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HISTOPATHOLOGICAL ALTERATIONS OF THE GILL, LIVER AND KIDNEY OF THE FISH *CHANNA PUNCTATA* (BLOCH) EXPOSED TO CHLORPYRIFOS 20% EC

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Abstract

The fish, *Channa punctata* exposed to an organophosphate Chlorpyrifos 20% EC in the laboratory for 10 days in sublethal concentration as 1/10th of 96h LC_{50} value (0.14 µg/L). The architectural damages of the entry point of the gill, the respiring organ was damaged that impairs the process of respiration. As heterotrophic organisms, the fish depend on the respiring gas for the metabolism which is affected and as a result the growth can be curtailed. The liver the site of metabolism because of its devastating manifestation in it as lesions, all the biochemical interactions are impeded. The exit point of the toxicant, the excretory organ kidney when was defaced in its internal structure, the aminotellic excretory aspect was effected.

Key words: Chlorpyrifos, 20% EC, *Channa punctata*, Histopathological changes, Gill, Liver, Kidney.

INTRODUCTION

The contamination of the aquatic environment by pesticides due to indiscriminate use of pesticides them is happening and is a global problem. Tang *et al*. (2021); Kelly Macnamura (2021); Zeshan and Praveen (2020). Even in India, the use and defilement of the aquatic environment is inevitable to raise the yield as production due to green, blue and white revolutions. As a result, the Indian and regional scenarios (Sucheta Yadav and Subroto Dutta (2019); Anamika Srivastava *et al*. (2018), Somayyah *et al*. (2017), Monali and Roy (2017) Indira Devi *et al*. (2017) are well explained.

Many biomarkers studies are suggested to determine the effects of these chemicals in the tissue level and such study of organs made of tissues, known as Histology and alterations as histopathological changes. The adverse effects need not be in lethal concentrations but even in sublethal concentrations, show such devastating manifestations as opined by Yanchova *et al.* (2015) and Anilava Kaviraj and Gupta (2014). Tissue deposition quantitatively pave the way for brining any qualitative changes as opined by Yanchova *et al*. (2019) and Javeed *et al*. (2017).

Such biomarker studies of histopathological nature for synthetic pyrethroids, Sana Ullah *et al*. (2019) and for pesticides in general by Ullah and Zorriezahara (2015) and for chlorpyrifos by Sunanda *et al*. (2016), Deb and Das (2013) are available for reference of study as the review articles. Javeed *et al*. (2017) mentioned about such study exclusively in the fish *Channa punctata* the present studied fish, and also by Stella Stryanova *et al*. (2020) in the fish Chlorpyrifos (the present studied toxicant). As such, the individual research reports for different fish using different class of pesticides, organophosphates only and also chlorpyrifos only, for the last 20 years due to more use of the present studied pesticides are reported.

By taking all into considerations of reports, as the users both in agriculture as well as to certain extent in aquaculture practices where in fish disease management use organophosphates that are commercially available, hence only EC formulation as 20% of chlorpyrifos, in sublethal concentrations for a limited period of exposure, 10 days as recommended by APHA, 1998, 2005 and 2012) guidelines $1/10^{th}$ 96 hrs LC_{50} value the present study is attempted.

MATERIAL AND METHODS

Fresh water fish *Channa punctata* (8-10 cm size, 8-10 gms. wt.) from local market, Guntur, A.P. India was acclimatized to the laboratory conditions for 10 days. 50 number of fish are exposed to 20% EC chlorpyrifos for 10 days by taking into consideration of LC_{50} value of 96 hours ($1/10^{th}$ of the 96h LC_{50}) value 0.14 µg/L as per the APHA guidelines(1998, 2005 & 2012).

After the exposure period of 10 days the fish are randomly selected for histopathological examinations. The exposed fish are sacrificed and tissues viz., gill, liver and kidney of the fish are isolated and also from that fish not exposed to the toxicant which serve as control to observe so as to differentiate the tissue changes in pathological conditions.

NaCl 0.85% saline solution was used to rinse and clean the tissues that were isolated. Then were fixed in the aqueous Bouine solution and kept for 48 hr to process further through the graded series of alcohols. Then cleared with xylene and later were embedded in the paraffin wax. The gills alone were processed by through double embedding technique (Humason, 1972).

6 µm thickness sections were cut using the microtome which were later stained with Ehlrich haematoxylin/Eosin that was dissolved in 70% alcohol for clarity in viewing and finally mounted on the Canada balsam (Humason, 1972). The sections were observed first through the digital microscope and later the occurred histopathological lesions were examined and the photographs are taken with the help of Intel Pentium Q x 3 Computer attached microscope, under 400x lens (made in China).

OBSERVATIONS

Gill structure (Control-Plate 1, Fig. A)

In *Channa punctatus*, there four pairs of gills that are semicircular gill arches also (curved) called are present. Each one has a growth zone, from which row of microscopic gill lamellae that are going to arise (radiate) from it only have a bilaterally arranged secondary gill lamellae that are present. All are embedded ones, as covered by an thick cuticular operculum. The secondary one have arrangement of perpendicular one, in their running as observed. Each lamellae, has a squamous Epithelium otherwise called as pavement Epithelium also with non-differentiated cells with apically located nucleus. Each of the lamellum is made up of two sheets of Epithelium which are separated by pillar cells (columnar cells) with basal nuclei. These cells are contracticle and are for supply of blood (capillaries – channels). They have a lumen where one or two erythrocytes are present which are nucleated unlike mammals. The lamella of adjacent, in between have cells, chloride, mucous and goblet cells and finally at the outer most squamous epithelium. In between, the pillar cells, squamous pavement cells there is round cells with centrally located nucleus – apical cells. The cells that are present on the secondary lamellae and the layers are also present in the primary gill filament but somewhat thicker and appear as microridges. They are primary gill lamellae (PGL), secondary gill lamellae (SGL), basal lamina (BL) and Microridges (MR), as layers with different cells. The difference between the primary and secondary lamellae lies not only in thickness, which is important for the gaseous diffusion but also in mucus producing cells, which more in primary, to secondary hence characteristically, mucus vacuoles are more in primary lamellae.

