CHAPTER -V

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1) GENERAL SUMMARY :

'Control of undesirable fishes with minimum side effects', was the basic aim of the present investigation. Besides the present investigation was aimed at getting complete phytochemical analysis of the fruits of A. concinna (DC), 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, T. mossambica in the fresh water bodies which create a number of problems in the pisciculture. 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 due to piscicide stress.

T. mossambica (Peters), an undesirable fish species used for the present investigation is readily available and easy to handle. It is undesirable because it compets with all the food fishes in the ponds. It also affects survival and growth of other food fishes like rohu and carp when cultivated together. Thus, it affects 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 expensive also. Therefore, indigenous plants of piscicidal nature have received considerable attention of researchers. The plant A. concinna (DC), selected for the present investigation is used by the natives as a substitute for shampoo and to wash silk clothes. In the preliminary search it was observed that it contained saponins which kill fishes. These earlier studies throw light on the nature of the active principle of this plant which affect the vital organs like buccal mass, gills, liver, kidney and intestine 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 any toxin whether synthetic or of natural origin, is secretion of large amount of mucus in most of the organs. It is well established that the mucins play significant role in the every physiological process in the vertebrates. Therefore, this chemical moeity was selected for the present investigation.

'Material and Methods', the second chapter of the thesis gives the detailed morphology and classification of both the plant and fish, selected for the present investigation. It also elaborately gives the four types of methods which have been employed for this study such as - i) Solvent extraction methods ii) Phytochemical analytical methods, iii) Water quality analysing methods and iv) Physiological response analysing methods. In the last type the procedures selected for the mucosubstance detection are well known and widely accepted.

The third chapter on 'Observations' provides the information on the phytochemical analysis and percentage composition of the fruits of A. concinna and melting points of five solvent extracts (E_1-E_5). It also gives information on UV, NMR and IR spectra of these extracts. From the spectral analysis it revealed that the active principle present in the fruits of A. concinna might be either saponin, glycoside or flavonids, but the confirmation further purification of extracts is necessary.

It was also found that the application of the ethanol extract (E_5) of the fruits of A. concinna in the experiment did not alter the pH, hardness and DO 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 the presence of potential piscicidal component in the fruits of A. concinna.

The mortality of T. mossambica at different concentrations of the ethanol extract (E_5) at different time intervals were observed in the present investigation. The lethal threshold concentration (LTC) for the fish was 200 ppm. The LC_{50} values for different hours have been worked out. The LC_{50} values for T. mossambica at 6, 12, 24, 48, 72 and 96 hours were observed which were 297.88, 245.206, 241, 210.481, 148.115 and 140.00 ppm respectively.

Behavioural changes of the fish T. mossambica, were observed during the treatment of ethanol extract (E_5) of the fruits of A. cocinna. E_5 induced changes were dose and time dependent. Such changes are similar to the earlier investigations wehich were observed in piscicidal chemicals and plant toxins, showing high excitability, muscular spasms, slugishness, increased

opercular movements, no response to external stimuli, loss of equilibrium and upside down posture.

A brief resume of the effects of A. concinna fruit toxin on histopathology and histochemistry of mucosubstances of buccal mass, gills, liver, kidney and intestine of T. mossambica is given below :

i) Buccal mass .

Due to the effect of phytotoxin of fruits of A. concinna, the normal histology of the oral cavity gets changed. These changes are observed in the goblet cells of 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 be ascertained any functional significance except providing protection against toxin by increasing mucin number of secreting cells.

The second type goblet 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 second type goblet cells mucosubstances increased with the higher doses, glycogen elaborating first type goblet cells 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 ocurred 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.

ii) Gills.

The histological structure of the gills of the fish T. mossambica gets changed during the treatment of fruit toxin of A. cocinna. 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 spaces, reduction in primary gill lamellae, displacement of epithelium from the basement membrane, initiation of 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 lamellae were ruptured, the reduction, shortening and bulging at the tips of secondary gill lamellae were prominent.

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

On the margins of secondary lamellae epithelial cells are present, some epithelial cells contained only 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 sulphated and carboxyl mucins. The basement lamina included acid moiety containing mucins along with the neutral mucins. The gill rachis secreted strongly sulphated mucins.

Elaboration of mucosubstance by gills was greatly influenced by plant toxin treatment. Mucosubstance elaborated by pillar cells and epithelial cells

showed alterations during the lower and higher toxin treatments, were just contradictory to each other. The epithelial cells secreted increased amount of mucins in low doses and reached maximum concentrations in the higher doses whereas the pillar cells showed maximum mucosubstances even at lower dose treatment.

Mucosubstances elbarated by the basement lamina and gill rachins showed identical alterations coincided with the pattern of epithelial mucosubstance elaboration during the toxin treatment.

Such effects shown by the plant toxin from fruits of A. concinna were similar to the metal and pesticide toxicity. The process of respiration is affected by this toxin and causes ultimately death of the fish, T. mossambica.

iii) Liver.

