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ORIGINAL ARTICLE

Effects of Pesticide on the Histology of Stomach and Liver of A Water Breathing Teleost, *Mystus tengara*

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ABSTRACT
In the present investigation the histopathological study was observed in the stomach & liver of Mystus tengara, exposed to sub lethal concentration of a hybrid pesticide (recombination of two classes of pesticides),Chloropyrifos 50% + Cypermethrin 5% EC(Organophosphate +Synthetic pyrethroid).The study revealed histopathological changes observed in the stomach damage of mucosal epithelial cell & mucous in the lumen, separation of the gastric gland cell, rupture in sub mucosa etc. Histopathological changes observed in the liver which include irregular hepatocytes cytoplasm vacuolation, nuclear hypertrophy, cytoplasmic nuclear degeneration etc. These observations are thus indicative of the toxic effects caused by this hybrid pesticide at cellular/histological level in the organs of the fish Mystus tengara.
Keywords: *Pesticides, Synthetic Pyrethroid, Organophosphate, Mystus tengara, Hepatocytes, Mucosal epithelial, Stomach, Liver.*

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INTRODUCTION

Pesticides are used on large scale to improve the production of food grains. Pesticides are used to kill and keep away the unwanted and harmful insects & pests. Through pesticides are used for better production of crop but at the same time these pesticides also enter in rivers, lakes and ponds through rain water. It affects the life of living things of water mainly fishes. On mixing with water it also changes the chemical composition of water & makes it harmful for animals that live in water.

Since fishes are fully dependent on water & breathe under water to keep them alive. These chemicals enter into fishes' body and bring changes in internal structure of the organs slowly & slowly.

These fishes are very sensitive & brings the effect of pesticides very fast even after very small quantity. Its mortality rate is very high. These pesticides affect the tissue of fishes including *M. tengra* & due to this many organs pass through the histological & structural changes.

Pesticides have been used in agriculture for decades to enhance food production by eradicating out diseases caused by insects & vectors [1]. These chemicals may reach the environment such as lakes & rivers through rains, winds or mishandling, thus affecting many non-targeted organisms. The significant increase in water resources has led to the deleterious effects on many aquatic organisms [2,3]. Fish are able to up take and retain different xenobiotics dissolved in water via active or passives processes. They can be used to detect and monitor pollutants released into their aquatic environments. Sub-lethal concentrations of pesticides in aquatic environments cause structural & functional changes in the body of affected fish [4]. Chronic exposure of sub-lethal concentration of pesticides has been found to cause moderate to severe histopathological changes in the tissues & organs of fish. Recent trend in the increased use of organophosphates (ops) and synthetic pyrethroids in agricultural practices is a major issue of concern. All the organophosphates are potent nerve inhibitors. They block the active sites of the enzyme acetyl cholinesterase (AChE) that breaks down and hydrolyses the neurotransmitter acetylcholine (Ach) from the nerve synapse. Chlorpyrifos is a synthetic organophosphate (OP), non systemic and broad spectrum insecticide, acting as a cholinesterase inhibiting and may get assess into the body via dermal contact, ingestion and respiratory pathway. Similarly, synthetic pyrethroids are also widely used in agricultural practices. Pyrethroids are several orders of magnitude more toxic to fish than the organophasphate and synthetic pyrethroids on the stomach and liver of the fish, *Mystus tengara* (family Bagridae) under laboratory condition.

MATERIAL AND METHODS

The fresh water fish *M. tengara* was bought from Muzaffarpur city market. These were disinfected by subjecting them to both of 0.1% aqueous potassium permanganate ($KMnO_4$) solution for 15 minutes to remove any dermal infection then fish were now transferred to a large tank containing water and kept for 20 days for acclimatization. During the period of acclimatization they were fed alternatively with pieces of chick's intestine. The average physic-chemical conditions were maintained optimum during this period. The water of the tank was renewed every two days to minimize contamination as well as maintain the average physio-chemical characteristics of the water.

BIOASSAY TEST

An acute toxicity LC_{50} test by the static renewal bioassay of chlorpyrifos 50% + cypermethrin 5% EC in the fresh water fish, *Mystus tengara* exposed to various concentrations of the pesticide till 96 hrs based on the mortality observed at different concentrations during 96 hrs. LC_{50} value was estimated for different periods such as 24 hrs, 48 hrs, 72 hrs and 96 hrs using straight line graphical interpolation method. For exposing the test animal to sub-lethal concentration of the pesticide, $1/10^{th}$ of the 96 hrs LC_{50} value was taken and fish were exposed to this concentration for 30 days. After this period, the fish were sacrificed and their organs were extracted. These organs were fixed in fixatives (formalin and bouin's solution aqueous). The tissues were then dehydrated, cleansed and embedded in wax. Thin sections were cut with microtome and observed under microscope after undergoing standard staining protocol (H&E Staining).

