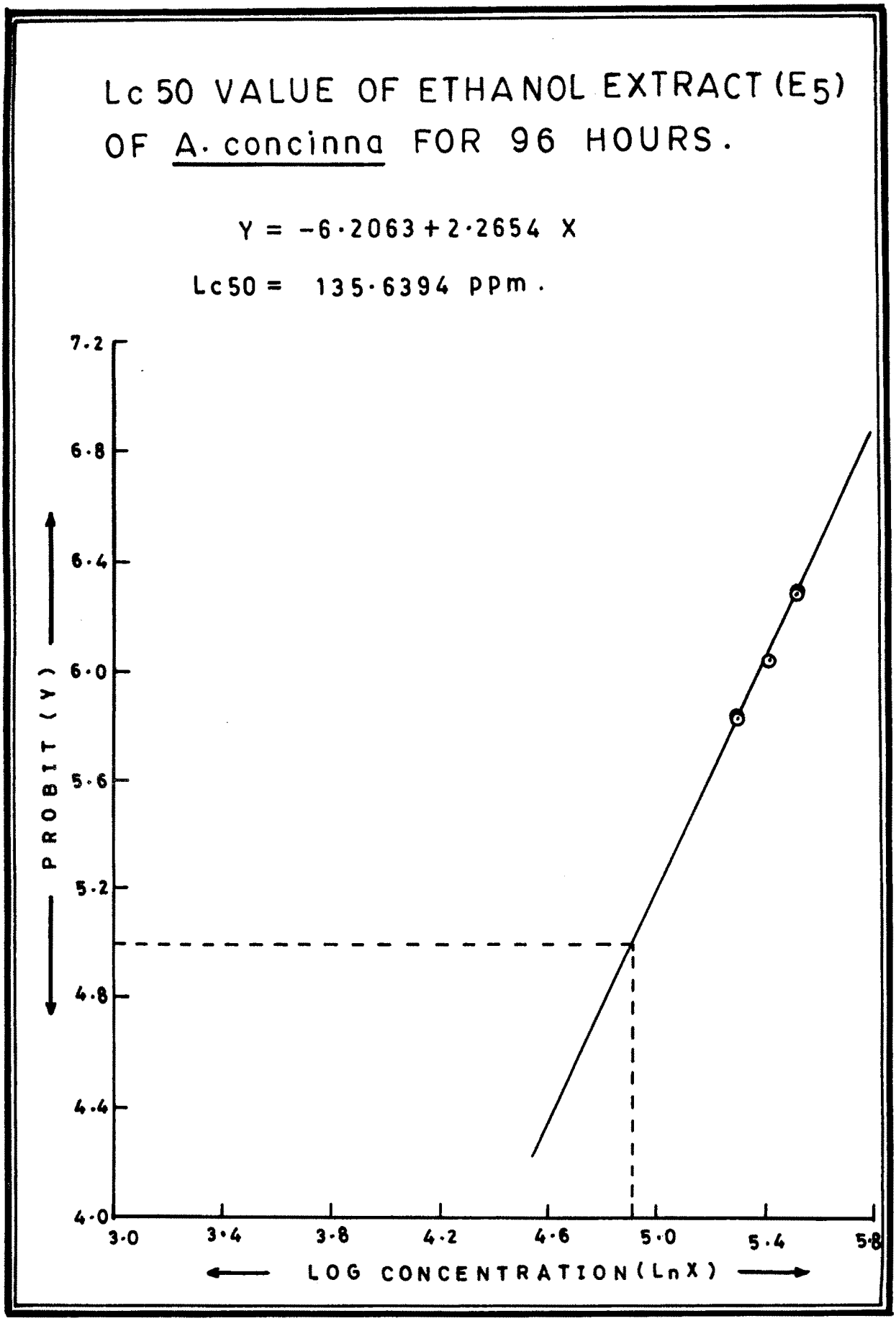
Lc 50 VALUE OF ETHANOL EXTRACT (E_5) OF A. concinna FOR 96 HOURS.
Y = -6·2063 + 2·2654 X
Lc50 = 135·6394 PPm.
PROBIT (Y)
7·2
6·8
6·4
6·0
5·6
5·2
4·8
4·4
4·0
3·0
3·4
3·8
4·2
4·6
5·0
5·4
5·8
LOG CONCENTRATION (LnX)

FIG. NO. 25

TABLE NO. 15

Values of 'b' and 'a' for different time intervals.

(These values used for calculation of Lc 50 value and expression of required regression equation for different time intervals).