Pathological observations (Plate 1, Figs. B, C & D)

The secondary gill lamellae are fused, (their surface are supposed to have diffusive function), hyperplasia (increase in number or cells in the gill Epithelium particularly the cells just below the squamous layer. The curling of the secondary Epithelium is very evident. The pathological term telangiectasia (capillaries-arteries-veins are bulged). Shortening of lamellae, disruption of the cartilaginous part of the primary gill lamella, the supporting structure of secondary lamellae, its disorganization as well as of atrophy reducing the reduction of the surface area sometimes radiated secondary structures fused. The squamous epithelium covering the outermost tissue is lifted (separated), not to have the functional coordination, curling also made an added disadvantage part from hyperplasia of cuboidal cells, the junction cells of columnar basal cells and squamous (serous) cells.

General Histology of Liver (Plate 2, Fig. A)

The liver of the fish is bilobed structure, five lobed in (mammals) and when dissected the lobe is larger than right and gall blader round shaped one which is well developed, by append it appears as intestine.

The cells of liver, hapatocytes whose arrangement is systematic, colour is reddish and shape is polygonal with a prominent spherical nucleus, which is by location of centrally placed. The chromatin of the liver cells is at its periphery and nucleolus shows characteristic pattern of RNA synthesis for its role in metabolism. The endoplasmic reticulum both with rough (RER) and smooth (SER) to have the functions of both secretory (acinar) as well as detoxification associated with more synthesis. Mitochondria are round and elongated for energy synthesis, more associated with rough endoplasic reticulum. The secretory part is with vacuoles and a sterna (RER) are concentrically arranged.

The liver surface is covered with serious membrane, connective tissue that extend upto thick cords called hepatic cords. It has fibers and the sinusoids, bile canilculi which are located at the centre of the hepatic cards extending upto sinusoids and each cord has glycolipid granules in large quantitative.

Pathological observations of liver tissue (Plate 2, Figs. B, C & D)

Mild necrosis, in the liver can only be observed in the compact structure, blood is congested as the condition of pathological term congestion can be applied, infiltration (gaps) that interfere in functional continuam, lymphocytes enter into the gaps, the hapatocyte cells showed pykenotic nuclei supposed to have more functional aspect of biochemical – (transcription – translation) disturbances, the observation of the inflicted aggregation of melanomacraphase (phagocytes - kuffer cells), cells of some component of section showed foamy nature, dialation of sinusoids is very prominent (BS). The appearance of vacuoles (V) and hypertrophy (H) of the tissue is also seen.

General Histology of Kidney (Plate-3, Fig. A)

Fish kidney, aminotellic in secretory function, conservation of salts Aglomerular in its function apart from haemopoietic nature. It is reticulo-endothelial endocrine as well as excretory role, it has to performs. It has glomerula filteration as well as salts conservation of reabsorption have with proximal as well as distal convoluted tubules (PCT & DCT). The renal corpuscle is near PCT and DCT ends into urinary bladder. Taller columnar cells and smaller columnar cells varied in its distribution. The glomenlar tissues consists of inner and outer layer of a single flattened epithelium (Renal Epithelium-squanous) and renal tubules too have covering. Mesangium is the space, that fills the space between loops of glomerular capillaries.

The renal tubules (RT) are thin and short in the neck segment which are divided into two parts, segment I and II consisting of cuboidal epithelium, densely arranged. The segment of the RT is having microvilli for absorption whereas they are absent in the segment I (Brush border).

Pathology of the Kidney – observed (Plate 3, Figs. B, C & D)

There are alterations related to circulatory component as hemorrhage and hyperemia, glomerular alterations as nuclear as well as plasma alterations. Atrophy hypertrophy and hyperplasia of tissue cells, in terms of pathological terms that can be applied. Necrosis of tubular part, focal necrosis in the glomerulus filtering point is very conspicuous. Damage in the filtering point, increase of number of cells of the tubule capsular membrane – hyperplacia, peritubular fibrosis leucocyte related abnormality all are the severe pathological alterations that are observed in the exposed fish which damage the organ in its functional aspect.

DISCUSSION

Gill

Yanchova *et al*. (2015) and Stryzewska *et al*. (2016) in their respective review articles and the lesions of the respiratory structure as well as excretory aspect of salts (secretion) is the reflection of the sensitivity nature of the fish, if any appreciable changes in the ambient waters of external as well as internal blood aspect of them. The fine capillaries described as arterial-arterial and arterial venous' for exchange of gases as blood to be oxysecreted. The chloride cells of the gills with mitochondria in them to provide energy for the important function of osmo-regulation. Excess production of mucus, oedema and necrosis discuss the functional status and as a consequence less consumption of oxygen while distortion.

Zeshan and Parveen (2020) in their review article mentioned that pesticides have/had impact on histology of the different organs of the fish. The gills the entry point, if any changes resulted by toxicants as pesticides because any life's physiological functions starts only with respiration.

Stoyonova *et al*. (2020) in their study report, the histopathological study of any organ as one of multi-biomarker of assessment in common carp due to the acute toxicity of chlorpyrifos exposure. When tissues/organs are damaged the other biochemical changes are imperative

Akter *et al*. (2020) in the stinging cat fish *Heteropneustes fossils* due to the exposure of the organophosphate, envoy 50% at sublethal doses which they followed a different criteria recommended dose of springing and half of the recommended dose of it for 7 days. The present study result apart from a lesion of clubbing (selling) of gills was reported. The methodology of exposure at a concentration is different (a deviation of the recommended) and the gills showed similar alterations.

