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Effect of Cartap hydrochloride (50% SP) insecticide on Liver and Kidney histology of the fish, *Cirrhinus mrigala* (Hamilton)

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ABSTRACT

Effect of Cartap hydrochloride on the liver and kidney of the Indian major carp, *Cirrhinus mrigala* was studied. Fingerlings were exposed to sub-lethal (0.0376 mg^{-1}) and lethal (0.376 mg-^{1}) concentrations of Cartap hydrochloride for 24 and 96 hours. The histological changes in the liver of fish *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of cartap hydrochloride were characterized by the formation of the prominent yellowish-brown pigment cytoplasmic vacuoles, pycnotic nuclei, degeneration of hepato-pancreatic tissue, eosinophilic granules, Melano macrophages aggregate, appearance of blood streaks, degeneration of hepatopancreatic tissue, hypertrophic nuclei, cytoplasmic degeneration change in the shape of the hepatocytes, disappearance of hepatic cells, hyperplasia of cells, hypertrophic nuclei and degeneration of cell membrane. The kidney of the treated fish showed the appearance of prominent tubular yellowish brown pigment, formation of vacuoles, swelling of glomerulus, separation of the tubular epithelium, tubular architectural loss, damage of bowmans capsule, tubular cytoplasmic degeneration, cloudy swelling degeneration, damage to renal tubules, damaged haemopoitic tissue, necrosis of cell and renal tubules, disorganization of connective tissue, degeneration of cytoplasm, pyknotic nuclei, hypertrophy of nuclei, disintegration of cell membrane were also seen. The results obtained were discussed at length with the available literature. By studying these histological deformities, it was concluded that the pesticide caused enough damage to the liver and kidney of the fish and can influence on the survival of the fish.

Key words: *Cartap hydrochloride, Cirrhinus mrigala, Liver histopathology, Kidney histopathology*

Introduction

One of the major concerns worldwide is aquatic contamination. Among these contaminants, pesticides are regarded as the most potent in aquatic environment (Ahmed *et al*., 2018; Abd *et al*., 2020 and Shahjahan *et al*., 2021). Fish accumulate these pollutants directly or indirectly from polluted waters and food chain (Jabeen *et al*. 2016). Carbamates are extensively used water-soluble pesticides in agricultural practices. In India, carbamate pesticide, Cartap hydrochloride which is considered as nereistoxin analog is extensively used in sugarcane and rice crops to control pests (Vani *et al*., 2020). Histopathological analysis can act as a very sensitive parameter and is helpful in determining cellular damage that may occur in target organs (Altinok and Capkin, 2007). In this viewpoint an attempt was made to study the effect of sublethal and lethal concentrations of Cartap hydrochloride on the Liver and kidney histopathology of freshwater fish, *Cirrhinus mrigala* exposed for 24 and 96 hours.

Materials and Methods

The fingerlings of the test fish *Cirrhinus mrigala* of size 6-8 ± ½ cm and weight 6-7 ± ½ gm were procured from local fish hatcheries of Nandivelugu, Tenali mandal, Guntur district, Andhra Pradesh. The fish were acclimated at (28±2 ^{o}C) in the laboratory conditions for two weeks. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms were followed (Annon, 1975). Fish were regularly fed with rice bran and one day prior to the experimentation feeding was stopped. Fingerlings were exposed to sub-lethal (1/10th of 96 h LC_{50} value 0.0376 mg^{-1}) and lethal (96 hr LC_{50} value 0.376 mg^{-1}) concentrations of Cartap hydrochloride for 24 and 96 hours. After exposure to each selected test period fish were sacrificed and were used for histopathological studies. The vital organ tissues, viz; liver and kidney were collected from normal and pesticide cartap hydrochloride treated fish.

Liver and Kidney were processed in laboratory for routine histological characterization by the double staining method using Haematoxylene and Eosin. All samples were fixed in 10% phosphate-buffered formalin for about 24 hours. The specimens were dehydrated in a series of graded ethanol (50%, 70% and 90%) and then embedded in paraffin. Five-microns thick sections were cut using an ultramicrotome (Leica, Japan) and deparaffinized by means of xylol. The sections were dehydrated in 90%, 70% and 50% ethanol followed by a 10 min wash in water and further stained with Ehrlich hematoxylin and Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in Canada balsam. Sections were observed in digital microscope (Intel Play QX3) at 400 x magnification.

