CHAPTER 4

DISCUSSION

IV. DISCUSSION

As has been pointed out earlier in the introductory chapter, some of the plants which are consumed by the animals are severely toxic to them. This discovery now constitutes a base for further research in many countries particularly the determination of the full extent of the plant tissues, finding chemical components of these tissues, detection of a toxin, use of these toxins for controlling and eradication of some unwanted species of plants and animals.

Therefore, natural toxins of plant origin have been used to clear the ponds from weed fishes and other undesirable acquatic animals, in the pisciculture. In India, it is gratifying to note that in recent years a growing interest is being taken to use indigenous variety of plants in fish-nursery management, in developing fisheries technology.

Although in this line some work has been reported in North Eastern India (Ramanujam and Ratha, 1980a, 1980b, and 1983) there are no studies available on the plant toxins on this aspect in the Western part of India. The Western Ghat region has many indigenous plant species with piscicidal potentialities. Therefore, knowing the vast scope for study on indigenous plants from Western Ghat of India, a commonlyused indigenous variety of piscicidal plant Lasiosiphon eriocephalus was selected for the present investigation. While selecting the plant emphasis was that it should belong to the family Thymelaeaceae or Euphorbiaceae. This is because most of the earlier work is on the plants of these families and secondly the toxin produced by these plants are piscicidal in character. The fish Tilapia mossambica, selected for

the present investigation, is also locally, easily available and it creates number of problems in nursery ponds in this region.

Moreover earlier investigators have used many plants for their study in pisciculture, they have not been investigated in detail the chemical compo sition of these plants. The present investigation first time provides the information not only on a biochemical composition of the indigenous piscicidal plant, L.eriocephalus but also its phytochemical analysis. As it is brought to the notice, the paucity of literature on this area of investigation, it was hoped that such a study will throw light on effects of plant toxins on vital organs of fish in a probable eradication studies to control undesirable fish variety which are harmful to food fishes and game fishes.

1) Chemical Composition and TLC :

The leaves of L.eriocephalus contains the three chemical components extractable in benzene, chloroform and alcohol. There are few inorganic ions particularly, Fe, Ca, Na, Zn, Cu and Co in it. Among the three components the ethanol extracted components seemed to contain active principle/toxin which exhibited piscicidal properties. The TLC showed that the benzene extract contained two components with 0.57 and 0.77 Rf values, the chloroform extract contained five distinct components of 0.34, 0.45, 0.61, 0.71 and 0.8 Rf values and the ethanol extract contained only two components of 0.37 and 0.6 Rf values. At a comparative level the chloroform soluble components were more than the other two solvents. But the active principle seemed to be water soluble and hence was detected in the ethanol fraction. The melting points of these three extracts were tested and found that their melting points ranged from 80 to 120^{o}C. The melting point of benzene

extract was 80°C, chloroform extract was 100°C and that of ethanol extract was in between 118 to 120°C. From the scanty literature of this and previous investigations on the melting points of the active principles of piscicidal in character found in the plants, no decisive conclusion could be derived.

2) UV Spectral Analysis :

UV spectra of the three compounds extracted with benzene, chloroform and ethanol showed intense peaks in between 190 nm to 350 nm. The two benzene components showed identical UV spectral patterns showing intense peak at 225 λ maxima. The five chloroform extracted components (C_1, C_2, C_3, C_4 and C_5) showed different but conjugated UV spectra showing intense λ nm peak values at 250, 255, 255, 260 and 250 respectively. In this spectrum the intense peaks were observed identical to those for two components of the benzene extract showing λ max. at 225 nm indicating remaining these two components might be extracted in the chloroform treatment. The two ethanol components showed distinct pattern of spectra from that of benzene and chloroform patterns. The component E_1 started to show increase in spectrum at 353 nm which increased steadily and reached a peak value at 351 and 346 nm. E_2 component has only one peak value λ max. at 346 nm These peaks were prominent. The E_1 component showed conjugated spectrum but E_2 showed clear single spectrum. From the nature of the UV spectra of ethanol soluble components of the leaves of L.eriocephalus it indicated that these components might be glycosides or flavonoids which might be affecting the fishes.