RESULTS

Physico-chemical characteristics of the test water

The result of the physical & chemical analysis of the test water estimated by using procedures as mentioned APHA [5] are given in given table

Table: Average physico - chemical characteristics of test water

1. Dissolved O_2 = 7.42±1.10 ppm
2. Temperature = 26^0±2.0^0C
3. pH = 7.12±0.14
4. Total alkalinity as $CaCO_3$ = 148.64±7.77 ppm
5. Chlorides = 14.42±1.05 ppm
6. Total hardness as $CaCO_3$ = 164.76±5.38 ppm

HISTOPATHOLOGICAL STUDIES OF STOMACH

Histology of normal stomach

Histologically the stomach wall of *Mystus tengra* (Control species) exhibited four general layers they were characteristic of the entire digestive tract-mucosa, sub-mucosa, muscularis and serosa (fig.1). The innermost layer mucosa was lined by simple columnar epithelium, extending into the gastric pits. The gastric glands were lined with a single layer of cells rested on a thin basement membrane. The sub mucosa was made up of loose connective tissue and contained blood vessels & lymph spaces.

Fig. 1. Histological slides of normal or control stomach showing serosa(a), submucosa(b), muscularis(c), mucosa(d).

HISTOLOGY OF THE STOMACH EXPOSED TO THE PESTICIDE

The observed pathological changes of the stomach were: damage of the mucosal epithelial layer which was detached at places from the basal layer (fig.2), separation of the gastric gland cell from the base

basement membrane forming cluster, rupture in sub mucosa and sometimes in muscularis layer, hypermic blood vessel, and presence of scattered blood cells in the submucosa & muscularis layer or all over the entire stomach tissue.

HISTOPATHOLOGICAL STUDIES OF LIVER

Histology of the normal liver

The liver is a solid glandular organ made up of rounded hepatic lobules or acini in the form of branched columns, separated from one another by the connective tissue(fig.3). Each acini or hepatic lobules contains roughly 5-10 hepatic cells which are penetrated by fine network of connective tissue & sinusoid vessels called as hepatic capillaries. Each hepatocytes contains a centrally located spherical nucleus with prominent nucleolus. The cytoplasm is grandular taking deep basophilic stain. Hepatic acini &hepatocytes are very distinct.Each hepatocytes is rounded and contains one or two nuclei. These hepatocytes are radially arranged around a central vein in inter connected lamina.

HISTOLOGY OF THE LIVER EXPOSED TO THE PESTICIDE

The toxicity of pesticides is also assessed by the extent of histological changes induced by them in the liver of fish Mystus tengara. The main alternations found in the liver were altered tissue architecture, irregular shaped placed laterally, nuclear hypertrophy, nuclear vacuolation and presence of megelanomacrophage aggregates(fig.4).Cytoplasm and nuclear degenerations were also observed.The tissues were slightly to moderately damaged as is evident by altered histological structure and cytoplasmic degeneration. The liver showed vacuolated hypatocytes. Bile stagnation was also identified as brown-yellowish granules in the cytoplasm.

Fig.2. Histological slide of treated stomach showing mucosal epithelial layer(MEL), gastric Gland cell(GGC), muscularis layer(ML),blood vessels(BV), blood cells(BC)

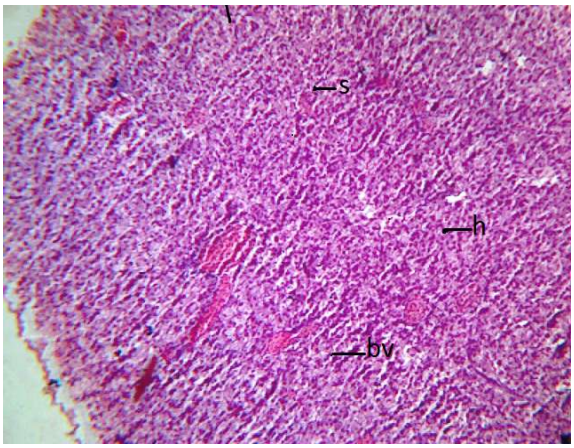

Fig.3.Histological slides of normal liver showing hepatocyte(h), sinusoid vessels(s), blood vessels(bv).

