# CHAPTER-IV

# Discussion

# IV DISCUSSION

In India, there are about 700 poisonous plant species belonging to over 90 families of flowering plants (Chopra et al. 1965), and there is no comprehensive review on these plants to show their toxic principles. Some of these plants are used as piscicides. Recently, Viswanathan and Joshi (1983) have published short but very important review on the toxic constituents of some Indian plants, which stimulated us to search for a natural piscicide of indigenous plant of Western Ghat, Sapindus laurifolius. In their review they have classified the toxic substances into three categories according to (i) its physiological manifestations e.g. as nerve and muscle poison, (ii) its chemical constitution e.g. alkaloid, glycoside or (iii) its botanical origin. But their survey gives information on the plants used in medicine or of potential use in medicine, on edible plants and those used as adulterants in food, on toxic plants and allergens. In recent years, due to the rising cost, the development of resistance and the pollution caused by the chemical piscicides used in the control of undesirable fish species in the pisciculture, call for the discovery of less expensive and less hazardous alternatives. With this view in mind, in few laboratories in India, the indigenous plants are explored to discover a plant product having potentiality to use as piscicide (Ramanujam and Ratha, 1980; Nanaware and Harold, 1987 a,b,c,d,e). But the active principles of all these plants have not been fully discovered. The present investigation is the first of this type to show the nature of the natural piscicide.

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 common used indigenous variety of piscicidal plant, Sapindus laurifolius (Vahl) was selected for the present investigation. The most of the earlier work is restricted to the plants belonging to the families Thymelaeaceae and Euphorbiaceae. But this plant belongs to the family Sapindaceae. 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. The reasons for the selection of this fish species have been elaborately described in the first chapter of introduction.

Moreover earlier investigators have used many plants for their study in pisciculture, they have not been investigated in detail the chemical composition of these plants. The present investigation first time provides the information not only on a biochemical composition of the indigenous piscicidal plant S. lauri folius but also on its phytochemical analysis. It has been brought to the notice, the paucity of literature on the effects of plant toxins on vital organs of undesirable fishes in the eradication studies, the effects of the toxin extracted from the fruits of this plant have been observed on the important vital organs like liver, kidney, intestine, gills and oral cavity of T.mossambica also.

## A. Discussion on Solvent Extraction and TLC

### 1) Solvent Extraction

The fruits of S.laurifolious were extracted using five different solvents-petroleum ether, benzene, ethyl acetate, chloroform and ethanol. These solvents

were used for extraction because these solvents are with increasing polarity, so the less polar components will move in less polar solvents and more polar solutes in more polar solvent systems. Among the five extracts (PE, BE, EAE, CE and EE) obtained in extraction procedures the ethanol extract seemed to contain piscicide component. Hence the active principle extracted in the fruits of S.laurifolius seems to be water soluble in nature.

### 2) Chemical Composition

The chemical composition of the fruit of S.laurifolius shows the more percentage of ethanol extracted component. At a comparative level also the percentage of this component is more than in the leaves of Lasiosiphone eriocephalus, which also contain a piscicidal active principle. In L.eriocephalus it is 3.25% whereas in S.laurifolius it is 15.05%, indicating these fruits are rich source of piscicide component. But on inspection of the doses required to 100% mortality of T.mossambica, the L.eriocephalus component (15 ppm) was much more toxic than the S.laurifolius (400 ppm) component. Hence it could be stated that the toxic compound of this plant is milder than the toxin of L.eriocephalus

### 3) TLC

The TLC analysis of five extracts showed the varied number of components in each extract. The petroleum ether, benzene, ethyl acetate, chloroform and ethanol extracts showed 3, 3, 4, 2 and 3 components respectively. Out of these, the ethanol components only contain the active piscicide principle. The isolation and characterization of each component do not come within the scope of the present dissertation. But full analysis of these components only could provide extract chemical configuration of the piscicidal compound, which is undertaken for further studies in this laboratory.

## B. Discussion on Phytochemical Analysis

### 1) Melting Points

The melting points of all the extracts ranged from 90-100°C. The melting point of ethanol extract of this plant is 99°C and it is 118-120°C in case of L.eriocephalus (Harold, 1987). But such a comparison could not throw any light on the nature of the active piscicide component. To come to a conclusion by us ing melting points as the criteria for identification for natural piscicides, vast number of toxic components of plant origine must be analysed.