3) NMR Spectral Analysis :

NMR spectrum of benzene soluble components showed identical pattern for compound I and II indicating possible identical chemical moeity in their structure. The values of the chemical shift expressed in δ ppm, showed unresolved multiplet at 0.9 which is due to presence of methyl ($-CH_3$) groups and a singlet at 1.25 indicates the presence of methelene ($-CH_2$) groups. The mean area under the peak i.e. integrated value indicate that the ratio of Methyl : Methylene groups is 1 : 8. δ 1.6 unresolved multiplet indicates presence of methine ($-\overset{|}{\underset{|}{C}}-H$) group, and δ 2.0 singlet indicates the presence of acetate ($-\overset{|}{\underset{|}{C}}-CH_3$) in the compound.

Thus, the NMR spectrum indicates that the benzene extract of L.eriocephalus leaves contain acetate of hydrocarbon or possibly triterpene acetates.

NMR spectrum of chloroform soluble components showed identical pattern of peak values to those for benzene extract having unresolved multiplet at δ 0.9 due to presence of methyl ($-CH_3$) groups, a singlet at 1.25 indicating the presence of methelene ($-CH_2$) groups, δ at 1.6 unresolved multiplet indicates presence of methine group ($-\overset{|}{\underset{|}{C}}-H$) and δ 2.0 singlet indicates the presence of acetate ($-\overset{|}{\underset{|}{C}}-CH_3$). Thus, the NMR spectrum of chloroform soluble components indicated the presence of Hydrocarbon compound in this fraction.

The NMR spectrum of the ethanol soluble components showed a multiplet (unresolved) centered at δ 1.2 - 1.3 indicated the presence of methylene groups ($-CH_2$), and unresolved multiplet at δ 1.5 - 1.65 indicated the presence of methine ($-\overset{|}{\underset{|}{C}}-H$) groups, δ 2.0 indicated the

presence of - $\overset{|}{C}$ - CH_2 grouping and δ 4.2 - 5.0 broad unresolved multiplet indicated the presence of hydroxy (- OH) group in the compound.

Thus, the above data of ethanol extracts of the L.eriocephalus leaves indicated the possible presence of either saponins or flavonoids. In the further work the purification of all the components of benzene, chloroform and ehtanol extracts is required for the confirmation of these results.

4) IR Spectral Analysis :

The IR spectrum of benzene soluble extract showed stretching band at 1850 cm^{-1} corresponding to - $\overset{|}{C}$ - H stretching and a stretching band at 1740 cm^{-1} indicated the acetate ($\overset{|}{\underset{/}{\backslash}}C = 0$) group.

The IR spectrum of chloroform soluble extract showed a broad band at 3400 - 3500 cm^{-1} indicating the presence of hydroxyl (- OH) group, and a band at 1708 cm^{-1} indicating the presence of $\underset{/}{\backslash}C = O$ or acetate group.

The IR spectrum of ethanol extract showed stretching broad band at 3500 cm^{-1} indicating the presence of -OH or hydroxyl group and a band between 1700 - 1720 cm^{-1} indicated the presence of ketonic group ($\underset{/}{\backslash}C = O$) in the compound.

Thus the NMR spectral results and IR spectral results of the leaf extracts of L.eriocephalus, in benzene chloroform and ethanol are complimentary to each other indicating the possible presence of hydrocarbon acetate derivatives in these compounds.

Since the stretching bands are broad indicating some impurities or complex nature of the compounds, in further work such impurities can be removed and then the new spectra can be obtained for confirmation of the results.

For the appearance of broad band of hydroxyl group in the ethanol extract, one of the reasons might be the presence of metalic ions, Cu, Co, Fe, Ca, Zn, Na and K which probably must have formed chelation with the ions in the components. But for confirmation further research is necessary.

5) Atomic Absorption Spectrophotometric Analysis :

The atomic absorption spectrophotometric measurement for many cations was carried out and found that the leaf extracts of L.eriocephalus contained Na, K, Ca, Cu, Co, Fe, and Zn. At a comparative level Calcium concentration was more in all the three components extracted in benzene, chloroform and ethanol, showing the values 0.68 $\pm$ 0, 10.51 $\pm$ 0.07 and 11.14 $\pm$ 0.04 in ppm respectively. Within the three components, the ethanol extract concentration for this cation was more than the others. The Fe concentration was more in chloroform extract, which was 0.49 ppm, next in the decreasing order was benzene extract 0.35 ppm and ethanol extract has lowest Fe concentration of 0.33 ppm. Zn and Cu concentration was higher in chloroform extract showing 0.40 ppm and 0.22 ppm values respectively. Benzene extract contained intermediate values whereas ethanol extract contained lowest concentrations in it.