Yanchova *et al*. (2019) reported due to chlorpyrifos intoxification in the fish *Cyprinus carpio* gills are damaged after 72 hrs of exposure. The report by them in terms of the alterations resulted were under the caption as circulatory disturbances (Vasidialation) regressive lesions (Epithelium-necrosis) progressive lesions (lamella lifting, proliferation of Cells) where in the present study as made of the changes as necrosis, hyperplasia, congestion, by the chlorpyrifos 20% EC.

Sathick *et al*. (2019) reported on the histopathological changes of the gill tissue of an esturine fish *Mugil cephalus*, that resulted after exposure 10, 20 and 30 days at 10% of 96h LC_{50} values of sublethal concentration. The changes that resulted due to more days of exposure were severe and majority of the present study results are similar, with their results of report.

Trivany Edwin *et al*. (2019), reported in freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* due to the exposure of chlorpyrifos in 10, 20, 30 days of exposure at three different concentrations gill was studied and observed be histopathological changes. The results that were time dependent and concentration dependent only, but the severity increases even the concentrations were at sublethal. Almost similar results in both the fish studied.

Arumugam Stalin *et al*. (2019) reported in the fish, *Channa punctata* exposed to chlorpyrifos in 10, 20, 30 days of exposure at sublethal concentration as (5 ppm). Except *Mucoid metaplacia* all other results were similar (30 days of exposure). Probably as in the present study that was only 8 days of exposure.

Somayyah *et al* (2017) in their review article of organophosphate Sunanda *et al*. (2016) Deb and Das (2013) in their review article of chlorpyrifos mentioned about the reports of the histopathological changes in different fish.

Channa punctatus fish due to exposure to 4-non-lphenol Madhu Sharma *et al.* (2018) and Butchiram *et al*. (2009) exposing to phenol reported alterations, in the gill tissue/organs and these manifestation due to toxic stress render the fish not to be normal in the respiration rate due to gill damage.

Sadia Sharmin *et al.* (2016) in the fish *Cyprinus carpio* exposing to Malathion another organophosphate, Ragade *et al.* (2015) in the fish *Channa punctata* exposed to Monocrotophos another organophosphate, Yogita Devi and Abu Mishra (2013) in the fish *Channa punctatus* exposed to Hilban (Chlorpyrifos).

Jyothinarendran (2012) in the fish *Channa punctata* due to exposure to dimethoate an organophosphate Ladipo *et al*. (2011) in the fish *Clarias gariepinus*, due to paraquat another organophosphate in the histopathological alterations of gills, even in sublethal concentrations. The chronic toxic action is severe and losts long than to acute toxic action resulting immediate death.

Japamalai (2017) in her study, reported the changes in the gill of the fish *Labeo rohita* exposed to an organo phosphate, Dichlorovos of 76% EC. After 8 days exposure and when sections are taken the tips of primary gill lamellae were bulged, the secondary gill lamellae lost their shape, necrosis, vacuoles in the gill epithelium and fusion of disorganized cells. Even though the toxicant belongs to different class of compounds i.e. OP compound the effect is in the similar line with minor changes.

Mandeep Kaur and Rajendra Jindal (2016), reported a study of the branchial architecture and its changes in the fish *Ctenopharyngodon idella*, after exposure to chlorpyrifos an organophosphate. After exposure to the toxicant at sub lethal concentrations for 15, 30 and 60 days duration which differs to the present work in methodology and the direct contact point, of the entry of the toxicant and the thin surfaces with larger surface area had shown damage.

The effect is duration dependent mucous secretion distorted secondary lamellae. They too observed changes of the primary lamellae which resulted denudation as the toxicant of different chemical exposure. Hyperplasia, damage of the squamous epithelium, of the compound tissue were the observations that were reported.

Khosravi *et al*., (2015) studied on the histopathological changes in the fish gills of *Rutilus rutilus*, a tautonym species which in the early stages can survive in fresh as well as brackish waters, a migratory fish for spanwing when exposed to diazonin. The sea water and freshwater has ex and endosmosis problems when effected histopathologically, the gills of the fingerlings of the fish is unable to cope the situation. They too observed oedema (accumulation of salts). Epithelial tissue lifting and damage of the secondary gill lamellae were also reported as in the present study.

Velumurugan *et al*. (2009) studied and reported also using the toxicant Dichlorvos an Organophosphate to observe the changes in the gill of the fish *Cirrhinus mrigala* apart from using the toxicant fenvalerate. The toxicant had a remarkable alteration particularly the secondary lamellae curling apart from aneurism. In the epithelium lining of the gill hyperplasia desquamation and localized death of cells, lifting and oedemic condition due to the imbalance of salt diffusion. They too observed the fusion of lamellae. The observations are similar mostly of the present study that had architectural damage resulting on the oxygen intake and carbon dioxide sending out while the fish is respiring and hence the process of the diffusion of gases is impaired. The toxicant exposure even though in sublethal concentration it had the adverse effect of the gills, the entry point of the toxicant.

Ayoola and Ajani (2008) reported on the fish *Clarias gariepinus* the effect of the glyphosphate, that are observed as alterations of the gill a histopathological study. The observations include cellular infilteration (curling) swollen tip of the gill filaments congestion and damage of gill similarly. The toxicant is a herbicide a different class of the chemical of the present study and exposure concentration are different even though some changes that were observed are correlating.

Ahmadirand *et al*. (2014) too reported in the fish rainbow trout *Oncorhynchus mykiss* after exposure to Butachlor. They reported that due to the toxicant stress hyperplasia, and lamella epithelium fusion and also is similar line of the present study. They too observed rod like structures which is not case as in the present study.

Mohammad *et al*. (2016) in their study using Deltamethrin as the toxicant in the fish *Nile tilapia* too observed changes as lesions, hyperplasia of secondary lamellae and joining of them termed as fusion which are also the same in agreement of the present study.

