CHAPTER 5

GENERAL SUMMARY AND CONCLUDING REMARKS

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1) General Summary

The present investigation was undertaken with a view to get a complete phytochemical analysis of an indigenous plant, Lasiosiphon eriocephalus Decaisne 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. To determine the damage caused by the L.eriocephalus toxin to the histology and histochemistry of muco-substances in normal and intoxicated fishes were studied in the vital organs including gills, liver and oral cavity which belong to different organ systems which are directly concerned and usually affected during the toxin treatment 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 phytochemical, histological, histochemical and biochemical work on the various organs with an emphasis of the work done on the fresh-water fishes.

The fish species used for present investigation is T.mossambica (Peters) which is readily available and easy to handle and large amount of physiologicaldata is available on every organ system and it is undesirable fish due to its high prolificity at an early age and small size. The pond fills with

wild "spawned fry", all competing for food 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 and when they 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. As it is brought to the notice, the paucity of literature on plant toxins. The plant Lasiosiphon eriocephalus Decaisne selected for the present investigation is used by the natives for killing the fish. It was hoped that such a study will throw light on the nature of the active principle of this plant affecting the vital organs, 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.

Since the mucosubstances play an important role in the every physio logical process in the vertebrate body and first and foremost effect of toxin is secretion of large amount of mucous in most of the organs, this chemical moeity was selected for investigation. The mucosubstances have been studied by employing the well-known and accepted histochemical techniques. The procedures for mucosubstance detection and other methods used for chemical analysis of the L.eriocephalus leaves for the detection of active principle have been described along with their interpretations in chapter second. The chapter also provides the information on the classification and pharmacological peculiarities of the plant, classification and physiological peculiarities of the

fish selected for the study, dose schedule and duration of experiment. Appropriate dosages for subchronic (low) and chronic (high) were selected and sufficient number of fishes of the same size were maintained throughout the experimental procedure. Histological and histochemical determinations for the mucosubstances and their alterations in the toxin treatment were made at intervals in the selected organs.

In the third Chapter, the biochemical analysis of L.eriocephalus along with percentage composition of each of the three solvent (benzene, chloroform and ethanol) extracts whose percentages were 0.84, 1.48 and 3.35 and melting points 80°C, 100°C and 118-120°C respectively. The spectral analysis made on UV, NMR and IR revealed that the active principle present in the L.eriocephalus leaves might be either saponin glycosides or flavonoids, but for confirmation further purification of extracts is necessary.

Studies on Atomic Absorption Spectrophotometer showed presence of Ca, Fe,Zn, Cu and Co in their decreasing order of concentrations in the Benzene, chloroform and ethanol extracts of L.eriocephalus leaves which form chelation with the compounds in the extracts hence no clear and marked IR, NMR, UV spectra were recorded. Therefore, after separation of these metals such spectral analysis would provide better results.

The application of either crude powder or extract of the L.eriocephalus in the experiment did not alter the pH, DO and hardness of the normal 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 L.eriocephalus leaves.

In the present investigation, the fish mortality of T.mossambica at different concentrations of the crude powder and of ethanol extract at different time intervals were observed, and found that the lethal threshold concentration (LTC) for this fish was 80 ppm and 4 ppm respectively. From the LC_{50} values of ethanol extract and the crude powder it seemed that the former one is more effective than the latter. This might be due to the presence of active principle of piscicidal in nature, which is extracted in pure form in the ethanol, making ethanol extract suitable for fish eradication.

During the treatment of ethanol extract of L.eriocephalus to the T.mossambica behavioural changes were observed but benzene extract and chloroform extract were without any visible behavioural changes. 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 L.eriocephalus toxin on histology and histochemistry of mucosubstances of oral cavity, gills, liver and kidney of T.mossambica.

i) Oral Cavity

The histology of the oral cavity revealed that it is lined by thin stratified squamous epithelium consisting of small and large cells supported by connective and muscular tissues. The mucus membrane has several fungi-

form and filiform papilla whose structure more or less resembled to those found in mammalian tongue. The L.eriocephalus toxin treatment showed changes in the number of small and large cells 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 except providing protection against toxin by increasing mucin secreting cell number.