The importance of these cations from the present investigations cannot be deduced. But these cations might be useful for the biochemical machinery of this plant.

As earlier pointed out these metalic ions might be forming chelation with the compounds extracted from the leaves which interfere in resolving the clear chemical structure and the functional groups in the compounds, as indicated by their IR, NMR and UV spectral patterns.

6) Physico-Chemical studies Analysis :

A great many of the physical and chemical factors influence directly or indirectly the productivity of pond (Srivastava, 1985). In the acquatic environment the polluants influence the physiology of the organisms (Warren, 1971; Cardwell et al., 1976). An ordinary pollutant harms a fish either indirectly or directly (Ellis, 1936).

A) pH :

In the present investigation, no significant changes were observed in the pH (Fig.No.13 and 14) A higher pH 11 is lethal to fish (Swingle, 1961) so also a pH below 4.5 is particularly injurious or unproductive (Srivastava, 1985). So the acquatic life Advisory Committee 1955) have suggested that at no time shall the pH below 5 or above pH 9, and that in-so-far as possible pH value be maintained between 6.5 and 8.5 to maintain the productivity of the water for acquatic life. In generalneutral or slightly alkaline 7-8 pH water is more productive (Srivastava, 1985). The pH of the "dilution water" used for the present study ranged between 6-7 pH for the extract as well as for the crude powder. During treatment no significant changes were observed in the pH, indicating that the extract and the crude powder did not have any effect on the water quality and its toxicity might be due to the direct effect of the active principle on the physiology of fish.

B) DO : (Dissolved Oxygen)

This is one of the most important factors limiting productivity in fresh waters (Srivastava, 1985) under a given set of conditions. There is a non-linear inverse relation between temperature and the dissolved oxygen. The latter always tending to maintain a normal value towards the saturation

point (Jhingran, 1972). When water is having lowered concentrations of oxygen, fish begin to rise to surface or crowd near inlets. In extreme depletions of dissolved oxygen level, fish may die of asphyxia. According to the Acquatic Life Advisory Committee (1955) the dissolved oxygen content of warm water fish habitats shall be not less than 5 ppm. during at least 16 hrs of any 24 hrs period. It may be less than 5 ppm for a period not to exceed 8 hrs. within any 24 hrs period. But at no time shall the oxygen content be less than 3 ppm. To sustain a coarse fish population the dissolved oxygen concentration may be less than 5 ppm for a period of not more than 8 hrs. out of any 24 hrs period, but at no time shall the concentration be below 2 ppm. DO of the dilution water used for the present study ranged between 6-7.5 ppm. for the ethanol extract as well as for the crude powder and there was no significant change during the treatment in the DO (Fig.No. 11 and 12), indicating that the ethanol extract and crude powder of the leaves of L.eriocephalus did not have any effect on the water quality and its toxicity may be due to - (1) Direct effect of the active principles in the plant toxin on the fish, (2) Indirect effect where in the DO is made unavailable to the fish by the toxin. Hence large haemorrhages and heavy secretion of mucous on the gills is formed, indicating that the plant toxin has an indirect effect on the fish.

C) Hardness of the water :

The total alkalinity depends on the geology of the region. In natural environment the water with very low value of alkalinity are generally less productive than with high values. The hardness expressed in $CaCO_3$ for the present study ranged between 50-55 ppm. There was no change in hardness during the experimental period (Fig.No.15 and 16). The present observations

are also agreeing with the studies of earlier investigation (Henderson et al., 1960). According to them pH and hardness did have no major effect on the toxicity to fish caused by chlorinated hydrocarbons. Similar observations on other indigenous piscicidal plants were noted by Chakraborthy et al.,1971, Virdi, 1982; Ratha and Ramanujam, 1983 and Bhuyan, 1967.