Results and Discussion

Liver

The transverse section of the liver tissue of the normal fish is covered by a serous membrane and some connective tissue extends inwards into the parenchyma. The parenchyma tissue is composed of parenchymal cells (hepatic cells) and lattice fibers to support the former. Hepatic cells are roundish polygonal in shape and contain clear spherical nucleus and granular cytoplasm. They are located among sinusoids forming cord-like structures called hepatic cell cords. A bile canaliculus is centrally located in each hepatic cell cord. These cords extended between the central and portal zones (Kendall and Hawkins, 1975 and Brusle and Anadon, 1996). Pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement (Fig. 1).

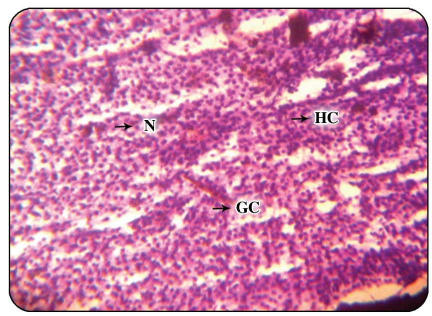

Fig. 1. Control : Normal structure of liver of *Cirrhinus mrigala* Bouin, HE x400
N : Nucleus
HC : Hepatic cell
GC : Granular cytoplasm

The changes in the liver of fish *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of cartap hydrochloride were characterized by the prominent yellowish-brown pigment (24 hr lethal), cytoplasmic vacuoles, pycnotic nuclei, degeneration of hepato-pancreatic tissue, eosinophilic granules are observed in the liver of 24 hr sublethal and lethal concentrations exposed fish. Pycnotic nuclei, Melano macrophages aggregate, appearance of blood streaks, degeneration of hepatopancreatic tissue, hypertrophic nuclei, cytoplasmic degeneration (96 hr sublethal and lethal), change in the shape of the hepatocytes, disappearance of hepatic cells. In addition to this hyperplasia of cells, hypertrophic nuclei and degeneration of cell membrane was also observed in the liver of 24 and 96 hr exposed fish.

Relatively less effect was observed in the liver of 24 hr sublethal exposure (Fig. 2, 3, 4 and 5). In the present study cartap has induced discrete pathological changes in the liver of fish *Cirrhinus mrigala*.

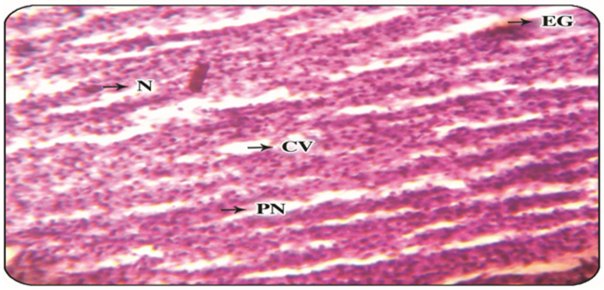

Fig. 2. Sublethal : Structure of liver of *Cirrhinus mrigala*, exposed to sublethal concentration of cartap hydrochloride for 24 hr. Bouin, HE x400
CV : Cytoplasmic vacuole
EG : Eosinophilic granules
PN : Pycnotic nuclei

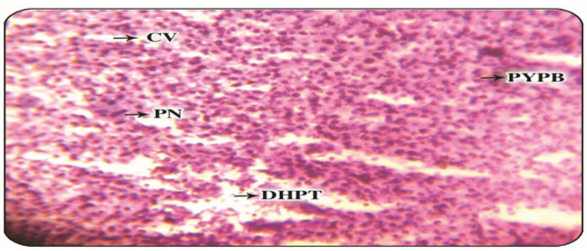

Fig. 3. Lethal : Structure of liver of *Cirrhinus mrigala* , exposed to lethal concentration of cartap hydrochloride for 24 hr. Bouin, HE x400
DHPT : Degeneration of hepato-pancreatic tissue
PYBP : Prominent yellowish brown pigment

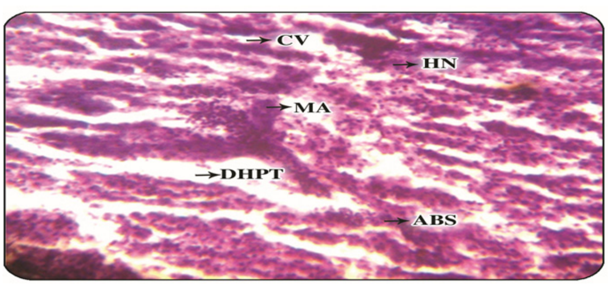

Fig. 4. Sublethal : Structure of liver of *Cirrhinus mrigala*, exposed to sublethal concentration of cartap hydrochloride for 96 hr. Bouin, HE x400
CD : Cytoplasmic degeneration
MA : Melano macrophages aggregate
ABS : Appearance of blood streak
DHPT : Degeneration of hepato-pancreatic tissue