No.	Time interval in hours	'b'	'a'
1.	6	4.5303	- 20.8082
2	12	3.0682	- 11.88
3	24	3.302	- 13.12
4	48	3.2098	- 12.1705
5	72	2.4785	- 7.3368
6	96	2.2654	- 6.2063

TABLE NO. 16

Regressiion equations with Lc 50 values for ethanol extract of A. concinna at different time intervals.

No.	Time interval in Hrs.	Regression equation Y = a+b X	Lc 50 value ppm		
			Observed	Calculated	Graphical
1	6	Y=-20.8082+4.5303 X	300.00	303.1415	301.8710
2	12	Y=-11.88+3.0682 X	275.00	245.2063	243.4715
3	24	Y=-13.12+3.302 X	250.00	241.5315	247.1511
4	48	Y=-12.1705+3.2098 X	222.50	202.3097	210.6082
5	72	Y=-7.3368+2.4785 X	-	145.1111	143.3085
6	96	Y=-6.2063+2.2654 X	-	140.6959	135.6394

The LC_{50} values for each time interval are also estimated by plotting the graph of the equation of line of regression of 'Y' on 'X' i.e. $Y = a + bX$ The graphs for 6 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs are respectively shown in the Fig. Nos. 20 to 25.

2) Behavioural responses :

Fish, T. mossambica shows some behavioural responses to the extract E_5. These behavioural responses were recorded as per visual observations during the experimental procedures. The behavioural response to the extracts E_1, E_2, E_3 and E_4 was not shown by the fish T. mossambica. The reactions to the extract E_5 were remarkable. The reaction time was different with different concentrations, such as 200, 225, 250, 275, 300, 350 and 400 ppm selected for experimental procedures.

The following summarised conditions of behavioural changes of T. mossambica were recorded when these fishes were exposed to E_5 extract.

A) At first the fish showed the excitment with violent movements and increased opercular movements. It showed response to touch, light and vibrations.

B) The fish became steady and tried to keep himself in the corner of acquarium. It showed bend in the region of tail. It showed response to touch. Although it was steady, it became excited on touch.

C) The fish became sluggish, losing its control over equilibrium. Fish tilted on one side but immediately it kept its normal position. Opercular movements got slow down. Fish did not show response for light and vibrations, but showed response for touch.

D) Fish tilted in position upside down. It showed only response for touch. On touch he moved in the same position. Finally he got collapsed in the upside down position and lost response for touch.

E) Fish secreted mucus with blood clots, which were entangled in the gills. Colour of gills became black-red and finally it died.

The above conditions of behavioural changes observed at different concentrations are as follows :

i) Behavioural changes in the conc. 200 and 225 ppm :

T. mossambica showed condition 'A' for the first hour of treatment. Then from one hour to one and a half hour of treatment it showed condition 'B'. It also showed condition 'C' during one and a half hour to two and a half hour, condition 'D' during two and a half hour to two hours and forty minutes of the treatment. While it had shown condition 'E' after two hours and forty five minutes of the treatment.

ii) Behavioural changes in the conc. 250 and 275 ppm :

Fish T. mossambica showed condition 'A' during the first hour, condition 'B' during one hour to one hour and twenty minutes, condition 'C' during one hour and twenty minutes to two hours, condition 'D' during two hours to two hours and ten minutes of the treatment. It showed condition 'E' after total period of two hours and fifteen minutes of the treatment.

iii) Behavioural changes in the conc. 300 ppm :

Fish T. mossambica had shown condition 'A' during the first thirty minutes, condition 'B' from thirty minutes to one hour and ten minutes,

condition 'C' during one hour and ten minutes to one hour and twenty five minutes, condition 'D' during one hour and twenty five minutes to one hour and thirty five minutes of the treatment, while it showed condition 'E' after the total period of one hour and forty minutes of the treatment.

iv) Behavioural changes in the conc. 350 ppm :

Fish T. mossambica showed condition 'A' during the first thirty minutes, condition 'B' during 30 to 40 minutes, condition 'C' during 45 minutes to one hour and condition 'D' during one hour to one hour and twenty minutes of the treatment, while condition 'E' was observed after one hour and twenty five minutes of the treatment.

v) Behavioural changes in the conc. 400 ppm :

Fish T. mossambica showed condition 'A' during the first thirty minutes, condition 'B' during thirty to forty five minutes, condition 'C' during forty five minutes to one hour and five minutes; condition 'D' during one hour and five minutes to one hour and ten minutes of the treatment, while condition 'E' was observed after the total period of one hour and fifteen minutes of the treatment.

3) Histopathological and histochemical observations :

i) Buccal mass.

a) Normal histology of buccal mass :

The anterior chamber of the digestive tract of T. mossambica is divided into an oral cavity and a pharynx. For the present study buccal mass from the earlier part is taken, since the latter possesses the gill clefts.