D) LC_{50} or LD_{50} :

One of the better known uses of dose-response relationships deals with the determination of the LD_{50} or LC_{50} (lethal dose or lethal concentration). LD_{50} used in determination of biological response, considered to be as 'yes-no' or 'binary' (on-off, present-absent, 0-1) response, in which it is determined. Whether the subject is dead or alive with no intermediate category. In other words, it is a point of separation.

LC_{50} can be estimated by three methods, (i) simple graphical method, in which linear response (per cent mortality) is plotted against linear dose, (ii) Use of Semilog paper method in which the dose-response (per cent mortality) is plotted on semilog paper against log dose because the response is more linear with the logarithm of the dose than with the arithmetic value of the dose, (iii) The third method is also a graphic method that requires logarithmic-probit paper, in which dose-response (per cent mortality curve plotted on probit paper which is with many variables like, dose percentage, % mortality, dose schedule, etc. For the present study, the first method is used for the calculation of LC_{50} values for the following reasons.

In studies on fish toxicity, a slight degree of variability is normally encountered between different individuals of the same batch or the same

species. Usually, there is the odd specimen that succumbs to the effect of the toxicant before the main batch or the odd individual that manages to survive a little time longer when all others are dead. This natural variability is allowed in routine tests by baring results on not less than 20 fish exposed at each concentration (Muirhead Thomson, 1976).

In the present study it was not possible to select the concentrations of the toxicant in geometric proportions to calculate the LC_{50} values for certain period by regression plot and probit analysis, because of short survival time and tolerance phenomenon exhibited by the fish.

The percent mortality increased with increase in concentration and 100 % mortality (LC_{100}) was reached within 3 hrs with 12 ppm for ethanol extract and with 150 ppm for the crude powder. The high values of LC_{100} (150 ppm) shown by the crude powder of L.eriocephalus leaves confirms that the crude form of the toxin is not very effective as a fish eradicant. The low values of LC_{100} (12 ppm) exhibited by ethanol extract make it suitable for fish eradication.

The lethal threshold concentration (LTC) for T.mossambica was found to be 80 ppm for the crude powder and 4 ppm for the ethanol extract (Table No. 5).

At lower concentrations (4, 5, 6, 7, 8 and 9 ppm) of the ethanol extract and (80, 90, 100, 110 and 120 ppm) of the crude powder, after a period of initial stress, the fish survived upto a period of 12 hrs. This might be due to the 'phenomenon of tolerance'. Such type of tolerance phenomenon was observed in case of detergents (Degens et al., 1950), hydrogen ions and

ammonium (Lloyd and Orr, 1969), Cyanide (Neil, 1957) and Zinc (Edward and Brown, 1964) and for Zanthoxylum aramatum fruit extract (Ramanujam and Ratha, 1983).

A comparison of the effect of the crude powder and the extract on T.mossambica showed that the crude powder LC_{50} for 3 hrs and 6 hrs. was similar. This might be due to the physiological resistance to particular active principles in the crude powder on the part of the test fish. This type of physiological resistance was shown by mosquito fish (Gambusia affinis) toward chemicals such as DDT, BHC and Dieldrin (Boyd and Fergusson, 1964).

The unescapable conclusion from the above studies is that the ethanol extract of L.eriocephalus leaves is very potent and can be used as a selective eradicant of undesirable fishes at concentrations of 10 and 12 ppm

7) Analysis of behaviour of the fish intoxicated with L.eriocephalus toxin :

The behavioural responses of T.mossambica to the benzene extract and chloroform extract were not distinct. But the responses to the ethanol extract of the L.eriocephalus leaves were prominent and easily could be noticed. The behavioural responses were concentration dependent. At lower concentrations (1, 3, 5 and 6 ppm) the fish did not respond but at higher concentrations (10, 12 and 15 ppm) these behavioural responses were noticeable. Such concentration dependent behavioural responses were observed in phenol intoxication in the carp, Cyprinus carpio (Lukyanov et al.,1984). These responses were shown to be linked to the disturbances in the cholinergic system of the brain. In the present investigation such changes have not

studied but studies on investigations in the cholinergic system of the brain would possibly give an answer for behavioural responses of Tilapia mossambica to the high concentrations of ethanol extracts of the plant toxin. Ramanujam and Ratha (1980a) have observed effect of ten different plants on behavioural responses of fishes, Danio dangila and Heteropneustes fossilis and have shown that the dose required for air-breathing fishes was more as compared to those for gill-breathers. The present observations are contradictory to their observations since the T.mossambica is a gill-breather even it requires more concentrations of this particular plant toxin to change the normal behaviour of the fish. But other observations like active movement in the initial phase of toxin treatment, erratic movements, turning upside down and finally collapsing to the bottom of the jar were almost similar to those observed by Ramanujam and Ratha (1980a).