Neelima *et al*. (2015) reported in the fish *Cyprinus carpio* due to exposure to cypermethrin 25% EC caused changes in the gill tissues. They mentioned about the primary lamellae necrosis, fusion of the secondary lamella clubbing and shortening wherein it had all the alterations that were also of the present study even though they belong to two different classes.

Kan, *et al.* (2012) in their study reported by using deltamethrin as a toxicant on the fish *Oreochromis niloticus* apart from causing, the micronucleus in the gill tissue of erythrocytes along with damage which is also the case of the present study.

Anitha Susan *et al*. (2012) studied and reported in the fish of the three major carps *Labeo rohita, Catla catla* and *Cirrhinus mrigala* after exposure to the synthetic pyrethroid of type II, fenvalerate. The tissues they studied, include gills also, a comparative study. The observations are in agreement of the present work even though the present tested toxicant belongs to different class of pesticide.

Velisek *et al*. (2014), reported on the fish effects by the pyrethroid and triazine pesticides mentioned also about the histopathological damages that resulted after exposure to the cypermethrin, a synthetic type II pyrethroid, Bifenthrin and also the triazines. When cypermethrin was studied in the fish rainbow trout after exposure to 96h lethal concentration, caused a pathological conditions called 'Teleagioectasia' in the secondary lamellae of the gills and pillar /columnar cells of the compound epitheliam. In the fish *Cyprinus carpio* hyperplacia of the respiratory epithelium of the chloride cells was also observed. Bifenthrin another toxicant as one of the triazines, severe Teleogioectasia was observed (dialation of capillaries) for the same fish.

Velisek *et al*. (2009 & 2007) when observed and reported in the fish rainbow trout *Onchorynchus mykiss*, exposed to biferthrin, type I synthetic pyrethroid, the localized tissue damage and also the weakened cell walls known by the term aneurysm in secondary lamellae, hyperplasia of the epithelium and fusion of cells in the gills. This is also similar in line of the present observation and is also of similar of other studies of type II synthetic pyrethroids (Velisek *et al.* (2007), Cengiz (2006) and Cengiz and Unlu (2006) in *Cyprinus carpio* after exposure to Deltamethrin.

Diana *et al*. (2007) opined that the damage of the chloride cells (Hyperemia) due to the exposure of the Deltamethrin at 2 µg/L concentration that was similar as in the present study and the resultant damage preclude the chloride cells to death, that make difficulty in the process excretion of the salts through the gills.

Balu Velumurugan *et al*. (2007) reported the study of the toxicant fenvalerate another synthetic pyrethroid, to the fish *Cirrhinus mrigala*. The study revealed that epithelial hyperplasia, necrosis of the epithelial surface desquamation and fusion of lamellar fusion hindering the diffusion pathway of the gases apart from curling. The present work even though it belong to the different class of the synthetic pyrethroid is of similar line.

Yildrin *et al*. (2006) study of the toxicant exposure of Deltamethrin to the fish *Gambusia affinis* for a long duration in sublethal concentration too revealed similar changes of fusion of secondary lamellae of the present study. Observation as above and in addition were also reported as hyperemia and aneurysm by Cengiz and Unlu (2006b).

Butchiram *et al*., (2013) reported in the fish *Labeo rohita* exposed to phenol for 8 days in both sub lethal and lethal concentrations. The observed changes in the gill in both concentrations of the toxicant were hyperplacia, hypertrophy, oedema and necrosis. They reported the mucus secretion and also the primary and secondary lamellae damage. The synthetic pyrethroids, group I or II are subjected to degradation and may be resulted in the phenol like chemical as metabolites. The methodology was the same of experimentation that reflect the architectural damage is most possible as opined in the present study.

Thus the fish gill, the entry point of nektonic fish when damaged even in sub lethal concentrations which are really lethal at one end and the commercial formulations of the pesticides that are marketed have to be viewed seriously when gills of the fish are effected and finally the respiration is impaired.

Liver – Discussion

Zeshan and Parveen (2020) in their review article mentioned that pesticides alter the architecture of the liver and thereby metabolic biochemical reactions got disordered. Elvin *et al*. (2020) too opined that due to Bisphenol xenoesterogen, as toxicant in the fish *Channa striate*, liver damage was dose dependent and the changes as of degenerative nature were noticed. While referring to the fish *Cyprinus carpio* due to chloropyrifos toxic action as a biomarker where granular deposition was conspicuous. Nucleus degenerative changes described in pathological terms karyolysis, karyorrhexis and karyopyknosis (Karyon mean nucleus, lysis, disintegration), such changes too are observed in the present study of the *Channa punctata*, fish liver.

Akter *et al*. (2020) in the fish *Heteropneustes fossils* due to Envoy 50 SC organophosphate toxic action, resulted pathological alterations which was time and concentration dependent. Vacuolation in the hepatocyte cytoplasm, hypertrophy, hemorrhage, pyknosis were some of the manifestation due to toxic action as in the present study that were reported.

Arumugam Stalin *et al*. (2019) in the fish *Channa punctata* due to toxic action of chlorpyrifos necrotic hapatocytes were reported as in the present study. The method of toxicity determination and duration of exposure were different of the present study.

Somayyah *et al*. (2017) in their report of organophosphorous pesticides effect on liver, opined that histomorphological alterations in the organ/tissue had biochemical alterations as a consequence the metabolism is impaired. In the review article of Sunanda *et al*. (2016) and Deb and Das (2013) mentioned about the histopathological studies of liver due to chlorpyrifos toxic action in different fish.

Golam Mohammad *et al*. (2015), in the fish *Barbonymus gonionotus* (silver barb) reported that due to the toxic action of Quinolphos an organophosphate, in the liver. Necrosis and damage of the sinusoids were reported as in the present study.