The pathological changes observed in the liver of fish might affect the physiological activity such as decreased enzyme synthesis (Sastry and Sharma,

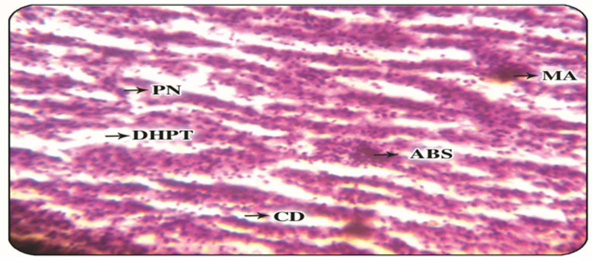

Fig. 5. Lethal: Structure of liver of *Cirrhinus mrigala*, exposed to lethal concentration of cartap hydrochloride for 96 hr. Bouin, HE x400
HN : Hypertrophic nuclei

1980 and Rashatwar and Ilyas, 1984). Due to this the functional ability of liver is reduced which indirectly affects all metabolic activities of the organism. The degenerative changes are more pronounced in lethal exposures. Liver is the main organ in the transformation of xenobiotics including pesticides. Hepatic histopathology revealed the induction of central necrosis and nuclear karyolysis in fish *Labeo rohita* in response to cartap hydrochloride (Vivek *et al*., 2016). Such hepatic lesions in response to xenobiotics and insecticides have been mentioned in some earlier studies (Eritja *et al*., 2016 and Jiang *et al*., 2016) as well. Similar pathological changes like formation of vacuoles, rupture in blood vessels, atrophy, necrosis and disappearance of hepatocytes cell wall and formation of vacuoles rupturing in blood vessels, disposition of hepatic cords, blood cognation, disappearance of hepatic wall was also observed by Anitha (2015) in the liver of the fish *Labeo rohita* exposed to pyraclostrobin, a carbamate pesticide.

Since liver plays a primary role in the digestion, storage, metabolism production of yolk protein and excretion of xenobiotic compounds, structural alterations can obviously occur in toxic conditions. Although liver has no direct contact with pollutants in water but because of its contact with blood it is affected indirectly. Due to this reason changes in the liver cell morphology close to blood vessels are being observed first. Increased vacuolization in the hepatocytes can be a signal of degenerative process which suggests metabolic damage, possibly due to exposure to contaminated water. Bile stagnation can be characterized by the presence of brownish-yellow granules in cytoplasm of hepatocytes (Pacheco and Santos, 2002). This accumulation of bile indicates possible damage in the hepatic metabolism (Fanta *et al*., 2003). The present study revealed that a strong link is present between liver damage and toxicants.

Pradeep Kumar Maurya *et al*., (2019) reported that the liver of the exposed fish *H. fossilis* exhibited slightly vacuolated hepatocytes showing evidence of fatty degeneration. Necrosis is observed in some portions of the liver tissue probably due to excessive work done by the hepatocytes to get rid of the toxicant during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may have led to necrosis of hepatic cells of sinusoids (Pradeep Kumar Maurya *et al*., 2019).

Similar histopathological changes in Liver were reported in *Cyprinus carpio* exposed to Benzene hexachloride (Sunita Kaser *et al*., 2018), in *Rasbora daniconius* exposed to sugar industry effluent (Kakade, 2019), in *Mystus gulio* exposed to monocrotophos (Sathick *et al*., 2019), in Mozambique tilapia, *O. mossambicus* exposed to chlorpyrifos (Subburaj *et al*., 2020), in O. niloticus exposed to chlorpyrifos (Farhan *et al*., 2021), in freshwater iridescent shark, *Pangasius hypothalamus* exposedto deltamethrin (Prabhanjan Kumar Reddy *et al*., 2023).

Kidney

The kidney is an important organ of osmoregulation and excretion and because of its high blood supply it is highly susceptible to toxic substances. Kidney is the important organ of body, which performs several functions like erythropoiesis, maintaining volume and pH of blood and body fluids and homeostasis (Hickman and Trump, (1969).

