

# HISTOPATHOLOGICAL DISTORTIONS IN THE FISH *CYPRINUS CARPIO* INDUCED BY THE DELTAMETHRIN 11% EC IN SUB-LETHAL CONCENTRATIONS

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## Abstract

The fish *Cyprinus carpio*, *in vivo* studies in the laboratory, exposure to  $1/10^{\text{th}}$  of  $LC_{50}$  value for 10 days, do have certain distortions that are observed through microscope and are photo-captured. The tissues/organs gill, liver and kidney, the entry point, the metabolic point and exit point, respectively, showed, some appreciable changes in their anatomical architecture rendering them not to have their normal function. The actions that the toxicant caused due to its impact, rendered even in sub-lethal concentrations in the laboratory and such situations might prevail in the natural conditions, hence they are real lethal. The commercial formulation, as 11% EC caused damage to the gill, both primary and secondary lamellae, hepatocytes of the liver, vacuoles appearance in the secretory part and in kidney the segments of proximal and distal convoluted tubules, thereby the respiration, metabolism and excretion was curtailed in the fish. Ultimately, caused the resultant action of mortality as the toxicant effect.

**Keywords:** Deltamethrin, 11% EC, Lethal, sub-lethal concentration, *Cyprinus carpio*, Histopathological effects.

## INTRODUCTION

Yanchava *et al.* (2015) and Kaviraja and Gupta (2014) recognized the histopathology as a tool of 'Biomarker' study in the possible effects of study tissue lesions in fish. Where such histopathological aspects are viewed with the aqueous environment, the hydrosphere, the subdivision of earth, largest, the fish are the good species as a candidate species of study. The contamination of the aquatic environment, by the use of pesticides is a global phenomenon and in India it is not an exception Kaushik *et al.* (2015) and Indira Devi *et al.* (2017). We know that there are four important class of compounds, viz., chlorinated hydrocarbons, organophosphates carbamates and synthetic pyrethroids all are reported to have changes in the fish tissues. They were mentioned in the review articles Ullah and Zallil (2015), Murthy *et al.* (2014) and also by Prusty *et al.* (2015) and Hasibur Rahman *et al.* (2014) for synthetic pyrethroids.

The individual research reports by Velisek and Stara (2011), Cenzig *et al.* (2006a& b) and Velmurugan *et al.*, (2007 and 2009) also made a point, such histopathological changes due to the toxic action of the chemicals. The specific reports of the Deltamethrin by Ullah *et al.* (2019), Parlak (2018); Cunha *et al.* (2018),

Lien *et al.* (2018), Guardiola *et al.* (2014), Ai-Ghanbousi *et al.* (2012), Kem *et al.* (2012), Velisek *et al.* (2009), Yildirim *et al.* (2006) and Dobsikova *et al.* (2006).

However, overall paucity of information that is available for the fish *Cyprinus carpio*, the present study is undertaken to observe the changes as the adverse effects that can happen in sub-lethal concentrations where the toxicity method by which the LC<sub>50</sub> value is determined and arrived 1/10<sup>th</sup> of it was not reported so far (Continuous flow through test)

## MATERIAL AND METHODS

Fresh water fish *Cyprinus carpio* was acclimatized to the laboratory conditions for 10 days. 50 numbers of the fish are exposed to 11% EC Deltamethrin for 10 days by taking into consideration of LC<sub>50</sub> value of 96 hours (1/10<sup>th</sup> of the 96h LC<sub>50</sub>) value 0.8µg/L as per the APHA guidelines (1998, 2005 & 2012).

At the end of the exposure, period of 10 days the fish are randomly selected for histopathological examinations. The exposed fish are sacrificed and the gill, liver and kidney tissues are isolated and also from the fish not exposed to the toxicant which serve as control.

Physiological saline solutions 0.85% NaCl was used to rinse and clean. They were fixed in aqueous Bouins solution for 48 hour processed through graded series of alcohols, cleared xylene and embedded in the paraffin wax. Gills alone were processed by through double embedding technique (Humason,1972).

Sections of 6 µm thickness were cut stained with Ehrlich haematoxylin/Eosin dissolved in 70% alcohol and mounted on Canada balsam, as recommended by Humason (1972). The sections were observed in digital microscope (Intel ply – Q x 3 at 200 x magnification). Histopathological lesions were examined and photographed with the help of Intel Pentium Q x 3 Computer attached microscope, under 400x lens (made in China).