The buccal mass 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 a flat top indicating fungiform type. Sometimes a few conical shaped filliform papillae are also observed.

The buccal mucosa is rich in mucous secreting cells and showed some superficial invaginations and some projections at the surface. These cells are known as the goblet cells which are of three types. In the first type these cells are oval, narrow and elongated in shape while in the second type these cells are club shaped and elongated and in the third type these are observed as a giant. cell

The normal histology of the buccal mass of T. mossambica has been described in detail by Harold (1987) and Bhosale (1988).

The histopathological and histochemical observations in normal and plant toxin treated tissues of oral cavity are photomicrographically illustrated in Plate Nos. 1 to 5.

b) Histopathological alterations due to A. concinna fruit toxin :

Histopathological changes were clearly seen in the epithelium particularly in the diameter of the cells.

i) Muscle cells :

There was no change in the muscle layer supporting the oral mucosa.

ii) Goblet cells :

There are three types of goblet cells, the cells of first type are small

and oval, the cells of second type are club shaped and elongated while the cells of third type are large. Small cells are scattered throughout the surface in the outermost layer and large cells form many layers of stratified epithelium. At the 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 (200 and 225 ppm) the number of small cells was found to be increased. The number, the height and the size of the large cells was doubled. The staining intensity of these cells was also increased. In higher doses (400 ppm), the similar enhanced reactivity of the cells and the cell proliferation were distinctly observed.

iii) Connective tissue :

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.No.1). The cells and their sizes were increased in the toxin treatment.

iv) Taste buds :

As pointed out earlier there are two types of papillae (fungiform and filliform) in the oral mucosa of the T. mossambica. During toxin treatment cells bordering these papillae showed remarkable changes in their number and staining reactivities. The number of small cells lying at top of these papillae was moderate in 200 and 300 ppm. toxin treatment. But it was increased enormously in 400 ppm. toxin treatment. Sometimes they migrated in the lamina propria. The large cells which were lying mainly on the sides

of these papillae, lost their columnar nature and became more or less round. Their number was increased and staining reactivities were enhanced. Sometimes cells lost their boundaries and compact mass was observed (Plate No.1, Fig.No.3).

v) Lamina propria :

Lamina propria of the papillae also showed increase in size and number of the cells. As in case of stratified epithelium, the connective tissue was thickened and vacuolization was prominent in this tissue.

c) Histochemical observations :

The histochemical data on some important staining reactions employed in the present investigation are recorded in the Table No. 17 according to the visually estimated intensity of staining and shade with four plus (++++) (representing the strongest activity. Histochemical observations requiring further description and consideration are presented hereafter along with their interpretations.

i) Muscles :

It is generally said that tongues of fishes lack skeletal muscles (Langer et al., 1962) but in the present investigation it was noted that bulk of T. mossambica tongue was made of connective tissue.

ii) Connective tissue :

The connective tissue was moderately PAS positive and their PAS reactivity resisted to diastase digestion. These initial reactions indicated absence of glycogen and neutral mucosubstances, but possible presence of

TABLE NO. 17

Histochemical observations on mucosubstances in the various tissues of the buccal mass in control and plant toxin (A.concinna) treated fish T. mosssambica (Peters)

No.	Histochemical methods	TISSUE									
		Epithelium						Connective tissue		Muscles	
		Goblet cells (Type-I)		Goblet cells (Type II)		Goblet cells (Type III)					
		Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1.	H-E	N-Blue E-ve	staining increased	N-Blue E-ve	staining increased	N-Blue E-ve	staining increased	++	++++	++	++++
2.	PAS	++	++++	++	++++	++	++++	++	++++	++	++++
3.	M-Diastase-PAS	++	++	++	++	±	++	++	+++	-	-
4.	Ab pH 1.0	-	-	++	+++	-	-	++	++++	-	-
5.	AB pH 2.5	-	-	++	++++	-	-	-	-	-	-
6.	AB pH 1-PAS	++P -	+++P +B	-P ++++B	-P -B	+P -B	+++P	++P	++++P	++	++++
7.	AB pH 2.5 -PAS	++P -B	+++P +B	++P ++B	++++P ++++B	+P -B	+++P ++B	++P	++++P	++P	++++P
8.	AF	-	-	++P	+++P	++P	+++P	-	-	-	-
9.	AF-AB pH 2.5	-	-	++B	+++B	++P	+++B	-	-	-	-

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

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

acidic mucosubstances. The mast cells were scattered in this tissue. These cells exhibited alcianophilia at pH 1.0. This indicates presence of sulphate esters in them presence of sulphate esters in the lingual mast cells further supported by their blue purple staining with AB pH 1-PAS, sequential staining reactions.

iii) Goblet cells :

The lingual mucosa was rich in mucous secreting cells and showed some superficial invaginations and some projections at the surface. All the goblet cells reacted positively towards PAS, the reaction being moderate in some but intense in others. At the onset, the goblet cells, thus, could be classified into three types, moderately PAS positive, intensely PAS positive and partly resistant and partly PAS sensitive after phenylhydrazine treatment.

