CHAPTER -V

General Summary and Concluding Remarks

V GENERAL SUMMARY AND CONCLUDING REMARKS

1) General Summary

The present investigation was undertaken with a view to get a complete phytochemical analysis of the fruits of Sanidus laurifolius (Vahl) an indigenous plant, from the Western Ghat region of Maharashtra to search for an active principle of the potential piscicide to control an unwanted common species of fish, Tilapia mossambica in the fresh-water bodies which create a number of problems in the pisciculture and its effects on histopathology and histochemistry of the various target organs of this fish. The reasons that lead us to take up the present investigation have been elaborately described in the introductory chapter, which also gives a detailed review of the previous work on the phytochemical analysis, histological, histochemical and biochemical localization and alterations in the nutrients in the various organs of fresh water fishes.

An undesirable fish species used for the present investigation is T.mossambica (Peters) which is readily available and easy to handle. It is undesirable because it competes with all the food fishes in pond. It also affects the survival and growth of other food fishes like Rohu and carp when cultivated together. Thus affecting total fish production. Therefore it is advised not to introduce this fish into commercial fish farms and to control of this species is a problem in pisciculture. The various chemicals are used in control of undesirable fish species but they upset the balance of nature or these are misused, have severe and prolonged residual effects, gets accumulated in biota, and are more costly also. Therefore indigenous plants of piscicidal nature have received

considerable attention of researchers. The plant Sapindus laurifolius (Vahl) selected for the present investigation is used by the natives as substitute for soap. In the preliminary search it was found that it contained saponins and kills fishes.. Hence it was hoped that such a study will throw light on the nature of the active principle of this plant affecting the vital organs like intestine, liver, kidney, gills and oral cavity of the fish. Thus this plant satisfies all the requirement for an ideal study of the type mentioned above. The first and foremost effect of toxin is secretion of large amount of mucous in most of the organs. The mucins play very important roles in the every physiological process in the vertebrate, therefore this chemical moeity was selected for investigation.

The second chapter of the thesis gives the detailed morphology and classification of both the plant and the fish selected for the present investigation. It also elaborately gives the four types of methods which have been employed for this study such as - 1) solvent extraction and TLC separation methods, 2) phytochemical analytical methods, 3) water quality analysing methods, and 4) physiological response analysing methods. In the last type the procedures selected for mucosubstance detection study are well-known and widely accepted.

The third chapter provides the information on the phytochemical analysis and the percentage composition of the fruits of S.laurifolius. The fruits have been subjected for solvent extraction procedures using five solvents and extracts' melting points have been recorded. The spectral analysis has been made on UV, NMR and IR, which revealed that the active principle present in the fruits of S.laurifolius might be either saponin, glycosides or flavonoids, but for confirmation further purification of extracts is necessary.

Studies on Atomic Absorption Spectrophotometer showed the presence of Ca, Fe, Zn, Cu and Mg in the various fruit extracts of S.laurifolius, which form chelation with the compounds in the extracts hence no clear and marked IR, NMR, UV spectra were recorded.

The applications of the ethanol extract of the fruits of S.laurifolius in the experiment did not alter the pH, DO and hardness of the water indicating that the active principle is responsible for the lethal effect that observed in the test fish. Thus, such studies are indicative of potential piscicidal compound in the fruits of S.laurifolius.

In the present investigation, the fish mortality of T. mossambica at different concentrations of the ethanol extract at different time intervals were observed, and found that the lethal threshold concentration (LTC) for this fish was 200 ppm. The LC_{50} values for different hrs. have been worked out.

During the treatment of ethanol extract of the fruits of S. laurifolius to the T. mossambica behavioural changes were observed. These ethanol extract induced behavioural changes were dose and time dependent. Such changes are similar to the earlier investigations which observed in piscicidal chemicals and plant toxins, showing high excitability, muscular spasms, sluggishness, increased opercular movements, no response to external stimuli, loss of equilibrium and upside down posture.

Following is a brief resume of the effects of S. laurifolius toxin on histopathology and histochemistry of mucosubstances of oral cavity, gills, liver, intestine and kidney of T. mossambica.

i) Oral Cavity

The normal histology of the oral cavity changed due to the phytotoxin of S. laurifolius which showed changes in the number of small and large cells in the epithelium and papillae. The staining reactivities had also changed, showing increased intensities. The connective tissue was thickened and showed vacuolization. The histological alterations were dose dependent which cannot ascertain any functional significance But the increase in the mucin secreting cells seems to be related with the protective function against the phytotoxin.