### 2) UV Spectral Analysis

UV spectra of the five extracts extracted with petroleum ether, benzene, ethyl acetate, chloroform and ethanol showed intense peaks in between 220 nm to 290 nm. The petroleum ether extract showed two $\lambda$ maxima, first at 230 nm and second at 260 nm. The benzene extract showed only one intense peak at 290 $\lambda$ maxima. The ethyl acetate extract showed a peak value at 245 nm. on UV spectrum. The chloroform extracted component showed UV spectrum showing only one intense $\lambda$ nm peak at 280-290 nm. The ethanol extract showed distinct spectrum from other extracts' spectra. The first $\lambda$ peak was at 220 nm whereas the other at 280 nm, where aromatic or conjugated ketones show $\lambda$ max. values. From the nature of the UV spectra of ethanol soluble components of the fruits of S.laurifolius, it indicated that these components might be glycosides which might be affecting the fishes.

### 3) NMR Spectral Analysis

NMR spectrum of petroleum ether extract showed peaks at 0.9, 1.3, 2.0 where $-CH_3$, $-CH_2$ and $\geq C-H$ groups of the compounds shows distinct peaks, respectively, and at 3.6 and 5.3 $\delta$ ppm indicating acetate and $>C=C(H)-\underline{H}$ grouping respectively. NMR spectrum of benzene extract showed more or less identical chemical moeity in their structure. The chemical shift 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 multiplet at 2.0 indicating $\geq C-H$ groups. $\delta$ 3.6 unresolved multiplet indicates presence of $>C(H)-O-$ $-C-CH_3$ $\delta$ 4.6 indicates $>C=C(H)H$ or $-O-CH_2$ - and $\delta$ 5.3 indicates $>C=C(H)-\underline{H}$. Thus, the NMR spectrum of the benzene extract of fruits of S.laurifolius contain acetate of hydrocarbon or possibly triterpene acetates.

From the nature of the NMR spectrum of the ethyl acetate extract also indicated the presence of $-CH_3$, $-CH_2$, $\geq C-\underline{H}$ and $>C(\underline{H})-OC(=O)-CH_3$ groups and possibly contain hydrocarbone acetate. NMR spectrum of chloroform soluble components showed identical peak values similar to the benzene extract and confirms the identical functional groupings in these two components. Thus, the NMR spectrum of chloroform extract also indicated the presence of hydrocarbon compound in this fraction.

The NMR spectrum of the ethanol soluble components showed a multiplets (unresolved) centered at $\delta$ 0.8, 1.2 - 1.3 and 2.0 indicated the presence of ($-CH_3$), ($-CH_2$), ($\geq C-\underline{H}$) and unresolved multiplet at $\delta$ 3.9 indicated the presence of $>C(\underline{H})-O-C(=O)-CH_3$ and $\delta$ 5.0 indicated the presence of $>C=C-\underline{H}$ grouping.

Thus, the above data of ethanol extracts of the S.laurifolius fruits indicated the possible presence of either saponins or flavonoids. In the further work the purification of the components of ethanol extracts is required for the confirmation of these results.

4) IR Spectral Analysis

IR spectrum of petroleum ether extract showed stretching bands at 1685, 1740 and 2900 $cm^{-1}$ corresponding to $-\underline{C}=0$, $-O-\underline{C}(=O)-CH_3$ and $>\underline{CH}$ stretchings. The IR spectrum of benzene extract showed exactly similar pattern of petroleum ether extract showing stretching bands at 1685, 1740 and 2900 $cm^{-1}$ and confirms the above groups indicating presence of acetate ethers. IR spectrum of ethyl acetate extract of fruits of S.laurifolius showed $>C=O$ stretching (1740$cm^{-1}$), $-O-\underline{C}(=O)-$ stretching (1790 $cm^{-1}$), $>\underline{C}-\underline{H}$ (2900 $cm^{-1}$) and -OH broad stretching at 3400 $cm^{-1}$, also indicating the presence of acetate and hydroxyl groups.

The IR spectrum of chloroform extract showed a broad band at 3400-3500 $cm^{-1}$ indicating the presence of hydroxyl (-OH) group, 2900 $cm^{-1}$ ($>\underline{C}-\underline{H}$) group, 1800 $cm^{-1}$ of $-O-\underline{C}(=O)-CH_3$ group and 1708 $cm^{-1}$ indicating the presence of $>C=O$ or acetate group.