The moderately PAS positive goblet cells (Type-I goblet cells) were oval, narrow and elongated in shape. Their mucosubstances resisted diastase digestion. It shows absence of glycogen in them. These cells reacted negatively with AB both at pH 1.0 and pH 2.5, thus indicating absence of acidic group in them. Flask shaped goblet cells of T. mossambica exhibited partly resistant PAS reactivity after phenylhydrazine treatment, it shows presence of acid mucosubstance in them.

The club cells and some elongated goblet cells (type-II goblet cells) showed intense alcianophilia with AB both at pH 1.0 and 2.5, indicating sulphated acid mucosubstances in them. This conclusion was further supported from their only blue staining with AB pH 1.00 and AB pH 2.5-PAS sequential staining procedures.

Giant goblet cells of T. mossambica (Type III goblet cells) showed different cytochemical reactions with the earlier two types of goblet cells. Their partly resistant and partly sensitive PAS reactivity after phenylhydrazine treatment indicated presence of acidic mucosubstances. These cells reacted negatively with AB pH 1.0 and remained PAS positive in AB pH-1-PAS sequence. At pH 2.5 these cells reacted moderately with AB and appeared purple-blue in AB pH 2.5-PAS sequence indicating presence of neutral and acidic mucosubstances in them.

iv) Taste buds :

The taste buds exhibit PAS positive reactivity which was diastase resistant. The hair processes region (neck) region of the taste buds showed weak to negligible alcianophilia only at pH 1.0. It showed presence of sulphate groups in them.

d) Mucosubstances alterations due to A. concinna fruit toxin :

The plant toxin studies revealed interesting alterations in the staining intensity and concentration of mucosubstances in muscular layer, in the epithelium and in the connective tissue of the oral mucosa of T. mossambica.

i) Muscle layer :

Muscle layer showed increased staining activity in the PAS positive cells.

ii) Goblet cells :

The first type goblet cells which contained 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 it had reached a minimum in the higher doses.

The second type goblet cells reflected variations in the intensity of staining and the concentration of mucosubstances synthesize by these cells. The cells synthesizing glycogen and acidic mucins showed minimum staining in the normal tissues. The staining intensity immediately after toxin treatment increased and reached its maximum, but after higher doses the mucosubstances were decreased remarkabley.

iii) Connective tissue :

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 mucosubstances were increased in the tissues treated with plant toxin.

ii) Gills.

a) Normal histology of gills :

Gill is formed by two rows of primary gill lamellae in T. mossambica. The primary gill lamellae internally supported by a bony structure and the spaces are filled with many blood cells (Plate No.2; Fig. Nos. 2,5). 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 eipthelium of the primary gill lamellae.

The secondary gill lamellae are separated from each other by interbranchial septum and are free at their distal ends. These gill filaments are supported by the cartilagenous tissue (Plate No.2, Fig.No.2). The secondary lamellae are also supported by internally by pillar cells (Plate No.2, Fig.No.3). 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 linning the secondary gill lamellae also consist of numerous mucous cells and the acidophilic cells are seen at the base of these lamellae.

The normal histology of gills of T. mossambica has been described in earlier work from this laboratory (Nikam, 1980; Harold 1987; Bhosale,1988).

b) Histopathological alterations due to A. concinna fruit toxin :

The effects of the ethanol extract (E_5) of fruits of A. Concinna showed very similar histopathological effects on the fish gills to those of L. eriocephalus (Harold, 1987) and S. laurifolius (Bhosale, 1988). These changes were observed only after the treatment of higher concentrations of the extract.

At lower doses (200-300 ppm) 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 summarised as 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 buldged (Plate No.2, Fig. No.6) 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 acidophill cells was very much increased. The remarkable histological changes in the primary and secondary gill lamellae were observed in the treatment of higher concentrations (325 to 400 ppm) of toxin. The primary gill lamellae were affected and they showed alterations including reduction in the supporting bony element indicating degenerative changes, enlargement of distal gill lamellar spaces, increased subepithelial spaces, ruptured lamellar capillaries and detachment of epithelium.