Ragadi *et al*. (2015) in the fish *Channa punctatus*, due to Monocrotophos toxic action reported that cytoplasm damage, hapatocytes atrophy, rupture of blood vessels (liver canalculli) all were reported.

Yogita Devi and Abha Mishra (2013) in the fish *Channa punctata* were treated with Hilban (chlorpyrifos) that resulted changes in the morphological and anatomical nature in the hepatic organ. Fibrosis, infiltration of lymphocytes as in the present study apart from necrosis and hypertrophy were all reported.

Clarias gariepinus due to paraquat Dichloride an herbicide too in the liver was reported by Ladipo *et al*. (2011) as a result of that the organ had damaged and histopathological changes were very prominent. Tilak *et al*. (2005) reported on the histopathological changes in the fish *Catla catla* due to chlorpyrifos and the present study reiterate the same observations.

Even the synthetic pyrethroids had impact on the liver of the fish as showed the following study reports.

Karim *et al*., (2016) too reported histological and biochemical study of liver of silver carp *Hypothalmichthys moltrix* after acute exposure to pyrethroid deltamethrin. The study revealed that necrosis, nuclear pyknosis hypertrophy of hepatocytes vacuolization nuclear atrophy and congestion of blood vessels.

Neelima *et al*. (2015) reported in the *Cyprinus carpio* after cypermethrin exposure and the result was of the similar lines of the present study.

Suvetha *et al*. (2015) reported that any biochemical alterations in the organs of the fish *Labeo rohita* viz., liver metabolically active and kidney for excretion which were due to intoxification of a synthetic pyrethroid deltamethrin the type II wherein histopathological changes finally manifested that lead alterations in the biochemical enzymes of tissues/organs.

Manjula Sree Vani and Veeraiah (2014) reported the effect of cypermethrin on the histopathology of the freshwater fish *Cirrhinus mrigala*. The toxicant induced changes, such as the cytoplasm of the hepatocytes degeneration, formation of vacuoles and the rupture in the blood vessels and the disappearance of hepatocytic cell walls and also the disposition of the hepatic cords.

Velumurugan *et al.* (2007) reported a histopathological study of different organs of the fish exposed to fenvalerate a synthetic pyrethroid *Cirrhinus mrigala.* The study result used the terminology of congestion, cloudy swelling of the hepatocytes localized death of cells as focal necrosis and epithelial cells damage atrophy, infiltrations of Immune cells mucosal epithelium being desquamation. The report coincided with the present study.

Velisek *et al*. (2006) reported for the fish rainbow trout that the periportal portions degeneration of cells and the hapatocytes showed pycnotic nuclei and the vacuoles in the cytoplasm. The fatty degeneration is very prominent, in the liver of the fish when exposed to the sub lethal concentration of Alimethrin, a synthetic pyrethroid. The result of the work as in the report, reiterate the present study observations.

Velisek *et al.* (2009) reported on histopathological changes in the fish rainbow trout, *Oncorhynchus mykiss* when exposed to bifenthrin another synthetic pyrethroid of type I of the present study. As per their result the degeneration of the liver cells are observed as in the present study.

Anitha Susan *et al*. (2012) too reported damage to the liver tissue when fenvalerate toxicant is tested on the three Indian major carps and it also coincides the present work.

Cytoplasmic vascularization that are observed in the present study also are in agreement with Sakar *et al*., (2005) with carbofuran in the fish *Labeo rohita*, Boran *et al*.(2012) in the fish rainbow trout *Onchorynchus niloticus*, by Indirabai *et al*., (2010) in the fish *Labeo rohita* exposed to endosulfan. Majority of the results mentioned the localized death of the cells, necrosis.

The inability of the fish to regenerate newer liver cells may also have led to necrosis which was earlier reported by Rehman *et al*., (2002) in the fish *Anabas testudenius*, when exposed to the diazinon an organophosphate.

Mohammed *et al*., (2016) reported on the histopathological changes while studying the toxicity of deltamethrin to the fish *Nile tilapia* wherein they also observed the damage of the liver.

Butchiram *et al*., (2013) reported changes in liver of the fish *Labeo rohita* exposed to the phenol exposed to sub lethal concentrations. The study revealed that the formation of number of vacuoles, enlargement of the nuclei of some cells, nuclear hypertrophy also and enlarged sinusoids with numerous blood cells and atrophic areas. Pesticides are organic chemicals some of them degrade in soil and water forming phenols which too are dangerous.

The above reports of the researchers and the present study clearly make a point whatever the class of pesticides and whatever may be the kind of fish pathological situation will arise. The damage of the severity depends on concentration and duration dependent. The basic cells of the liver, hepatocytes are damaged and the metabolism and blood cells regeneration was delayed or stopped. The vital organ of the fish got damaged as a result, the failure of the metabolism, glycolytic cycle, glycolysis, glycogenesis and gluconeogenesis and cori cori cycle all get disturbed and that will be detrimental to the growth of the species.

Kidney – Discussion

Zeeshan and Parveen (2020) in their review article mentioned, the histopathological alterations in the excretory junction organ kidney, the haemopoietic one was also as earlier reports that are studied. Its main function through excretion of ammonia, which maintains the pH, quality of blood in its volume and also the body fluids.

The changes it were there in the glomerulus, filteration was disturbed, degeneration of tubular cells which leads to non-absorption and atrophy causes osmatic imbalance, homeostatis is lost.

Akter *et al*. (2020) due to envoy 50% SC an organophosphate, in the kidney of the fish *Heteronensteus fossils* reported that the kidney tissue degeneration, glomerular expansion that increased the diameter of the renal tubule necrosis, pyknosis, vacuolation and hemorrhage. Majority of the reported observations reiterate the present study.

Javed *et al*. (2017) while on the study in *Channa punctata*, the present studied one due to heavy metal toxic action, kidney showed, degenerative changes due to oxidative toxic stress. The hyperplacia pyknotic nucleus were other some of the pathological alterations that were resulted similarly as in the present study.