The IR spectrum of ethanol extract also showed stretching broad band at 3500 $cm^{-1}$ indicating the presence of -OH or hydroxyl group and bands between 3000-2900 $cm^{-1}$ ($>\underline{C}-\underline{H}$) and 1700-1720 $cm^{-1}$ indicated the presence of ketonic group ($>C=O$) in the compound.

Thus the UV, NMR and IR spectral results of the fruit extracts of S.laurifolius are complimentary to each other indicating the possible presence of hydrocarbon acetate derivatives in them.

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.

The very broad band of hydroxyl group in the ethyl acetate, chloroform and ethanol extracts of the bivalent metalic ions such as $Cu^{++}$, $Mg^{++}$, $Fe^{++}$, $Ca^{++}$, and $Zn^{++}$ which probably might be forming chelation with the ions in the components. But for confirmation further research is necessary.

5) Atomic Absorption Spectrophotometric Analysis

The resdults of atomic absorption of many cations indicated that the fruits of S.laurifolius contained measurable amounts of divalent $Ca^{++}$, $Cu^{++}$, $Mg^{++}$, $Fe^{++}$, and $Zn^{++}$. At a comparative level calcium concentration was more in all the five extracts of the fruits indicating 0.72 ppm (pethroleum ether extract), 1.63 ppm (benzene extract), 2.34 ppm (ethyl acetate extract), 1.09 ppm (chloroform extract) and 0.69 ppm in ethanol extract. Within the three components, the ethanol extract concentration for this cation was more than the others. The Fe concentration was more in petroleum ether extract but absent in chloroform extract, which was 0.06 ppm and still less concentration in other extracts. Cu concentration was more or less same, ranging from 0.03 to 0.05 ppm in all the five extracts. Zn concentration was highest in chloroform extract showing 0.73 ppm and in the decreasing order following 0.31 ppm in ethyl acetate extract, 0.25 ppm in petroleum ehter extract, 0.22 ppm in benzene extract and 0.16 ppm in ethanol extract. Mg concentration was highest in benzene extract (0.32 ppm) but remaining four extracts contained only about 0.22 ppm concentration of this element.

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 fruits 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.

## C. Discussion on Water Quality Analysis

### (1) Temperature

Water temperature is one of the most important factors in the environment of aquatic organisms and plays a vital role in determining their distribution, growth, reproduction, metabolism and behaviour. Because it is one of the most easily measurable factors in the natural environment, and one which can be readily controlled in the laboratory, perhaps more is known about the reactions of fresh water animals to this one factor than to any other in the environment (Muirhead-Thomson, 1971).

In the various studies envolving evaluation of pesticide impact, due recognition is given to this major role of water temperature by carrying out all tests at controlled temperature, either at one constant temperature for all tests or at 2 or 3 constant temperature ranges in the laboratory. In trying to assess the effect of temperature on the impact of pesticide on fresh water life it is important to recognize some of the many different facets which have to be taken into account. Differences in the temperature, or changes in the

temperature, can affect the general activity, metabolism and behaviour of fresh water forms. Temperature also influences the chemical and physical states of the pesticide. The actual rate of uptake of the toxic chemical by the fresh water organism may be strongly influenced by prevailing temperature conditions. Some of the variations and vagoris of temperature effect on pesticide impact are well brought out in the extensive fish toxicity studies carried out in different laboratories (Applegate et al. 1961, Muirhead-Thomson,1971). Most of these compounds show increasing toxicity at higher temperatures. With some of these compounds susceptibility of the fishes increases with the increase in temperature (Macek et al.,1969).

To avoid the complications, the toxicological experiments in the present investigation were performed nearly at uniform temperature, which was nearer to the natural environment of the test fish. All the experiments were performed in the range of 24-27$^{o}$C. There was hardly difference of 3$^{o}$C in the experimental temperature. As far as the present studies are concerned, the effect of temperature on the impact of natural piscicide present in the fruit extract of Sapindus laurifolius on fresh water fish T. mossambica was neglegible. But to test the influence of temperature on the chemical and physical state of the plant toxin, experiments with increasing and decreasing temperatures must be performed and to assess the relative increase in susceptibility of fishes, data on $LC_{50}$ should be worked out. Such work is in progress in this laboratory.