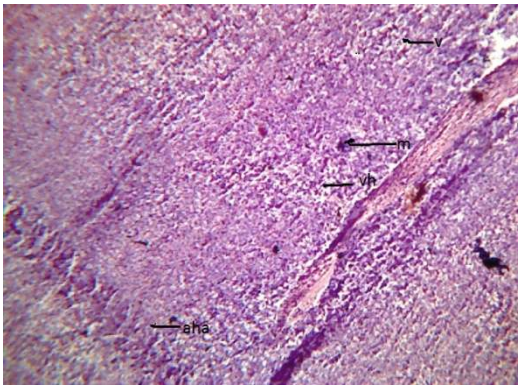

Fig.4. Histological slide of treated liver showing vacuolation(v), melanomacrophage aggregate(m), vacuolated hepatocytes(vh), altered hepatic architecture(aha).

DISCUSSION

The toxicity of pesticides is dependent on the physical & chemical characteristics of water. Therefore, the physical and chemical analysis of the test water becomes essential before performing experiment. In the present study, marked histopathological alternations were observed in the stomach and liver of the fish exposed to pesticide.

Amminiukutty and Rege [6] observed swelling, vecuobation and pyknosis of the mucosal epithelial cells of stomach. The most notable histopathlogical changes observed in the present work was the damage of mucosal epithelial cells and gastric gland cells of the stomach. Such damage was recognizable from the hypertrophied cells which were pyknotic with clear cytoplast in exposed fish. The situation was more severe with pesticides exposed fish where the epithelial cells were highly damaged & even some were completely ruptured. The gastric gland cells were damaged to form a cluster of cells in the middle of gland. Separating off of the epithelial cells or mucous in the stomach lumen were also observed. The hypertrophy of the epithelial cells of the mucosal membrane and digestive gland is known to be a protective mechanism that an animal usually shows in response to chemical or toxic stresses. However, under severe toxic conditions chypertophie cells undergo rupture & damage as found in the present findings when exposed to the pesticides.

Vacuolation of submucosa and muscularis layer has been reported from different test cases [7,8]. In the present investigation, conspicuous spaces and raptures n the lamina propria, submucosa and muscular is layer of stomach were observed in test fish when exposed in most of the pesticides. These layers were haemorrhagic as revealed by the presence of excess blood cells.

Besides, the stomach tissue showed clumping of blood cell in the lamina propria just beneath the mucosal epithelium in the fish exposed to pesticides.

Due to its function position and blood supply [9],it is also one of the organ most affected by contaminants in the water.Irregular shaped hepatocytes cytoplasmic vacuolation,nucleus in lateral positions were some of the s alteration observed during the experiment.cytoplasmic and nuclear degeneration were common. Such anomalies (irregular shaped hepatocytes, cytoplasmic vacuolation etc,)were also described in the siluriform corydoras palcatus contaminated with organophosphate pesticides [10].Vacuoles in the cytoplasm of the hepatocytes may contain lipid and glycogen. In present study, evidence for bile stagnation was also seen. Bile stagnation in the form of brownish-yellow granules in the cytoplasm of the hepatocytes [11]indicated that the bile is not being released from the liver.

CONCLUSION

The present investigation have thus revealed the degenerative effects of the pesticide, chlorpyrifos 50% + cypermethrin 5% EC on the stomach and liver of the fish. *Mystus tenara* exposed to the sub-lethal concentration of the pesticide. Histopathological changes in the stomach were damage of the mucosal epithelial cells and mucus in the lumen of the stomach hypertrophied epithelial cells of the mucosa and gastric glands with pyknotic nuclei and clear cytoplasm, separation of the gastric gland cells from the basement membrane forming cluster, rupture in sub mucosa and sometime in muscularis layer, hyperemic blood vessels and presence of scattered blood cell in the sub-mucosa & muscularis layer or all over the entire stomach tissue. . The main alternations found in the liver were altered tissue architecture, irregular shaped placed laterally, nuclear hypertrophy, nuclear vacuolation and presence of megelanomacrophage aggregates. Cytoplasmic and nuclear degenerations were also observed. The tissues were slightly to moderately damaged as is evident by altered histological structure and cytoplasmic degeneration. The liver showed vacuolated hypatocytes.

Our findings are also well in agreement with the finding of may previous researchers. Thus, it can be concluded that stomach and liver of the fish, *Mystus tengara* undergo severe histopathological alternations when exposed to the sub-lethal concentration of the pesticide, chloropyrifos 50% + cypermethrin 5% EC.

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