Due to the effect of this plant toxin the secondary gill lamellae also showed noticeable changes. The main alterations include the reduction and shortening of secondary gill lamellae (Plate No.2, Fig.No.3), accumulation of blood cells in intercellular spaces, loss of pillar cells, formation of haematomass, overlapping of the filaments, uneven curling, discontinuation of the epithelium at the basal regions, increasing mucous secreting cells in the epithelium and increase in number and size of the acidophill cells. In secondary gill lamellae there were many degenerative changes occurred suggesting the histolysis in this tissue.

c) Histochemical observations :

The histochemical data on some important staining reactions employed in the present investigation of the gills of T. mossambica are recorded in Table No. 18 according to the visually estimated staining intensity and shade

TABLE NO. 18

Histochemical observations on mucosubstances in various tissues of the gills in control and plant toxin (A. concinna) treated fish T. mossambica (Peters).

No.	Histochemical methods	TISSUES							
		Epithelial cells		Mucous cells		Basement lamina		Gill rachis	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
1.	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
3.	M.Diastase-PAS	++P	+++P	+++P	++++P	+++P	++++P	++P	++++P
4.	AB pH 1.0	+B	+++B	+++B	++++B	++B	++++B	++B	++++B
5.	AB pH 2.5	+B	+++B	+++B	++++B	+++B	++++B	++B	++++B
6.	AB pH 1.0-PAS	++P +B	++++P +++B	+++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	++++P ++++B	++P ++B	++++P ++++B
8	AF	+P	++P	++P	++++P	±P	+P	++P	++++P
9.	AF-AB pH 2.5	+P	+++P	++P	++++P	±	+	++P	++++P

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

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

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. 2.

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

i) Mucosubstance elaboration by epithelial cells :

PAS reactivity was shown by epithelial cells, 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 a 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.

ii) Mucosubstances elaboration by mucous cells :

Different cells on the gill lamellae stained deeply with various mucin staining 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 1.0-PAS and AB pH 2.5-PAS, some of these cells stained only purple (Plate No.2, Fig.No.6) 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 1.0-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 1.0 and pH 2.5 (Plate No.2, Fig. Nos.3,4). AF and AF-AB pH 2.5 also showed mixed staining in these cells. Thus, these cells have capacity to synthesize and elaborate mixed, neutral and acidic (sulfated) mucosubstances.

Acidic mucosubstances are secreted by some cells. These cells showed PAS reactivity resistant to diastase digestion and intense alcianophilia at pH 1.0 and pH 2.5. They stained blue with sequential staining techniques (AB pH 1.0-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.2, Fig.No.3).

iii) Mucosubstances elaboration by the basement lamina :

Epithelial cells resting on the basement lamina, simultaneously, showed moderate alcianophilia both at pH 1.0 and pH 2.5. In the combined sequential staining procedures like AB pH 1.0 -PAS, this tissue stained bluish-pink indicating acid moieties along with the neutral mucins in it (Plate No.2, Fig.No.4).

iv) Mucosubstances elaboration by the gill rachis :

Strong PAS ractivity was shown by the cartilagenous cells present in the gill rachis deep alcianophilia both at pH 1.0 and pH 2.5, stained only blue in the combined sequential staining techniques like AB pH 1.0-PAS and AB pH 2.5-PAS, AF staining was prominent indicating sulfated acid muco-substances in them (Plate No.2, Fig.No.5).

d) Mucosubstance alterations due to A. concinna fruit toxin :

The application of plant toxin A. concinna revealed several interesting alterations in the staining intensity and concentration of the mucosubstances in the epithelial cells, pillar cells, basement lamina and in the gill rachis of T. mossambica.

The mucous elaborating cells and epithelial cells reflected variations in the intensity of staining and in the concentration of the mucosubstances elaborated by these cells. During lower doses of the plant toxin their mucin secretion was increased and during the treatment of higher doses their elaboration reached maximum covering the whole tissue (Plate No.2, Fig. No.6).

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 mucosubstances while in the higher concentration due to the reduction in the number of pillar cells, their concentration and staining intensities were maximum.

iii) Liver.

a) Normal histology of liver :

The liver of T. 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.3, Fig.No.1). In some sections the pancreatic tissue contains big and differently staining esletcells.

b) Histopathological alterations due to A. concinna fruit toxin :

Both crude powder and 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 were distinct. The histopathologically distinct observable changes in the liver are summarised below :

The foremost effect of the plant toxin on the liver is the displacement of hepatocytes. The cells with the increasing doses of toxin started to enlarge. There was aggregation of cytoplasmic contents. Due to this

precipitation black patches could clearly be seen in this tissue (Plate No.3, Fig.No.9). Some hepatocytes were swollen and showed large vacuoles, with higher concentrations (350 ppm). Due to the swelling and vacuolization it was difficult to mark the cell boundaries. Disruption of sinusoids was also evident (Plate No.3, Fig. Nos.4,7,8).