(2) pH

The interrelation between pH and its role in determining toxicity of certain toxic chemicals is well brought out in studies or piscicide antimycin (Walker et al.,1964). As part of the comprehensive evaluation program the basic laboratories were followed by seminatural test in 1000 gallon wading pools,

provided with the bottom soil and sand or loam, and furnished with various introduced plants. As the mass of plant growth increases in the pools pH of the water changed. pH as an indicator of the acid base shift - rose from 7.5 upward to a value of 10 or more. In contrast to this relationship it appears that pH has no major effects on the toxicity to fish of chlorinated hydrocarbons (Henderson et al.,1960). This also applies to most organophosphorous compounds with the exception of Dipterex.

The pH of the water has been recognized as a factor which by itself can affect the impact of some pesticides. But according to Acquatic Life Advisory Committee (1955),pH be recognized as a poor criteria for the expression of the toxicity. According to them pH range of 5 to 9 was known to be non toxic. Therefore, pH values be maintained between 6.5 to 8.5 to maintain the aquatic life.

In the present study the pH of the water ranged from 6.9 to 7.3. The changes occured in the percent mortality seems not to be due to pH changes but due to the direct, effect of the phytotoxin. The concentration of the plant piscicide produces the kill of T.mossambica. This action seems to be similar to that of antimycin. Berger et al. (1969) showed the selective toxicity of piscicide antimycin with regard to pH relationships. More concentration required to produce a complete kill of fingerling goldfish in 96 hours at $12^{o}C$ was 0.20 ppb at pH 5 1.10 ppb at pH 8 and 60 ppb at pH 10. In the field it is suggested that an application of antimycin to soft water would be more effective if treatment were made at daybreak. This would allow substantially greater exposure time before the rapid diurenal rise in pH begins to cause degradation of the antimycin (Burress et al., 1969).

Paul and Raut (1987) recently have studied comparatively the toxicity of endosulpan in fresh water fishes under different pH. They observed, with the increase of pH the lethal dose of the pesticide also increase gradually with exposure to 24, 48, 72 and 96 hours and concluded that a pH of water do play a role in the toxicity of endosulpan in fresh water. It is clear that a higher pH value demands more pesticides.

### (3) Hardness

It has long been recognized that the action of toxic compounds on fresh water animals such as fish may be influenced by the quality of the water with regard to such characters as pH, alkalinity and hardness. In recognization of this, the majority of laboratories concern with routine studies on fish toxicity or routine screening of potential piscicides, either carry out test at consistantly uniform condition of water quality or duplicate all tests in hard as well as soft waters. The general concept of what is understood by the terms "hard" and "soft" water is exemplified by the composition of the dilution water used in standard tests with insecticides in the U.S.A. (Hendorson et al.,1960) and in molluscicide studies in South Africa (Meyling et al., 1962). A good example of the way in which water hardness may affect pesticide impact is provided by observations on the molluscicide, Bayluscide, especially in view of the fact that studies were made at both laboratory and field levels in quite different context in different countries (Meyling et al., 1962). Bayluscide at all exposure periods was found to be more toxic to Rainbow trout and other test fish in soft water than in hard and the greatest increase in toxicity was between medium and hard water which suggested degradation of Bayluscide at high pH level and at higher alkalinities (Marking and Hogan, 1967).

The toxicity of TFM to larval lampreys and to Rainbow trouts is strongly influenced by water hardness and pH. This chemical is most effective in soft acid waters in which minimum lethal concentration can be as low as 0.5 ppm. As pH, conductivity and alkalinity of the water increase the doses requirement of TFM to effect 100% kill of larval lampreys increases. In the hardest and most alkaline waters tested, the minimum lethal concentration for the larvae was 8.0 ppm. Changes in the toxicity of TFM to rainbow trout were comparable and the differential toxic effect of this compound was retained regardless of its level of activity in any given water.