Kidneys are elongated bodies extending along the whole length of the visceral cavity in fish. They are situated on the dorsal side of the body wall of fish above the swim-bladder. They are distinct anteriorly but become partly fused in the middle region. Teleostean kidney consists of head and body kidney. The anterior portion of the kidney is the head kidney and it consists of lymphoid tissue. Body kidney consists of numerous nephrons, functional excretory units, and interstitial lymphoid tissue (hematopoietic tissue). Each nephron consists of two parts, a renal corpuscle and a long convoluted uriniferous tubule. The renal corpuscle is made up of a double walled cup shaped.

Bowman's capsule and a knot of arterioles termed glomerulus. The Bowman's capsule is lined by an outer parietal layer and an inner visceral layer of epithelial cells. Renal tubules are short and thin in the neck segment. The proximal convoluted segment is divided into two parts, segment I and segment II. The renal tubules in segment II are made up of cuboidal epithelial cells showing densely arranged microvilli in thetubular lumen. Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells do not have microvilli. The cells of distal convoluted segment are stained with eosin more faintly than those of proximal convoluted segment. Thus, under light microscopy, it is easy to distinguish between proximal and distal convoluted segments (Oguri, 1982). The normal structure of kidney of *Cirrhinus mrigala* is shown in the Fig. 6.

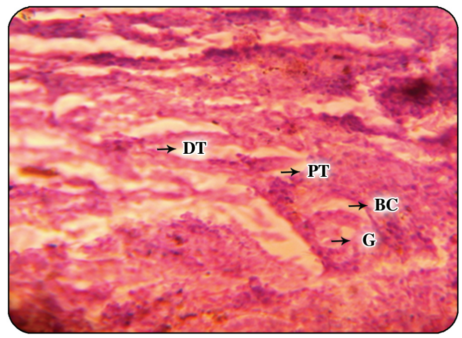

Fig. 6. Control: Normal structure of kidney of C*irrhinus mrigala* Bouin, HE x400.
BC: Bowman's capsule
G: Glomerulus
PT: Proximal tubule
DT: Distal tubule

On microscopic examination of kidney of the fish, *Cirrhinus mrigala*, treated with the sub-lethal and lethal (1/10th of Static, 96hr LC_{50} Static, 96 hr LC_{50}) concentration of cartap hydrochloride for 24, and 96 hr, prominent tubular yellowish brown pigment, formation of vacuoles, swelling of glomerulus, separation of the tubular epithelium, tubular architectural loss, (24 hr sub-lethal and lethal), damage of bowmans capsule, tubular cytoplasmic degenera-

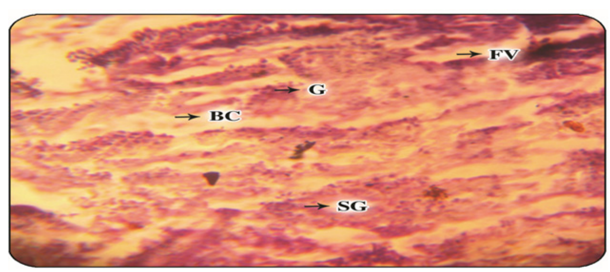

Fig. 7. Sub-lethal: Kidney of *Cirrhinus mrigala,* exposed to sub-lethal concentration. of cartap hydrochloride for 24 hr. Bouin, HE x400.
FV: Formation of vacuole
SG: Swelling of glomerulus

tion, separation of the tubular epithelium, tubular architectural loss, cloudy swelling degeneration, damage to renal tubules, damaged haemopoitic tissue (96 hr sub-lethal and lethal), necrosis of cell and renal tubules, aggregation of cells, disorganization of connective tissue, degeneration of cytoplasm within pyknotic nuclei, were observed. The hypertrophy of nuclei and disintegration of cell membrane were also seen (Figs. 7, 8, 9 and 10).

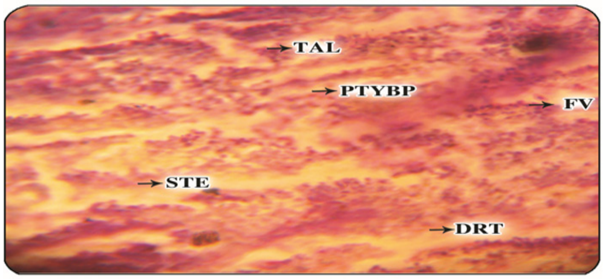

Fig. 8. Lethal: Kidney of *Cirrhinus mrigala*, exposed to lethal concentration of cartap hydrochloride for 24 hr. Bouin, HE x400.
PYBP: Prominent yellowish-brown pigment
TAL: Tubular architectural loss
STE: Separation of tubular epithelium
DRT: Damage to renal tubule