The large cells elaborated glycogen and acidic mucins, the small cells and muscular layer showed only glycogen in them, the connective tissue contained the neutral mucins alone. During the toxin treatment the large cell mucosubstances increased with the higher doses, glycogen elaborating small cell also showed synthesis of neutral mucosubstances in them. Such transformations occurred during the toxin treatment. Although increased intensity was observed in the mucosubstances elaborated by the connective and muscular tissues, but no prominent changes were occurred in these tissues during the toxin treatment.

The role of mucosubstances in the protection of oral mucosa against chemical injury is expected and discussed in the light of existing literature.

2) Gills

S. laurifolius toxin changed the histological structure of the gills completely. The changes were reflected in the staining intensities and in the number of the different cell types and architecture of the gill filaments. At

lower concentration of the toxin there was increase in interlamellar space, reduction in primary gill lamellae, displacement of epithelium from the basement membrane, initiation of the histolysis and increase in number of mucous secreting cells. The secondary gill lamellae were unevenly curled, staining reactivities of pillar cells and acidophilic cells were enhanced. During higher treatment the degenerative changes instituted and gill lamellae were ruptured, the reduction , shortening and bulging at the tips of secondary gill lamellae were prominent.

It seemed that these plant toxin induced changes were due to the mechanical injury and also because of the heavy secretion of mucous adversely affecting the process of respiration. In higher doses many cells were lost and finally fish dies due to these changes.

Some epithelial cells contained only neutral mucosubstances on the other hand, others contained acidic mucins in them. One type of the mucous cells elaborated mixed including neutral mucins as well as acidic mucins whereas other type elaborated only acidic mucosubstances including both sulfated and carboxyl mucins. The basement lamina included acid moiety containing mucins along with the neutral mucins. The gill rachis secreted strongly sulfated mucins.

Mucosubstance elaboration by the gills was greatly influenced by plant toxin treatment. The epithelial and pillar cell elaborated mucosubstances which showed alterations during the lower and higher toxin treatments, were just contradictory to each other. The epithelial cells started increasing in low doses and reached maximum concentrations in the higher doses whereas the pillar

cells showed maximum mucosubstances even at lower dose treatment which with the dose concentration decreased and reached minimum at the highest dose.

The elaboration of mucosubstances by the basement lamina and by the gill rachis showed identical alterations which coincided with the pattern of epithelial mucosubstance elaboration during the toxin treatment.

These effects were similar to the metal and pesticide toxicity and mainly affected the process of respiration, causing ultimately death to the fish.

3) Liver

Liver histology of T. mossambica is like other teleost fishes. Histological alterations in the lower and higher doses of plant toxin were identical. The changes mostly showed the aggregation of cytoplasmic granules forming patches of material in hepatocytes, showed vacuolization and loss of cell membranes, disruption of sinusoids, enlargement of cell nuclei, showing picnosis, binucleated forms, and few mitotic divisions. Ultimately the cordal arrangement showed deformation of liver histology with large gaps.

Glycogen and few carboxyl group containing acidic mucosubstances were mainly elaborated by the hepatocytes and Islet cells of pancreatic tissue. The plant toxin altered the mucosubstance staining reactivities of these hepatocytes. During phytoxin treatment the glycogen in these cells was considerably depleted and some remaining glycogen containing granules were aggregated in the form of patches in this tissue.

The function of these mucosubstances seemed to play roles in cell-nutrition. Therefore, the glycogen and its storage was depleted during the toxic treatment because the food intake of fish was greatly reduced during phytotoxin intoxication.

4) Kidney

Normal histology of kidney of T. mossambica revealed that it consisted of Malpighian bodies with Bowman's capsule and glomeruli, proximal and distal convoluted tubules, and collecting tubules. The proximal tubule cells were columnar which showed brush border towards luminal side, distal tubules lined by only columnar cells without brush border. There were some patches of haemopoeitic tissue in the Tilapia kidney.

Many histopathological changes were observed in the kidney in the plant toxin treatment. These changes were dose dependent and showed enlargement of the different parts of the nephrons. Even at lower concentrations kidney capsular size increased considerably and glomeruli showed shrinkage, capillaries were damaged and at higher doses the glomeruli were diffused, in some of the cells, nuclear material was lost and edema of tubules was prominent. In the distal tubules cells were swollen and lastly necrotic changes were instituted. The collecting tubular size was also enlarged.