Thus, at present it seemed that (1) The fish T.mossambica showed behavioural changes during the treatment of ethanol extract of L.eriocephalus leaves (2) But the benzene and chloroform extracts were without any visible changes. (3) The ethanol extract induced behavioural changes were dose and time dependent. (4) The behavioural changes showed by T.mossambica were identical to the responses of different other fishes to piscicidal chemicals and plant toxins.

8) Histology and Histochemistry :

i) Oral Cavity

A) Discussion on Histology :

The first organ exposed to the toxin, if the toxin is mixed in the water is oral cavity. Eventhough this organ has not been studied in the toxicological investigations from the histological as well as histochemical

point of view. This is the first attempt in such studies involving plants toxin induced histochemical alterations in the oral cavity of a fish, T.mossambica. Histology of oral cavity showed that an outer stratified epithelium, internally supported by connective and muscular tissues. There were several papillae having lamina propria, a vascularized connective tissue core and epithelial covering. Some papillae showing shape of fungiform type while others were showing filiform type similar to that of mammalian tongue papillae. Probably for the first type this histological observation has been recorded in case of the fish, T.mossambica (Plate No.1, Fig.Nos.5,6,7). Secondly the histologically differentiated two types of cells have also been observed and recorded. The epithelial lining of the oral cavity consisted of (i) the large columnar cells and (ii) the small cuboidal cells, their number, size and distribution were affected during the plant toxin treatment. The small cell population increased while the size of the large cells almost doubled in lower doses of the toxin treatment. In higher doses the staining reactivities and number of these cells increased and the columnar form changed to the rounded form. The thickening and vacuolization in connective tissue were prominently observed. These histological alterations seemed to be dose dependent and probably providing more cells for mucin secretion and for providing resistance to the toxin action. Other functional relationships cannot be ascertained with the help of present observations.

B) Discussion on histochemistry :

The whole epithelium of the oral cavity is filled with large quantities of mucosubstances in response to plant toxin treatment. It is well known that the mucins in the oral cavity of the vertebrates perform several functions in binding food particles, providing lubrication for swallowing the food, create

least friction along the gastrointestinal tract, provide antibactericidal action for killing bacteria in the food, and provide protection against mechanical and chemical injury, etc. (Jensen, 1976). But their enormous production in the several mucin producing cell types, in all the possibilities seemed to be involved in the protection against chemical injury caused by the plant toxin. Other functions of these mucosubstances are not known.

ii) Gills

A) Discussion on Histology :

It is well known that the gill histology is greatly affected due to the chemical intoxication (Metelev et al.,1971; Studnicka et al., 1983; Rojik et al., 1983; Delela et al., 1979). The piscicidal compounds whether of synthetic origin or of plant origin, the gills are the next organ which get damaged (Metelev et al., 1971). Among the chemicals, organic and inorganic elements are involved for production of ill changes in the gill histology. The inorganic ions decreases the pH of water, concommitantly increasing the acidity of the water body. The fishes exposed to such acidic environment secrete large amount of mucus by the gills and by the skin for protection (Metelev et al., 1971). It has been observed that the size and number of mucus secreting cells was increased in T.mossambica and R.daniconius in the sublethal doses of spent wash, which contains both organic and inorganic substances causing production of large amount mucus (Nikam, 1986). This volumenous thick mucus inhibits normal respiratory process and fishes die. Gill histological alterations include loss of cells due to sea bloom (Shimada, 1983), due to hydrocarbons (Lopez, 1981), due to spent wash (Nikam, 1986), loss of chloride cells due to other effluents in Fathead minows and

Pimephales promelas (Leino and Mccormic, 1984), curling of secondary lamellae due to loss of pillar cells (Skidmore and Tovell, 1972; Haniffa and Sundarvathanam, 1984 and Nikam, 1986), separation of gill epithelium from basement membrane, fusion of adjacent gill lamellae, erosion at the distal end of gill filaments, loss of cell membrane in Channa gachua due to endosulfan (Dalela et al., 1979), swelling and thickening of gill lamellae, necrosis and sloughing off in gill epithelium and interlamellar filaments (Ahmed and Ghufran, 1984).