## OBSERVATIONS

### Gill – Normal (Plate: 1A)

There are four pairs of gill arches which is made of cartilage. Each one has paired double rows that radiate termed, as primary lamella and secondary lamellae respectively whose number is more. Each arch of the primary lamella covered by epidermis which is thicker containing mucous cells whose number is very high in them. Below this there is a lymphoidal tissue and the arrangement is specific. The primary lamella of the arch covered by mucoidal epidermis within which it contains chloride cells whose function is salt secretion. At the base, such cells are numerous (at the proximal part) and main function in salt/ion transport.

The secondary lamellae play the role of gaseous exchange (diffusion) and baths in blood, and such that it can have counter current mechanism which operates. Squamous epithelium, followed by columnar pillar cells next to the pavement epithelium to give support and as such it is a compound tissue, all of it is ectodermal in origin. Blood that comes from the venous heart, which flows with high pressure and it requires support, for, not to have any distending structural deformity so that it should not house any dysfunction. The surface of the secondary lamellae have microvilli providing multifold functions. Goblet cells are scattered in the primary and secondary lamellae.

### Pathological changes – Observed Plate:I-B)

Toxic action resulted in the gill of the fish viz., and changes observed as;

- (1) Secondary lamellae shape – instead of long-erectile providing more surface area for diffusion of the gases (CLB labeled part) reduced and we can say club shaped (round-circular) reducing the surface area.
- (2) SEC – Separation of the secondary lamella that was noted not have any contact with the main primary lamella where in for the exchange of ions and diffusion of gases might be not possible as in the case of normal fish not exposed to the toxicant because of lack in structural cohesion.
- (3) EEC – Epithelial layer got separated and in pathological terms, as exfoliation, and

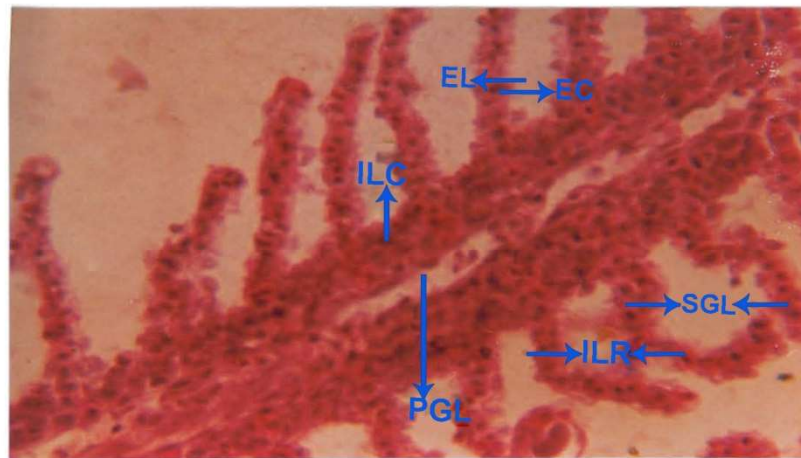
(4) H (Haemorrhage) - appearance of blood deposition as group of cells (aggregation), impairing of the exchange of gases ( $O_2/CO_2$ ) of the dissolved state of oxygen with carbon dioxide of the blood by the help of the enzyme carbonic dehydrase.

(5) Overall reduction of the surface area of the gill thereby a possibility of reduction in the gaseous exchange (1-A & I-B) which was due to the resultant damage of the local tissue, probably the supporting columnar cells that are damaged.



# PLATE-1

## FIG-A



## FIG-B

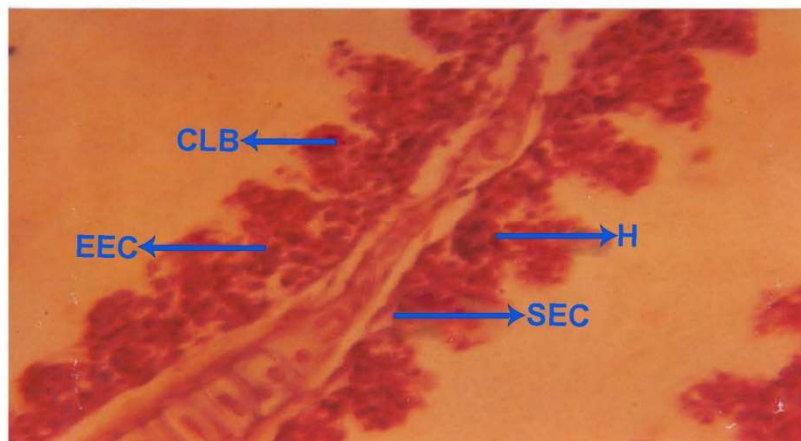


FIG. A Normal Gill Lamella of *Cyprinus carpio* Haematoxylin / Eosin Stain (HE), X400 PGL: Primary Gill Lamella SGL : Secondary Gill Lamella ILR : Inter Lamellar Region ILC : Inter Lamellar Cells EL: Epithelial cells EC : Erythrocyte