Absence of nuclei in the hepatocytes is another detectable change in the liver, whereas, in some the size of the nuclei was increased. The nuclei pycnosis was observed. There were some binucleated hepatocytes. In some cells nuclei with mitotic divisions were observed (Plate No.3, Fig. Nos. 5,8).

The net result of the plant toxin showed changes in the cordal arrangement, leading to deformation of liver histology (Plate No.3, fig.Nos. 7,8,9) showing large gaps in the hepatic tissues.

c) Histochemical observations :

The histochemical data on some important staining reactions employed in the present investigation of the liver of T. mossambica is recorded in Table No.19 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.3.

Intense PAS reactivities were exhibited by the liver cells (Plate No.3, fig.No.1) to test the nature of the PAS staining some of the sections were

TABLE NO. 19

Histochemical observation on mucosubstances in various tissues of the liver in control and plant toxin (A. concinna) treated fish T. mossambica (Peters).

No.	Histochemical methods	Hepatocytes	
		Control	Treated
1.	H-E	++++H	++++H
		+++E	+++E
2.	PAS	++P	++++P
3.	M-Diastase-PAS	-	-
4,	AV pH-1.0	+B	+++B
5.	AB pH 2.5	+B	+++B
6.	AB pH 1.0-PAS	++P	++++P
		+B	+++B
7.	AB pH 2.5-PAS	++P	++++P
		+B	+++B
8	AF	-	-
9	AF-AB pH 2.5	-	-

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

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

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. The hepatic tissue contained glycogen and a very few acidic mucosubstances.

The few patches of cells showed PAS positivity resistant to diastase digestion and moderate alcianophilia at pH 2.5 and all other reactions showed negative reactivities. Such reactivities indicated the presence of acidic mucosubstances in these cells.

It was interesting to note that the cells bordering the blood sinusoids were highly PAS positive and contained high concentration of glycogen in them (Plate No.3, fig.Nos.1,2).

d) Mucosubstance alterations due to A. concinna fruit toxin :

The application of plant toxin A. concinna to the fish T. mossambica revealed many striking changes in the intensity of staining and concentration of the mucosubstances of the hepatic cells only.

The liver sections in the lower concentrations (200 and 225 ppm) of plant toxin produced decrease in the staining intensity (Plate No.3, fig. Nos. 2,3). Whereas in concentration 300 ppm it was found gradually decreased staining activity reaching lowest at 400 ppm (Plate No.3, fig.No.6).

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 a number of glycogen patches throughout the hepatic tissue.

iv) Kidney.

a) Normal histology of kidney :

The kidney of T. mossambica is composed of many functional units, the nephrons. The malphigian bodies are clearly seen (Plate No.4, fig.No.2) with Bowman's capsule in which glomeruli are enclosed. 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.

b) Histopathological alterations due to A. concinna fruit toxin :

Due to effect of plant toxin from A. concinna the histology of the kidney was totally changed even at lower doses also. The initial toxin dose affected the Malphigian body. In the low concentration (225 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. The glomeruli became diffused (Plate No.4, fig. Nos.6,7,8).

The lumen of the proximal tubules was reduced and the brush border showed disintegration. There was loss of cytoplasmic material in a 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.

Epithelial cells show swelling and damage in the distal tubules. The concentrated substances were observed towards the luminal side showing honeycomb like structure. The hypertrophy and edema led to disorientation in the distal tubules, thus causing distortion in the kidney structure. The intertubular space was increased whereas lumen size was very much reduced. The severe necrotic changes were prominent in these tubules.

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

c) Histochemical observations :

The histochemical data on some important staining reactions employed in the present investigation of the kidney of T. mossambica are recorded in Table No. 20 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 alongwith 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.4.

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

TABLE NO. 20

Histochemical observations on mucosubstances in the various tissues of the kidney in control and plant toxin (A. concinna) treated fish T. mossambica (Peters).

No.	Histochemical methods	TISSUES					
		Malphigian body		Proximal tubules		Distal tubules	
		Control	Treated	Control	Treated	Control	Treated
1.	H-E	+++H	++++H	++++H	++++H	++++H	++++H
		+++E	++++E	+++E	++++E	++++E	++++E
2.	PAS	+++P	++++P	+++P	++++P	+++P	++++P
3.	M-Diastase-PAS	++P	++P	++P	++++P	-	-
4.	AB-pH 1.0	-	-	++B	++++B	++B	++B
5.	AB-pH 2.5	+++B	+++B	++B	++++B	++B	++++B
6.	AB ph 1.0 - PAS	+++P	+++P	++P	++++P	++P	++++P
		-	-	++B	++++B	++B	++++B
7.	AB pH 2.5-PAS	+++P	++++P	++P	++++P	++P	++++P
		+++B	++++B	++++B	++++B	++++B	++++B
8.	AF	-	-	+P	++++P	++P	+++P
9.	AF-AB pH 2.5	-	-	++P	++++P	++P	+++P
		++B	+++B	++B	++++B	++B	+++B