Although the gill histological changes have been studied by using chemical substances, no investigation is available on the plant toxin to show such changes. Kiptoon et al. (1982) studied the histology after plant toxin treatment in gastrointestinal tract and liver but there is not a single investigation to show histological alterations caused in the gills of the fishes. Here in the present inves-tigation we report for the first time that the gills of fresh water fish, T.mossambica show histological changes due to ethanol extract and crude powder of the leaves of L.eriocephalus. In the low concentrations, the histological changes include the increase in interlamellar space, reduction in the primary gill lamellae, displacement of the epithelium from the basement membrane, increase in the number of mucous secreting cells and acidophil cells, curling of secondary gill lamellae, pillar cells enlarged whereas at higher concentrations of the extracts these histological changes included the reduction in supporting bony elements, enlargement of distal gill lamellar blood spaces, increased subepithelial spaces, ruptured lamellar capillaries and detachment of gill epithelium. Such results were more or less similar to the changes caused by chemical substances in the earlier investigations.

B) Discussion on Histochemistry :

The gills of the fish T.mossambica, selected for the present study, elaborate a variety of mucosubstances, including neutral mucosubstances, acidic mucosubstances (both sulfated and carboxyl group containing MPS). At a comparative level the concentration of the acidic mucosubstances was more than the concentration of neutral mucosubstances. The different gill elements contained different types of mucosubstances in them. (i) The most of the epithelial cells elaborated only neutral mucosubstances but a few epithelial cells also contained both neutral and acidic (mixed) mucosubstance in them, indicating inter conversion of both these types in these cells, (ii) The mucus cells contained either neutral mucins, mixed mucins (acidic + neutral) sulfated mucins or carboxyl containing acidic mucins in them. (iii) The basement lamina contained only mixed mucosubstances in which both neutral and acidic mucins were present. (iv) The gill rachis contained only strong sulfated acid mucosubstances in them.

There observed an interesting change in the mucosubstance elaboration by the gill elements during the treatment of lower and higher concentrations of the plant toxin of L.eriocephalus to the fish, T.mossambica. Initially epithelial cells, basement lamina and gill rachis contained very low concentration of mucosubstances in them (Plate No.3, Fig. No.5, 10) which increased moderately in the lower doses of plant toxin (Plate No.3, Fig. Nos. 4, 1, 11) whereas in higher doses it became maximum in them (Plate No.3, Fig. Nos. 6, 8 and 9). But this pattern was not observed in the pillar cells of the secondary filaments of the gills. These cells contained moderate con-

centrations in the normal conditions which immediately even after a low dose treatment of plant toxin increased to maximum (Plate No.3, Fig.No.3) and in the higher doses their number and concentrations fall down to a minimum (Plate No.3, Fig.7). This might be due to the loss of pillar cells from the filaments (Skidmore and Tovell, 1972), or due to disappearance of cytoplasmic organelles as in copper sulfate and zinc chloride treatment (Rojik et al., 1983).

The localization of particular type of mucosubstance within a specific cell type of the gill in T.mossambica seemed to be related with their certain functions. Rosen and Cornford (1971) found correlation between the mucus and its friction reducing properties. Gill mucins also function as a protective layer in checking bacterial and fungal infections (Vanoosteen, 1957 Jansen, 1976). Such a protective function to the mucins has been attributed to invertebrate mucosubstances (Gottschalk, 1960). It is known that the muco substances of the lungs surface help in exchange of gases by keeping surface epithelium moist (Hoar, 1965). Similarly at the surface epithelial cells of gill, these mucosubstances might be involved in gaseous exchange. This view has been supported by the observations that the acidic mucins have water binding capacity, so that the film of water spreads over the gill surface, which in turn facilitates the exchange of gases (Munshi, 1979; Hughes, 1979; Hughes and Munshi, 1979). The gills epithelial acidic mucins might be useful in the transfer of cations across the epithelium (Kirschner, 1978) and hence might be helping in osmoregulation and electrolyte excretion as suggested by Ingale (1981).