As in case of pH the hardness of the water has no major effect on the toxicity to fish of chlorinated hydrocarbons with the exception of Dipterex (Henderson et al.,1960). Since the hardness of the water definitely affects the water quality and consequently impact of the toxic compounds on the fish mortality, during the present investigation, toxicological experiments were performed at consistantly uniform conditions of the water quality maintaining the hardness of water in the range 40 to 55 ppm. Hence it can be conclusively proved that the fish mortality was due to the ethanol extract of fruits of S.laurifolius and was not due to the change in the hardness of the water. The ethanol extract of fruits of S.laurifolius did not change the hardness of water at measurable level.

4) DO (Dissolved Oxygen) :

This is one of the most important factors limiting productivity in fresh waters (Srivastava, 1985) under a given set of conditions. 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. DO of the water used for the present study ranged between 4.6 - 7.0 ppm during the experimental procedures involving the ethanol extract of the fruits S.laurifolius and there was no significant change during the treatment in DO, indicating that the ethanol extract of the fruits of S.laurifolius 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 wherein 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.

## D. Discussion on Physiological Responses

### 1) $LC_{50}$ 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 a '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 weords, 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 simplest statistical numerical 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, 1971).

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 350 ppm for ethanol extract and hence it was thought that the ethanol extract of fruits of S.laurifolius is most suitable for eradication of undesirable fishes. The lethal threshold concentration (LTC) for T.mossambica was found to be 200 ppm for the ethanol extract (Table No.9).

At lower concentrations (200, 225 and 250 ppm) of the ethanol extract intoxication the fish survived upto a period of 60 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). OR this might be due to the physiological resistance to particular type of active principles of the fruit extract. Such type of physiological resistance was shown by mosquito fish (Gambusia affinis) towards chemical such as DDT, BHC and Dieldrin (Boyd and Fergusson, 1964) and by chichilid fish (T.mossambica) toward leaf phytotoxin of Lasiosiphon eriocephalus (Harold,1987).

The unescapable conclusion from the above discussion is that the ethanol extract of the fruits of S.laurifolius contains potent piscicide which can be used as a selective eradicant of undesirable fishes. The 24 h $LC_{50}$ value is 200 ppm, and further concentrations decreased the duration of the $LC_{50}$ values. Thus at 12 h, 9 h, 6 h and 3 h, the $LC_{50}$ values are 225 ppm, 250 ppm, 275 ppm and 350 ppm respectively (Table No.9).

## 2) Behavioural Responses

The behavioural responses of T.mossambica to the petroleum ether extract, benzene extract, ethyl acetate extract and chloroform extract were not distinct. But the responses to the ethanol extract of the fruits of S.laurifolius were prominent and easily could be noticed. The behavioural responses were concentration dependent. At lower concentrations (200, 225 and 250 ppm) the fish did not respond much but at higher concentrations (275, 300 and 400 ppm)

the behavioural responses were noticeable. Such concentration dependent behavioural responses were observed in phenol intoxication in the carp, Cyprinus carpio (Lukyanov et al., 1984) and in Channa orientalis (Nanaware and Mane, 1987). These responses were shown to be linked to the disturbances in the cholinergic system of the brain. In the present investigation such relationship has not been studied but the studies on 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 observations of the present study 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, change in colour, swelling of eye balls, keeping mouth open, turning upside down and finally collapsing to the bottom of the jar were almost similar to those observed by Ramanujam and Ratha (1980a) in Heteropheustes fossilis and Danio danglia and by Nanaware and Harold (1987) in Tilapia mossambica.

Thus, at present it seemed that (1) The fish T.mossambica showed behavioural changes during the treatment of ethanol extract of fruits of S.laurifolius. (2) The petroleum ether, benzene, ethyl acetate and chloroform extracts were without any noticeable behavioural 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 other plant toxins.

### 3) Histopathology and mucosubstance secretion

#### i) Oral Cavity

A) Histopathological alterations:

The oral cavity is the first organ exposed to the toxin, if the toxin is mixed in the water. Except very few investigators (Nanaware and Harold, 1987; Harold,1987) 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 plant toxin induced histochemical alterations in the oral cavity of a fish T.mossambica. Probably for the first time the histological observation has been recorded in case of the fish, T.mossambica to show fungiform and filiform papillae in the oral cavity of this fish (Plate No.1, Fig.Nos.4, 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. 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) Mucosubstance alterations :

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 injuriy, 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) Histopathological alterations :

It is well known that the gill histology is greatly affected due to the chemical intoxication (Studnicka et al., 1983; Rojik et al.,1983). 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 all changes in the gill histology. It has been observed that the size and number of mucus secreting cells were 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 of 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 hydro-

-carbons (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) and similar alterations in the T.mossambica in phytotoxin intoxication (Harold, 1987).