Both glomeruli and Bowman's capsule contained glycogen and acidic mucosubstances in them. The proximal tubules showed faint staining reactivities only the brush border showed the presence of neutral or acidic mucosubstances, whereas the distal tubules showed glycogen and weakly sulfated acid mucosubstances in them.

Toxin treatment showed increased and intensifying staining especially in the glomeruli and Bowman's capsules. At lower concentrations maximum elaboration was observed in these sites which diminished with increasing doses. As against the malphighian bodies, the proximal and distal tubule mucosubstances steadily increased with higher doses. The mucosubstance alteration pattern of these tubules was very much identical in the toxin treatment. The occurrence and alterations of the acidic and neutral mucosubstances seemed to be involved in the osmoregulatory function of the kidney.

5) Intestine

Histological picture of Tilapia mossambica is similar to the other teleostian fishes showing usual four layered i.e. serosa, muscle coat, sub-mucosa and mucosa. There are only two cell types in the mucosal epithelium - the columnar and goblet cells. During the phytotoxin treatment the mucosal layer showed severe damage and different types of degenerative changes leading to complete destruction of the columnar cells and enlargement of the goblet cells.

Histochemical architecture of the intestine revealed that the serosa contained neutral mucins, muscle coat contained glycogen, submucosa elaborated neutral plus sulfomucins and goblet cell contained only acidic mucosubstances including both the types i.e. sialomucins and hyaluronic aicd. During phytotoxin treatment t he intestines and number of mucosubstance secreting cells were increased showing the large amount of mucin production. The possible significance of the histopathological and mucosubstance elaboration alterations in the various parts of the intestine due to the phytotoxin treatment have been elaborately discussed.

The fourth chapter deals with the interpretations of the results of the present investigations with the existing literature. The various functional roles have been attributed to the various mucosubstances in the different organs in their physiological functions. The fifth chapter provides the general summary and the concluding remarks on this dissertation.

2) Concluding Remarks

The present investigation helps in understanding the chemical composition of the fruits of an indigenous piscicidal plant, Sapindus laurifolius (Vahl). Although, in India, there are 150 plants of piscicidal property and none of these especially from the Western Ghat region have been studied from their phytochemical analysis point of view. Since it was not possible to investigate more plants for their chemical analysis in an M.Phil. dissertation such as the present one, only one plant had selected for the study. The results of present investigation along with their interpretations promise that such studies may be of intense help in understanding the nature of the active principle of the piscicidal plant species in a far better manner. Isolation of the selective active principle shall provide the answer for the eradication of a particular variety of undesirable fish in the pisciculture.

The present investigation provides a guideline for the future phytochemical, biochemical and histochemical work on the different plants of piscicidal potentiality and on the action of phytotoxin on the vital organs of the other undesirable fish species and also for the development of new natural piscicides.

In the present investigation, five organs (oral cavity, gills, liver, kidney and intestine) have been selected. But other organ systems like circulatory system, neuromuscular system including brain and skeletal muscles and excretary system which are also affected by the toxin and their studies would give the plausible mechanism of action of the toxin on the fish.

There are various biological and chemical factors influencing the toxin action in the animals. There exists many marked differences among species metabolism. Different fish species show variations in toxin tolerance, toxin metabolizing enzymes, toxin binding plasma proteins, localization of toxins in body tissues etc. The studies imparting these factors would give more information on the influence of the toxin on the animals under test.

Action of plant toxin on haemolysis and on cholinestarase activity in the brain have not been touched in the present investigation. Such studies are usually performed in order to understand the mechanism of toxin action. Such studies would provide still better picture about the mechanism of the fruit toxin of S. laurifolius on the fish, T. mossambica. Some preliminary work in this direction is in progress in this laboratory.

The findings of the present studies provide good natural piscicide of plant origin with no residual effect and other side effects on fish life in the control of specific variety of undesirable fish in the commercial fisheries management. This laboratory has recently undertaken a research program to study this aspect on the introduction of indigenous plant toxins to eradicate undesirable fish varieties which creat number of problems in the pisciculture.

The work on some of the aforementioned problems is in progress in this laboratory, the results of which will be published elsewhere in due course of time.