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

The histology of gills of T.mossambica consisted of gill filaments which look like a lady's fan. Internally at their bases these were supported by bony elements. The outfoldings on their lateral sides called as the gill

lamellae which are functional parts of the gills. The gill lamellae consisted of a single layer of squamous epithelial cells supported by a basement lamina. The epithelial cells occasionally consisted of goblet cells and large acidophilic cells. Acidophilic cells occurred most densely between the bases of adjacent lamellae and formed clusters closest to the afferentbranchial artery. The histological structure resembled with any teleost fish.

L.eriocephalus 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 and acidophil 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 and shortening in 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 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 patterns of mucosubstances of basement lamina and of the gill rachis were identical and coincided with the pattern of epithelial pattern of 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 like other fishes showed hepatocytes containing granules of various size with centrally placed nucleus. There were number of blood sinusoids scattered in the hepatic tissue. Besides hepatocytes there were few Islet cells with different staining reactivities of pancreatic tissue.

Histological alterations in the lower and higher doses of plant toxin were identical. These 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 carboxy 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 indicating increased production of mucins during the toxin treatment. But during high doses glycogen in these cells was considerably depleted and some 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 because during toxic treatment food intake of fish was greatly reduced as observed in the present investigation.

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 histological 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 wee 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. The glomerular size was increased ten times at their maximum activity. 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.

The fourth chapter deals with the interpretations of the results of the present investigations with the existing literature concerned with this aspect. The various functions to the mucosubstance in the various physiological functions have also been attributed.

2) Concluding Remarks

The present investigation was aided at to get the phytochemical composition of one indigenous piscicidal plant, Lasiosiphon eriocephalus Decaisne only. Although, in India, there are 150 plants of piscicidal property and none of these from the Western Ghat region have been studied from their phytochemical analysis point of view. Since it was not possible to investigate more plants from their chemical analysis point of view in a M.Phil. dissertation such as the present one. Such more investigations would bring about the toxin which is of selectively toxic to a specific variety of fish. The present observations 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 than the earlier studies. Since pure and extracted toxin may give better action on the fish toxicity. Isolation of the spective active principle shall provide the answer for dose which selectively kill a particular variety of undesirable fish.

The present investigation provide a guideline for future phytochemical, biochemical and histochemical work on the different plants of piscicidal properties, and vital organ systems of the other undesirable fish species and also for the development of new synthetic piscicides.

In the present investigation we have included only four organs (oral cavity, gills, liver and kidney). Biochemical studies on other organ systems like circulatory system including heart, neuromuscular system including brain and skeletal muscles and excretary system would give the plausible mechanism of action of the toxin on the fish.

There are various biological and chemical factors affecting the toxins in the animals. There exists many marked differences among species metabolism within species, certain breeds or strains vary in tolerance, toxin metabolizing enzymes, enzymes and their interactions with hormones at various ages may also vary differential toxicity within the sexes, hepatic disease, the nutritional status, decreased body temperature, route of toxin administration, time of administration, interaction or competition with endogenous substances protein binding in plasma, localization of toxins in body tissues, enzyme inhibition, enzyme induction, etc. influence toxins in the animals. Such studies imparting these factors would give proper justification on the influence of the toxin on the animals under test.

Haemolysis and enzymatic studies on cholinestarase have not been touched in the present investigation since these studies are usually concerned with the plant toxin treatment but such studies would provide still better picture about the mechanism of L.eriocephalus toxin on the fish T.mossambica. Some preliminary work in this direction is in progress in this laboratory.

The findings of the present studies may form a good basis for the introduction of piscicides of plant origin with no residual effect and other side effects on fish life to control specific undesirable variety of 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 creating 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.