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 to gastrointestinal tract and liver and leaves toxin on gills of T.mossambica by Harold (1987), but there is not a single investigation to show histological alterations caused in the gills of the fishes. In the present investigation for the first time, the effects of fruit toxin of S.laurifolius on histopathology of the gills of fresh water fish, T. mossambica have been recorded. 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) Mucosubstance alterations :

The gills of the fish T.mossambica, selected for the present study, elaborate a variety of mucosubstances, including neutral mucosubstances, acidic mucosubstances. (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 wehich both neutral and acidic mucins were present. (iv) The gill rachis contained only strong sulfated acid mucosubstances in them.

There were interesting changes in the mucosubstance elaboration by the gill elements during the treatment of sub lethal concentrations of the phytotoxin. Initially epithelial cells, basement lamina and gill rachis contained very low concentration of mucosubstances in them which increased moderately in the lower doses of plant toxin whereas in higher doses it became maximum in them (Plate No.2, Fig.Nos.3, 6, 8 and 10). But this pattern was not observed in the pillar cells of the secondary filaments of the gills. These cells contained moderate concentrations in the normal conditions which immediately even after a low dose treatment of plant toxin increased to maximum (Plate No.2, Fig.No.5) and in the higher doses their number and concentrations fall down to a minimum (Plate No.2, Fig.10). This might be due to the loss of pillar cells from the filaments (Skidmore and Tovell, 1972 and Harold, 1987), 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 becterial and fungal infections (Vanoosteen, 1957; Jensen, 1976). Such a protective function to the mucins has been attributed to invertebrate mucosubstances (Gottschalk, 1960). It is known that the mucosubstances 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 ( Hughes, 1979; Hughes and Munshi, 1979). The acidic mucins in the gill epithelium might be useful in the transfer of cations across the epithelium (Kirschner, 1978) and hence might be helping in osmoregulation and electrolyte excretion (Ingale, 1981).

In the present investigation the mucosubstances during the toxin treatment get increased and when the fish die, the mucus with blood clots entangled in the gills. The elaboration of such bulk quantities of mucosubstances 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) Histopathological alterations :

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 histopathologic changes 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 -HCH (Hexochlorocyclohexane) (Studnicka et al., 1983). In copper sulfate and zinc chloride intoxication, organelles of the cells disappeared (Rojik et al.,1983) whereas Aroclor 1254 poisoning showed vacuolation of hepatocytes of Salmo gairdheri (Sivarajah et al., 1978). EM study, upon Aroclor 1254 treatment showed enlargement of rough endoplasmic reticulum of hepatocytes (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 investigators 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.

In the present investigation, the first effect of plant toxin was the displacement of hepatocytes (Plate No.3, Figs.2,3,4,6), then aggregation of

cytoplasmic contents of hepatocytes (Plant No.3, Fig.8), swollen hepatocytes with vacuolization with loss of cell boundaries (Plate No.3, Figs.3,4) disruption of sinusoids (Plate No.3, Fig.3,6) and lastly leading to deformation of liver histology (Plate No.3, Fig.3,6). These observations coincide with the observations of Sivarajeh 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 repture causing deformaties in the liver histology. The vacuolization and aggregation are 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 throw some light on the action of plant toxin on hepatocytes, such work have not been included in the present investigation, although some work on enzymes concerned with such toxicological functions is in progress in this laboratory. In the preliminary investigation it has been observed that the carbohydrate digesting enzymes of the liver are much affected due to phytotoxin.

B) Mucosubstance alterations :

PAS reactivity and malt diastase digestion tests proved the presence of glycogen in hepatocytes, whose concentration varied with different concentrations of the plant toxin. In the low doses mucosubstance changes were moderate but during higher doses maximum glycogen was lost. This change might be due to the fact that as the fish accumulates the poison in the liver

and become lethargic, 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) and 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 glycogen might be deplited in the liver during high doses of the toxin treatment.

The acidic mucosubstances in the liver did not show any appreciable change during the phytotoxin treatment to fish. The function of these acidic mucosubstances with present knowledge cannot be ascertained.