During the treatment of phytotoxin from fruits of A. concinna, histological alterations are observed in the liver, thus alterations were identical during the treatment of lower and higher doses of plant toxin. These changes mostly showed the aggregation of cytoplasmic granules forming patches of material in hepatocytes, showed vacuolization and loss of cell membrane, disruption of sinusoids, enlargement of cell nuclei showing pycnosis, binucleated forms and few mitotic division. 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 hepatocyes and islet cells of pancreatic tissue The plant toxin altered the mucosubstance staining reactivities of these

hepatocytes. During phytotoxin 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.

Mucosubstances play functional role in the cell nutrition. Therefore, glycogen and its storage was depleted during the toxin treatment because the food intake of fish was greatly reduced during phytotoxin intoxication.

iv) Kidney.

Histologically malphigian bodies with Bowman's capsule and glomeruli, proximal and distal convoluted tubules and collecting tubules are present in the kidney of fish T. mossambica. The proximal tubule cells were columnar which showed brush border towards luminal side, distal tubules are lined by only columnar cells without brush border. These were some patches of haemopoeitic tissue in the Tilapia kidney.

During the plant toxin treatment many histopathological changes were observed in the kidney. These changes were dose dependent and showed enlargement of different parts of the nephrons. Even at lower concentrations kidney capsular size was 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 distal tubules cells were swollen and lastly necrotic changes were instituted. The collecting tubule size was also enlarged.

Glycogen and acidic mucosubstances were observed in both glomeruli and Bowman's capsule. The proximal tubules showed faint staining reactivities only the brush border showed the presence of neutral and acidic muco-

sdubstances, 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 Malphigian 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.

v) Intestine.

Intestine of T. mossambica showed similar histological structure of intestine to that of other teleosts. It has usual four layers, serosa, muscle coat, submucosa and mucosa. There are two types of cells, columnar and epithelial cells observed in the mucosal layer. During the phytotoxin treatment the mucosal layer showed degenerative changes leading to destruction of the columnar cells and enlargement of the goblet cells.

Some histochemical changes were also observed in the intestine during toxin treatment. Histochemical architecture of the intestine revealed that the serosa contained neutral mucins, muscle coat contained glycogen, submucosa elaborated neutral and acidic sulphomucins and goblet cells contained only acidic mucosubstances. Including both sialomucins and hyaluronic acid. During phytotoxin treatment intensities of staining reactions and number of mucosubstance secreting cells were increased showing the large amount of mucin production. The possible significance of the histopathological alterations

and increased mucosubstance elaboration in the various parts of the intestine due to the phytotoxin treatment have been elaborately discussed.

The fourth chapter deals with interpretation of the results of the present investigation with the existing literature. The various functional roles have been attributed to the various mucosubstances in the different organs in their physiological functions.

2) CONCLUDING REMARKS :

Although in India there are about 150 plants of piscicidal property and only some of these especially from the Western Ghat region have been studied from their phytochemical analysis point of view. Some plants studied are L. eriocephalus (Harold, 1987) and S. laurifolius (Bhosale, 1988). Since it was not possible to investigate more plants for their chemical analysis in M.Phil. dissertation, only one plant had selected for the present study.

The present investigation helps in understanding the chemical composition of fruits of an indigenous plant A. concinna and observe their effect on vitality of fish T. mossambica to study the behavioural changes and the LC_{50} value. It also deals with the histopathological and histochemical changes in the target organs like buccal mass, gills, liver, kidney and intestine during toxin treatment. The results of the 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 undesirable fish in the pisciculture. This

also provides a guide line for the future phytochemical, biochemical and histochemical work on the diferent plants of piscicidal potentiality and on the action of on the different organs of the other undesirable fish species and also for the development of new natural piscicides.

Only five organs (oral cavity, gills, liver, kidney and intestine) hve been selected for the present investigation But other organ systems like circulatory system, neuromuscular system including brain, and skeletal muscles are also affected by the toxin and their study would give the possible mecha nism of action of the toxin on the fish.

Toxin action in the animals gets influenced by various biological and chemical factors. There exists many marked differences among metabolism in different species. 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 the test. Action of phytotoxin on choline esterase activity in the brain have not been touched in the present investigation. Such studies performed in order to understand the mechanism of toxin action. This type of study will provide the better idea about the mechanism of the fruit toxin of A. concinna on the fish T. mossambica. Such preliminary work is in progress in this laboratory having above line of investigation.

The present investigation provides a better natural piscicide of plant origin. This natural piscicide from fruits of A. concinna has no residual effect and other side effects on the biota during its application to control the specific undesribale fish in the pisciculture.

It is a matter of pride to note that this laboratory has recently undertaken a research programme to study this aspect on the introduction of indigenous plant toxins to eradicate undesirable fishes, creating number of problems in the pisciculture. This investigation will be a significant and valuable addition in the fishery management of India.

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