Somayyah *et al*. (2017) for organophosphates, Sunanda *et al*. (2016) and Deb and Das (2013) in their review article of chloropyrifos too, mentioned, the manifested alterations in the kidney of the fish.

Madhu Sarma *et al*. (2018) using 4-nonylphenol as toxicant, Butchiram *et al*. (2013) phenol as toxicant, in the fish *Channa punctatus* and *Labeo rohita* respectively, reported most of the present studied degenerative changes of kidney.

Golam Mohammad *et al*. (2015), in the fish *Barbonymus gonionotus* (silver barb), by using Quinolphos an organophate reported the alterations in the kidney due to toxic stress. Renal lesions, defect in Proximal Convoluted Tubule (PCT) also of damage that was extended from glomerulus to distal convoluted tubule (DCT) and all such as were time and concentration dependent.

Ragada *et al*. (2015) in the fish *Channa punctatus* using monocrotophos as toxicant reported necrosis, cellular hypertrophy and also granules in the cytoplasm which are similar as in the present study also.

Tilak *et al*. (2005) reported in the fish *Cirrhinus mrigala*, degenerative changes of kidney and observations also support and reiterate the present findings.

Even the synthetic pyrethroids showed alterations in the kidney of the fish, which are as follows:

Neelima *et al* (2015) also reported in the fish *Cyprinus carpio* after exposure to the cypermethrin a commercial formulation in the kidney and present work coincides with their report.

Velumurugan *et al* (2009) also reported that the kidney of the fish was effected with Cypermethrin as the toxicant in the fish *Clarias gariepinus*. Much of the observations were similar to the present study. Anitha *et al* (2012) in the three major carps using fenvalerate a synthetic pyrethroid of type II and Sree Veni and Veeraiah (2014) in the fish *Cirrhinus mrigala* after the exposure to the cypermethrin of 10%, too, reported the degenerative *changes* in the kidney.

In the fish, *Cirrhinus mrigala*, on exposure to two sublethal concentrations of lambda cyhalothrin of 0.3 and 0.6 ppb by Velumurugan *et al.* (2007) reported necrosis, pyconotic nature of the nuclei in the haemopoietic tissue, the hypertrophy of the cells in the epithelium of tubules, its lumen was narrowed and the space in the Bowman's capsule and glomerulus was expanded. Except the severity of the situation was not that much in the present study as described by them.

Similarly, Cengiz (2006) and Cengiz and Unlu (2006) reported in the fish *Cyprinus carpio* on exposure to deltamethrin of Synthetic Pyrethroid deltamethrin of 50% and 70% of 96h LC_{50} value. As observed by them that showed a regional damage and degeneration of the cells of the renal tubules, pyconotic nuclei in the haemopoitic tissue, dilation of glomerullar capillaries and intra cytoplasmic vacuoles in the epithelial cells apart from hypertrophoid and narrowing of the much of it and results of such were not observed in the present study.

Yildrim *et al* (2006), studied histopathological effects of deltamethrin and also apart from Staicu *et al.* (2007) too reported in the fish *Carassu sauradus* using the same toxicant.

CONCLUSION

The important tissues gills, liver and kidney of the fish, can be termed as vital when exposed to the toxicants like chlorpyrifos 20% EC that showed pathological degenerative changes which culminate the biochemical changes and the very survival of the fish in questionable even in the sub-lethal concentrations and apprehend that sub lethal concentrations are really lethal to the fish. If any, representation of the toxicant is going to be given for its usage, the sublethal, chronic devastations that are going to manifested be thoroughly studied and the actual mechanisms of toxicant and such lesions needs to be understood.

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PLATE 1

HISTOPATHOLOGICAL CHANGES OBSERVED IN THE Gills OF THE fISH *Channa Punctata* after exposure to chlorpyrifos 20% Ec in Sub Lethal Concentration