### iv) Kidney

A) Histopathological alterations :

The nitrogenous waste products and the toxins (Poisons) after detoxification 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 in low concentrations while these are drastic in 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 (Duble and Shah, 1984) and deve lopment of lesions in the kidney (Chaturvedi and Saxena, 1978).

One observaion 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 (Buck et al., 1976). Exactly similar results were obtained in the toxicological investigation on L.eriocephalus plant toxin during intoxication to fish, T.mossambica. The histological alterations observed in glomeruli size, degeneration of basement membrane and development of intertubular space, histologic alteration in proximal and distal tubules and malphighian bdies, which showed swelling of distal tubular cells and severe necrosis, dilation of Bowman's capsules and shrunken glomeruli.

In the present investigation the dilation of Bowman's capsules were not as observed by Harold (1987). But the formation of ring surrounding the glomeruli and others histopathological alterations in the various kidney elements were similar to those observed in this fish during intoxication due to toxin of leaves of L.eriocephalus.

Such histologic alterations must have been occurring in the kidney as an initial shock by the active principle of the S.laurifolius circulating through the blood. After enetering into the kidney, it accumulates and increase in concentration causing necrotic effects. Secondly the active principle present in the fruits of this plant must be of larger size with high molecular weight which must have caused such histologic ill effects in the kidney.

B) Mucosubstance alterations :

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 is the kidney tubules of adult vertebrates is well known due to the weorks 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 of mucosubstances in the kidney during the phytotoxin treatment to the fish.

With this view in mind the present investigation on mucosubstances in the kidney tissues of T.mossambica in the fruit toxin of S.laurifolius treatment was undertaken. Such study revealed that the glycogen and acidic mucosubstances are in the Bowman's capsular cells which immediately increased maximum even with low doses of toxin treatment (Plate No.4, Fig.2,5,8) and at higher doses steadily diminished (Plate No.4, Fig.6,9) which might be due to shrinkage of glomeruli and dilation of the Bowman's capsule during the treatment.

The proximal tubules contain 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 occurrence 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.

## V) Instestine

### A) Histopathological alterations :-

In the intestinal lumen mucosal folds projecting deep into the lumen of T. mossambica (Plate No.5, figs.1 to 8) are well placed for effective absorption of the product of digestion as indicated by Martin and Blaber (1984). During the plant toxin treatment some cells were destroyed. The goblet cells were enlarged and showing increase in number also. The high concentration of goblet cell secretion in the intestine may serve as lubrication and defication in forming mucum sheaths in casing the fecal ropes (Martin and Blaber, 1984). Nuclear and cellular hypertrophy, cellular disorganization in tissue, rupture of mucosal cell and vacuolization in tissue of the gut have been observed under urea stress (Srivastava and Srivastava, 1979 and 1982). Such changes also have been observed in the present study, but the significance of the increase in the number of

goblet cell is not clear. The nuclear size of the columnar cells increases. Whether these changes reflect any increase in the water absorptive function of the intestine during intoxication is not established.

B) Mucosubstance alterations :-

Yamada (1975) showed the intestinal goblet cells exhibit neuraminic acid containing mucopoly-saccharides like the goblet cells of epidermis in the cell Anguilla japonica. Whereas Florey (1962) stated that the mucosubstances secreted by the intestinal goblet cell are sulfated acid mucopoly saccharides in many teleostean species.

In the present study the nature of acidic mucosubstances have been confirmed and it was found that the goblet cells have capacity to elaborate sulfomucin whereas some have capacity to elaborate carboxyle group containing acid mucosubstances. Their function in the intestine seems to be the protection against the plant toxin.

But according to Reifel and Travill (1979) although small amounts of sulfomucins were in the rectum of E. americanus but no sulfomucins could be demonstrated in the proximal intestine of Perca flavescens.

According to Kaushik and Kapoor (1978), in Cirrhina mrigala, the columnar mucosal cells were devoid of polysaccharides. In contrast to this observation, the columnar epithelial cells of Tilapia mossambica showed the presence of simultaneous occurance of acidic and neutral mucosubstances in them.

Although the importance of the mucus in the intestine is now well recognized, the specificity of the role of various mucopolysaccharides is still uncertain. The lbycogen in the muscular coat provides a substrate for energy reaction in the cells.