In the present investigation the mucosubstances during the toxin treatment get increased and when the fish die, the mucus with blood clots

oozed out from the mouth. The elaboration of such bulk quantities of muco-substances might be having some protective function upto certain limit of intoxication against chemical injury to the gill filaments and if that limit exceed the protective function against such injury must have lost, and the haemolysis occurred.

iii) Liver

A) Discussion on Histology :

Among several functions, the important function of detoxification is performed by the liver in most of the vertebrates and many exogenous and endogenous toxic compounds are broken down by this organ (Lagler et al., 1977). Under normal conditions hepatocytic functions are not overburned but during intoxification in various toxic treatments hepatocytic functions are under stress. Therefore several histologicalchanges occurred in the liver due to the pollutants, inorganic ions and toxins, at gross tissue level as well as at cell level also (Eller, 1971; Baktavathsalam et al., 1982; Dubale and Shah, 1979). Liver histology was greatly altered in carps subjected to DDT, Lindane and α -HCH (Hexochlorocyclohexane) (Studnicka et al.,1983). In copper sulfate and zinc chloride intoxification, organelles of the cells disappeared (Rojik et al., 1983) whereas Aroclor, 1254 poisoning showed vacuolation of hepato-cytes of Salmo gairdheri (Sivarajah et al., 1978). EM study, upon Aroclor, 1254 treatment showed enlargement of rough endoplasmic reticulum of haepatocytes (Sivarajah et al.,1978). Proliferation of fibroblasts (fibrosis) in hepatocytes were evident in Cadmium treatment to Garra mullya (Wani and Latey, 1983), with mercury toxicity to Sarotherodon mossambicus (Naidu et al., 1983) and with plant toxin of Lasiosiphon latifolium treatment to bull

calves (Kiptoon et al., 1982). The earlier investigator except Kiptoon et al. (1982) have studied the changes with reference to chemical toxicity but not a single reference was found to show the histological changes due to plant toxin, but their results are helpful in interpreting the present results.

In the present investigation, the first effect of plant toxin was the displacement of hepatocytes (Plate No.4, Figs. 5 to 8), then aggregation of cytoplasmic contents of hepatocytes (Plate No.4, Fig.8), swollen hepatocytes with vacuolization with loss of cell boundaries (Plate No.4, Figs.9, 10) disruption of sinusoids (Plate No.4, Fig.11) and lastly leading to deformation of liver histology (Plate No.4, Fig.12). These results are tallying with the results observed by Sivarajah et al. (1978) and Wani and Latey (1983).

It appears that during intoxication, the plant toxin might be entering into the hepatocytes and R.B.Cs. and therefore, these cells enlarge. During higher doses, more toxin enter and cells get further enlarged, exceeding certain limit and cells rupture causing deformaties in the liver histology. The vacuolization and aggregation and consequent effects due to the swelling and rupturing of the hepatocytes. Similar necrotic and cirrhosis formation effects have also been observed by other investigators (Sastri and Sharma, 1978; Gupta and Singh, 1982; Chatterjee et al., 1983). The enzymatic studies would reflect some light on the action of toxin intoxification and on action of plant toxin on hepatocytes, which we have included in the present investigation, although some work on enzymes concerned with such functions are in progress. But logically these liver enzymes must be affected because of the plant toxin.

B) Discussion on Histochemistry :

PAS reactivity and malt diastase digestion tests proved the presence of glycogen in hepatocytes, whose concentration varied with different concen trations of the plant toxin. In the low doses mucosubstance changes were not significant but during higher doses maximum concentration of glycogen was reached. This change might be due to the fact that as the fish accumulates the poison in the liver and become lithargic, probably due to impairment of cholinestarase enzyme system of the nervous tissue as indicated by Ramanujam and Ratha (1983), Chopra et al. (1958) and Virdi (1982), food intake is greatly reduced. During this stress period, great amount of energy required might have come from this stored glycogen in the liver and that is why the glyco-gen might be getting accumulated in the liver during high doses of the toxin treatment.