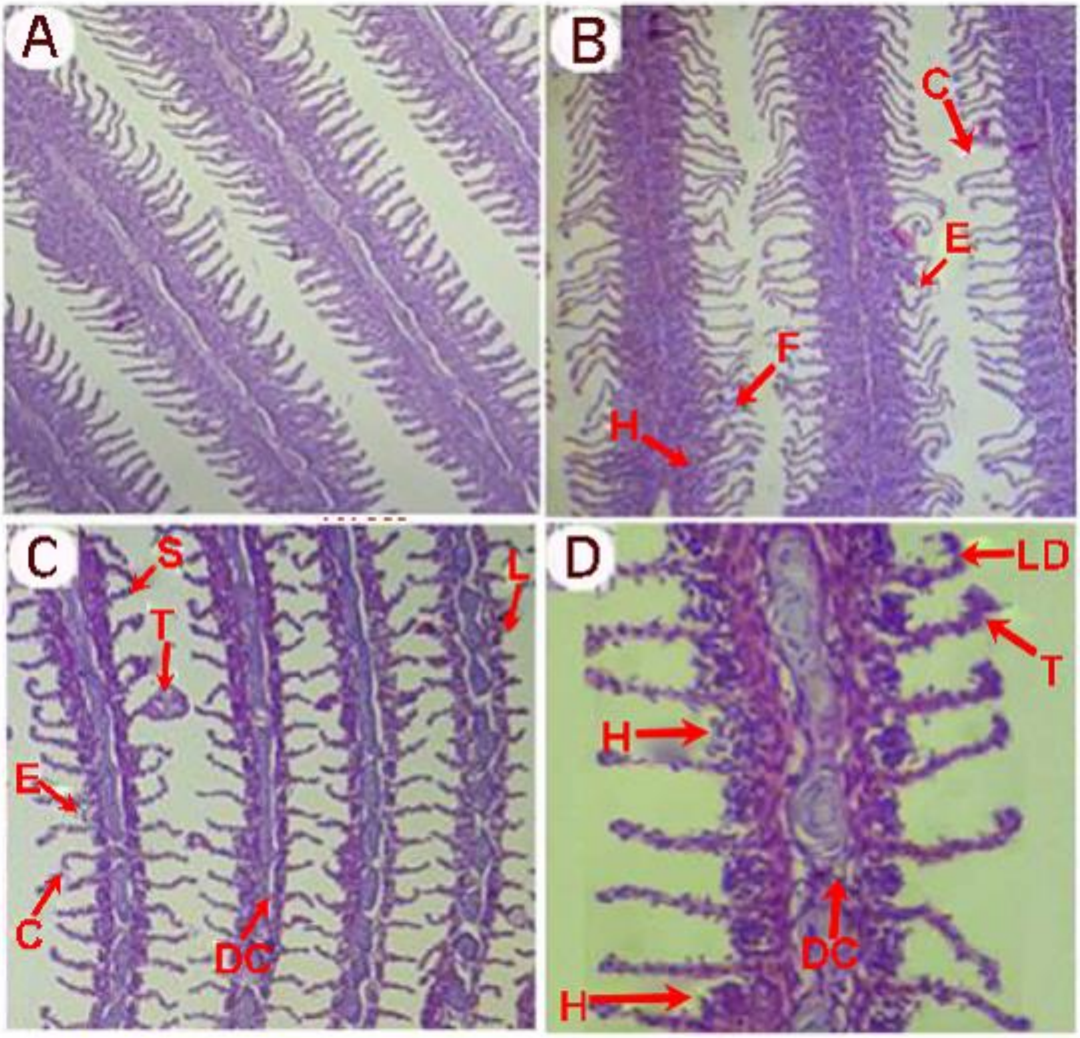


FIG A ⟶ Control group gills showing normal structure.
B,C,D ⟶ Treated groups gills showing various histopathological alterations including fusion of secondary gill lamellae.
F ⟶ Epithelial uplifting of secondary gill lamellae.
E ⟶ Hyperplasia of gill epithelium.
H ⟶ Curling of secondary gill lamellae.
C ⟶ Telangiectasia.
T ⟶ Shortening of secondary gill lamellae.
S ⟶ Disruption of cartilaginous core.
DC ⟶ Lamellar disorganization.
LD ⟶ And lamellar atrophy

PLATE 2

HISTOPATHOLOGICAL CHANGES OBSERVED IN THE lIVER OF THE fISH *Channa punctata* after exposure to chlorpyrifos 20% Ec in Sub Lethal Concentration