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

Abbreviations H-E = Haematoxyline-Eosine; PAS = Periodic Acid Schiff.; M.Diastase = Malt diastase; AB = Alcian blue 8 GX-300; AF = Aldehyde fuschin; P = Pink; B = Blue.

i) Elaboration of mucosubstances by Malphigian bodies :

Intense PAS reactivities were shown by both glomeruli and Bowman's capsule (Plate No.4, fig. No.1) which were partly susceptible 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 alcianophilia with AB pH 2.5 gave a moderate staining reaction (Plate No.4 fig. Nos.3,4). In AB pH 1.0-PAS and AB 2.5-PAS sequential staining procedures these parts showed mixed (bluish) staining. Thus Malphigian bodies indicated the presence of glycogen and acidic mucopolysaccharides in their cells.

ii) Elaboration of mucosubstances by proximal tubules :

The luminal brush border of proximal tubules showed intense PAS reaction and cellular staining was slightly faint (Plate No.4, fig.5). PAS positivity with prior diastase digestion had no effect indicating absence of glycogen in these tubules.

The luminal brush border showed intense alcianophilia at pH 2.5, indicating presence of acidic mucins in these sites. AB pH 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 indicating the presence of either neutral mucopolysaccharide or acidic mucins in these tubules (Plate No.4, fig. No.4).

iii) Elaboration of mucosubstances by the distal tubules :

Uniform PAS reactivity shown by distal tubules had a slight supressive effect with prior diastase digestion indicating the presence of glycogen in

these tubules. Alcianophilia at pH 1.0 and pH 2.5 was uniform but with very low intensity. AB (pH 1.0, 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 synthesizing and elaborating glycogen and slightly sulfated acid mucosubstances.

d) Mucosubstance alterations due to A. concinna fruit toxin :

The application of plant toxin 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 mucosdubstances. These structures contained glycogen and acid mucopolysaccharides in them. The concentrations of these components reached maximum even in low doses of plant toxin (Plate No.4, fig Nos.3,4) and their concentrations started decreasing as the concentration of the plant toxin in the treatment increased (Plate No.4, fig.6). The mucosubstance form a ring surrounding the glomeruli near the Bowman's capsule (Plate No.4, fig. No.7). In the higher concentrations of plant toxin these mucosubstances slowed and steadily diminished.

Mucosubstances in proximal tubules were also increased immediately after the treatment of low doses of the plant toxin which were distinctly observed towards luminal brush borders. As against the Malphigian bodies the increasing trend of mucosubstance accumulation or concentration in the sites of these tubules continued in the higher doses of the plant toxin treatment.

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

v) Intestine

a) Normal histology of intestine :

Intestine of T. mossambica shows usual four layered structure. The outermost serosa is followed by a muscular coat, comparatively better developed, consisting of an outer longitudinal and an inner circular layer. The submucosa is divisible into an outer stratum compactum, a dense connective tissue arranged in a wavy pattern and an inner stratum granulosa rich in capillary network. The latter merges with the tunica propria of the underlying mucosal coat, there being no muscularis mucosa. The epithelial lining of the mucosa consists of prismatic cells with basal nuclei. The nuclei of the intestinal cells are round with 2 to 3 nucleoli. The mucosal layer is thrown into many folds.

Only two main types of cells are present in the intestinal epithelium of mucosal layer. Goblet cells concerned with secretion and columnar cells which are generally lacking any definite secretory inclusions and are assumed to be concerned with the absorption. The goblet cells are of typical shape with a slender base, and permanent opening through the striated border. The columnar cells are tall and cylindrical having striations, and oval nuclei near the free borders.

b) Histopathological alterations due to A. concinna fruit toxin :

The mucosa of intestine was severely damaged in the phytotoxin treatment and different types of degenerative changes leading to complete the destruction of the cells (Plate No.5, fig.No.6) were evident. In some areas the cells, were considerably swollen with granular cytoplasm and in others they had become extensively vacuolated. In higher doses of toxin the cells in mucosa were separated from one another. Sometimes some cells were completely destroyed and the dead cell debris was seen lying in the lumen (Plate No.5, fig. No.5). The goblet cells and the blood capillaries were considerably enlarged and congested. Other layers were not much affected.

c) Histochemical observations :

The histochemical data on some important staining reactions employed in present investigation of the intestine of T. mossambica is recorded in Table No. 21 according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The distribution and alteration in the various cellular elements and their mucosubstances in the intestine are photomicrographically illustrated in Plate No. 5.