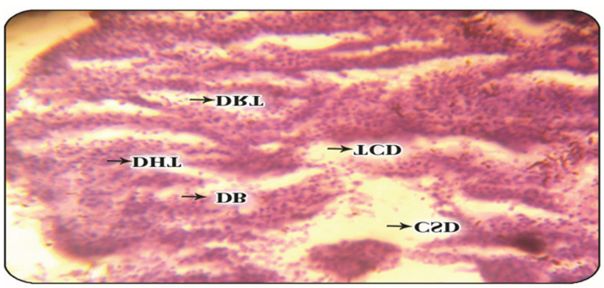

Fig. 9. Sub-lethal: Kidney of *Cirrhinus mrigala*, exposed to sub-lethal concentration of cartap hydrochloride for 96 hr. Bouin, HE x400.
CSD: Cloudy swelling degeneration
DB: Damage to bowman's capsule
DHT: Damage of hematopoietic tissue
TCD: Tubular cytoplasmic degeneration

The elimination of undetoxified toxicant molecules through urine is the cause for kidney damage. Cytoplasmic granules may be formed inside the renal cells, or the reabsorption of plasma proteins lost in the urine, indicate damage in the corpuscle (Takashima and Hibya, 1995). The results of the present study agree with the findings of Vivek *et al*., 2016), who reported similar degenerative changes such as contraction of bowman's capsule, reduction

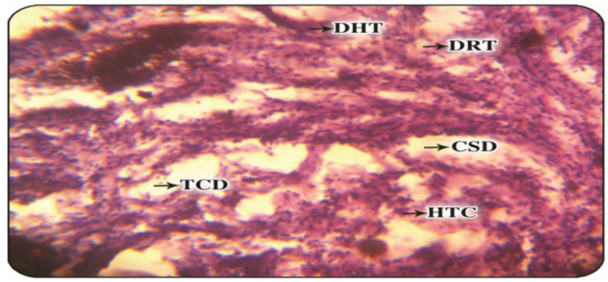

Fig. 10. Lethal: Kidney of *Cirrhinus mrigala*, exposed to lethal concentration of cartap hydrochloride for 96 hr. Bouin, HE x400.
HTC: Hypertrophied cells.

of tubular lumen, and necrosis of renal epithelial cells in the kidney of fish *Labeo rohita* on exposure to sublethal concentrations of cartap hydrochloride for 24, 48, 72 and 96 hours. This study revealed that sublethal concentration of cartap is moderately toxic to *Cirrhinus mrigala* at the histological levels.

The present observations agreed with the reports of Mariya Das, (2014), Anitha, (2015), and Vivek *et al.,* (2016) who observed rupture in the glomeruli, renal damage, reduced renal tubules and its lumen in different fish exposed to carbomate pesticides.

Similar histopathological changes in kidney were reported in in the kidney of *Labeo rohita* exposed to Lead nitrate Brraich and Kaur, 2017), in fish, *Mystus tengara* exposed to Cypermethrin (Haque *et al*., 2017), in *Rasbora daniconius* exposed sugar industry effluent (Kakade, 2019), in *Channa punctatus* exposed to Dithane M-45 (Rita Choudhury and Puranjit Das, 2020), in *O. niloticus* exposed to sumithion (Sharmin *et al*., 2021), in freshwater iridescent shark, *Pangasius hypothalamus* exposed to deltamethrin (Prabhanjan Kumar Reddy *et al*., 2023).

Conclusion

In conclusion, the present study showed that histopathology can act as a useful biomarker for environmental contamination, since all the two vital organs, liver, and kidney are moderately affected by pesticide pollution. The histopathological alterations resulting from the exposure to cartap hydrochloride in *Cirrhinus mrigala* may affect the functional efficiency of liver and kidney which may lead to malfunctioning of several organ systems of the fish. Thus, the exposure of fish, *Cirrhinus mrigala*, to the pesticide cartap hydrochloride led to irreparable architectural changes in the vital organs liver and kidney thus making the fish less fit for better survival. Pesticide toxicity may possess a serious health concern of consumers due to their bioaccumulation. So to control pesticides contamination in aquatic environment, regular monitoring programs are necessary which ensures healthy aquatic environment, safe fish production and sustainable aqua culture industry.

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Conflict of Interest: None

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