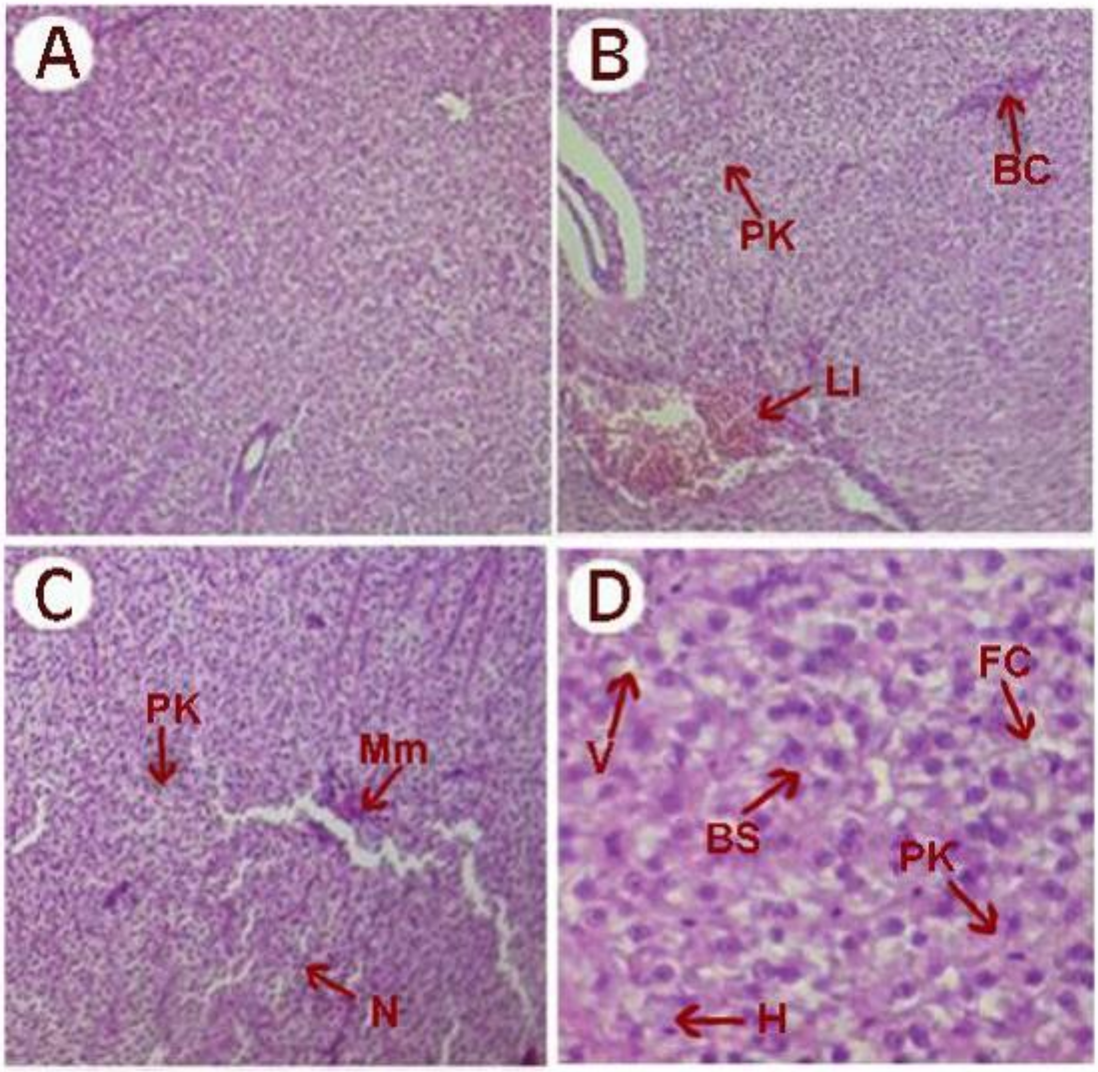
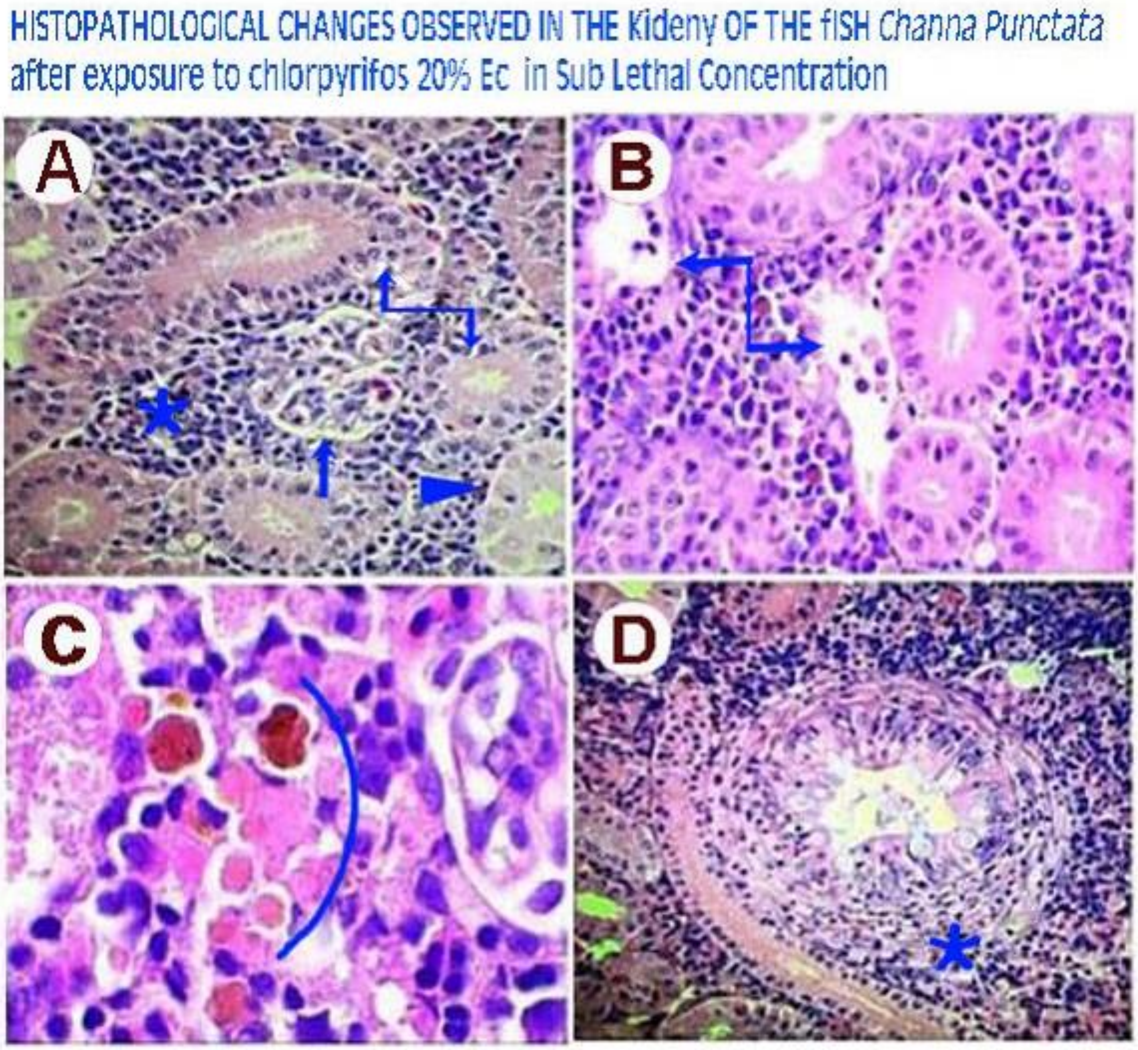


FIG-A ⟶ Control group liver showing normal structure,
B,C,D ⟶ Treated groups liver showing various histopathological alterations including blood congestion
BC ⟶ Lymphocytes infiltration.
LI ⟶ Pyknotic nuclei.
PK ⟶ Aggregation of melanomacrophage.
Mm ⟶ Necrosis
N ⟶ Foamy cells.
FC ⟶ Blood sinusoid dilation.
BS ⟶ Vaculation
V ⟶ And hypertrophy(H).

PLATE 3



A. Normal Kidney of the fish
B,C,D = Histopathological changes observed in the KIDNEY of the fish

Rupture of an artery and hemorrhage Blood congestion hyperemia intercellular edema of hematopoietic tissues tubule with architectural and structural cell alterations, plasma alterations and nuclear alterations. Tubules showing atrophy and necrosis Glomerulus with architectural and structural variations, alterations of nucleuses of epithelial endothelial cells and podocytes Atrophy and necrosis of glomerular cells plasma alterations hyaline degeneration of interstitial tissue Hypertrophy of tubular cells. Increase in number of tubule cells known as hyperplasma Hypertrophy of glomerulus cells occupying all real corpuscle thickening of Bowman's capsular membrane Hyperplasia of hematopoetic leukocyte cells and infiltration of fibroblasts, neutrophils monocytes and macrophages known as pertubular fibrosis.