The histochemical studies on the intestine reveal the following significant facts about the elaboration of mucosubstances - (1) The mucosubstance elaborating cells were along the inner mucosal margin of the intestine (2) Goblet cells show distinct mucosubstance content in them. (3) The plant toxin treatment show induction to elaborate more substances by the different cellular elements of the intestine.

i) Mucosubstances elaboration by epithelial cells :

The PAS reactivity was shown by columnar epithelial cells which was resistant to prolonged diastase digestion and reacted weakly with alcian blue at pH 2.5 and in the combined (AB pH 2.5-PAS) staining method, indicating probable simultaneous occurrence of acidic mucosubstances along with the neutral mucins in them.

Strong PAS reativity was exhibited by goblet cells, which was resistant to prior diastase digestion. They reacted with AB both at pH 2.5 and pH 1.0. They stained blue with sequential staining techniques (AB pH 1.0, 2.5-PAS). Some of the mucosubstance secreting cells were AF positive and in the combined sequential staining procedure (AF-AB pH 2.5) showed pink staining in them. Thus, some of the mucosubstance secreting goblet cells have capacity to elaborate sulfomucins whereas other contained carboxyl containing acid mucosubstances in them.

ii) Mucosubstance elaboration by submucosa :

PAS reactivity shown by submucosal layer of intestine, was resistant to malt diastase digestion. In AB pH 1.0 and AB pH 2.5-PAS techniques, this layer showed mixed staining. Other reactions showed the capacity of submucosa to synthesize and elaborate mixed, neutral and acidic (sulfated) mucosubstances.

iii) Mucosubstances elaboratioin by muscle coat :

Intense PAS reactivities were exhibited by muscle cells (Plate No.5, fig No.3) which were labile to malt diastase digestion. Such enzymatic digestion tests indicated presence of glycogen in these cells.

TABLE NO. 21

Histochemical observations on mucosubstances in the various tissues of the intestine in control and plant toxin (A. concinna) treated fish T. mossambica (Peters).

No.	Histochemical methods	TISSUES									
		MUCOSA				'Submucosa		Muscular coat		Serosa	
		Columnar cells		Goblet cells							
		Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1.	H-E	+++H	++++H	+++H	++++H	+++H	++++H	++H	++++H	++H	++++H
		++E	++++E	++E	++++E	++E	++++E	++E	+++++E	++E	++++E
2.	PAS	+++P	++++P	+++P	++++P	++P	++++P	+++P	++++P	+++P	++++P
3.	M.Diastase-PAS	++P	++++P	+++P	++++P	++P	+++P	-	-	++P	+++P
4.	AB pH 1.0	+++B	++++B	++B	++++B	++B	++++B	-	-	-	-
5.	AB pH 2.5	+++B	++++B	+++B	++++B	+++B	++++B	+B	++B	-	-
6.	AB pH 1.0-PAS	++P	++++P	++P	++++P	++P	++++P	+++P	++++P	++P	++++P
		++B	+++B	++B	++++B	+++B	++++B	-B	-B	-B	-B
7.	AB pH 2.5-PAS	+++P	++++P	++P	++++P	++P	+++P	++P	++++P	++P	++++P
		++B	+++B	++B	++++B	++B	+++B	-B	-B	-B	-B
8.	AF	++P	+++P	-	-	++P	++++P	-	-	-	-
9.	AF-AB pH 2.5	++P	+++P	++P	+++P	++P	+++P	-	-	-	-
		++B	+++B	++B	++++B	++B	+++B	-	-	-	-

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

Abbreviations H-E = Haematoxyline-Eosine; PAS = Periodic Acid Schiff.; M.Diastase = Malt diastase; AB = Alcian blue 8 GX-300; AF = Aldehyde fuschin; P = Pink; B = Blue.

iv) Mucosubstances elaboration by serosa :

PAS reactivity shown by serosa was resistant to prolonged malt diastase digestion indicating absence of glycogen in them. Other techniques for demonstration of acid mucosubstances showed negative reactivities indicating their absence from this layer. Thus the serosa of the intestine elaborated only neutral mucosubstances in them.

d) Mucosusbtances elaboration due to A. concinna fruit toxin :

The application of plant toxin of A. concinna reveal several interesting alterations in the staining intensity and concentration of mucosubstance in the four layers of the intestine.

The number of the mucosubstance secreting cells in the intestine were enlarged during phytotoxin treatment and showed large amount of mucosubstance production both in lower and higher doses of the phytotoxin. Especially, the columnar epithelial cells and the goblet cells showed maximum concentration and elaboration of mucosubstances, comparatively serosa, submucosa and muscular coat showed not much variations about their mucosubstance elaboration.