The presence of acidic mucosubstances did not show any appreciable change during the plant toxin treatment. The function of these acidic muco-substances with present knowledge cannot be attributed.

iv) Kidney

A) Discussion on Histology :

The nitrogenous waste products and the toxins (Poisons) after detoxi-fication are brought into the circulation and through blood to the kidney for their excretion. Since this unusual work performed by the kidneys, certain structural changes ought to occur. Such changes are less acute in low concen trations while drastic changes take place under high concentrations of toxin

treatment, leading to kidney failure and death of the fish (Mathur, 1969; Holden, 1965). Many chemical toxicity produce drastic changes in the kidney histology. Copper sulfate and zinc chloride produce necrosis of the glomeruli (Kumar and Pant, 1981; Rojik et al.,1983). Organic pollutants produce enormous swelling of the glomeruli, thickening of the endothelial wall of the capillaries and increases in the haemopoitic mass in Widow tetra (Amininkutty and Rege, 1978). Thiodon toxication and malathion produces necrosis in C.punctatus (Dubale and Shah, 1984) and development of lesions in the kidney (Chaturvedi and Saxena, 1978).

One observation is interesting due to Amaranthus retroflexus poisoning to swine and pigs. The histologic lesions in the kidneys of affected animals were characterized by hydropic degeneration and coagulative necrosis of both proximal and distal convoluted tubules. Glomeruli were shrunken and were apparently increased in cellularity. There was dilation of Bowman's capsules. Many tubular proteinaceous casts were observed in the distal and collecting tubules (William and Gray, 1976).

Exactly similar results were obtained in the present investigation of L.eriocephalus plant toxin intoxication to fish, T.mossambica. The histological alterations observed in the lower doses showed immediate increase in glomeruli size, degenration of basement membrane and development of intertubular space. In the higher doses, histologic alteration were shown by proximal and distal tubules and malphighian bodies. The tubules were distorted, swelling of distal tubular cells and severe necrosis. Dilation of Bowman's capsules and shrunken glomeruli were prominent (Plate No.5, Fig.7). Such histologic alterations must have been occurring in the kidney as an initial shock by the active principle of the L.eriocephalus circulating through the

blood and entering the kidney tissues and as it accumulates increase in concentration causing necrotic effects. Secondly this active principle (organic substance) must have larger size with high molecular weight which must have caused such histologic ill effects in the kidney.

B) Discussion of Histochemistry :

Kidney is the target organ of biochemists and histochemists working on toxicological problems. It is because after liver, degraded toxic compounds are brought to the kidney for their disposal to the outside of the body. The presence of mucosubstances in the kidney tubules of adult vertebrates is well known due to the works by Longley and Fisher (1954, 1956) and Longley et al. (1963). Longley and his coworkers suggested the role of mucins as protective or hydrophilic colloids in preventing the precipitation of solutes from the urine or in maintaining its fluidity or mobility. But there is not a single investigation to show the role mucosubstances in the kidney during the plant toxin treatment to the fish.

With this view in mind the present investigation on mucosubstances in the kidney tissues of T.mossambica in the L.eriocephalus toxin treatment was undertaken. Such study revealed that the malphighian bodies contained glycogen and acidic mucosubstances in the Bowman's capsular cells which immediately increased maximum even with low doses of toxin treatment (Plate No.5, Figs. 4 to 6) and at doses steadily diminished (Plate No.5, Fig.9) which might be due to shrinkage of glomeruli and dilation of the Bowman's capsule during the treatment.

The proximal tubules demonstrates intense reactions with PAS, AB (pH 2.5) at the brush border confirming acidic mucosubstances in them, Davis (1954) stated that some protein which escape into urine is reabsorbed by the proximal tubules. This reabsorption function might possibly be performed by these acidic mucosubstances found in the present investigation. Longley and Fisher (1963) and Butt and Hauser (1952) have suggested the functional role for mucosubstances as protective colloids in the prevention of stone formation. In the light of existing literature and with the help of theoretical background, an attempt has been made in this contribution to correlate the occurrence of acid mucosubstance with the osmoregulatory function of the kidney. But the role of mucosubstances which increased during the initial phases of toxin treatment and decreased after treatment of high doses so also the role of sulfomucins in the distal tubules cannot be interpreted with the observations of the present investigation. It requires further extensive histochemical and biochemical studies at a comparative level on the renal mucosubstances of different fishes subjected to different toxins of plant origin.

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