FIG. B Gill Lamella of *Cyprinus carpio* exposed for 10 days to sublethal concentrations of Deltamethrin 11% EC , Haematoxylin / Eosin Stain (HE) , X 400 CLB: Club-Shaped Secondary Lamella H: Haemorrhage EEC: Exfoliating Epithelial Cells SEC: Separation of epithelial layer from the central sinus of the filament

## General Structure of the Liver (Plate: 2A)

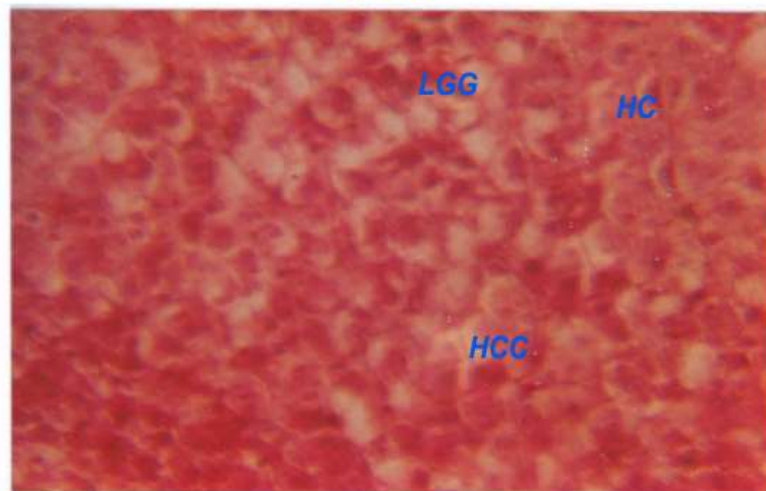
The organ of the largest gland, which shows two colors either reddish brown or lighter brown in herbivores and the fish being omnivorous, it shows much reddish brown. Hepatocytes of fish vary in number and is different with mammals and cannot form a distinct hepatic cords / lobes as in homeotherms. The passage of blood in the form of 'triads' are not obvious. It has distinct endothelial cells lining the border of sinusoids. They are irregularly arranged in their distribution in between the polygonal cells of the liver, which have prominent nuclei due to its metabolic role, to be active, in the process of transcription, always, and as such nucleolus is very prominent. Because, the tested fish is a cultured one, the cells are swollen. The liver, had a secretion of the pancreas being received and had many metabolizing enzymes. Lipid as well as glycogen granules are also prominent.

The Atrophy of the individual cells is very specific when Plate-2.A and 2.B (10% days exposed one) are compared. The structural deformity is observed due to differential staining, hetero-pyknosis due to lesions, making the nuclei hypertrophied. All these alterations culminate the other related aspects of histopathology.



# PLATE-2

## FIG-A



## FIG-B

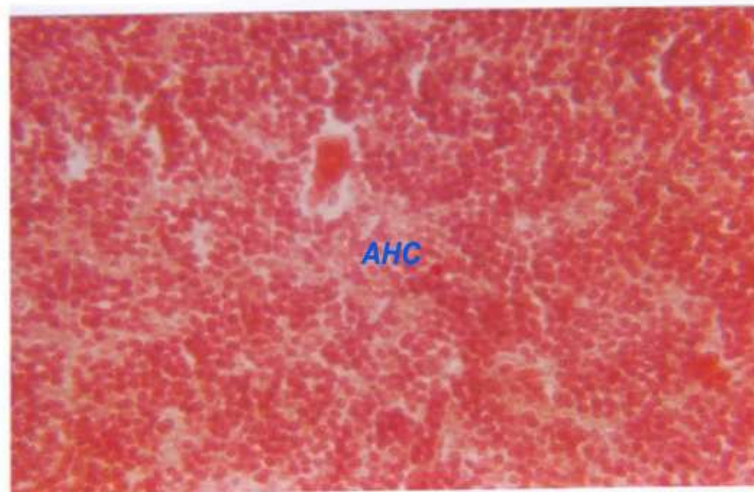


PLATE I :FIG. A Normal Structure of Liver in *Cyprinus carpio* Haematoxylin / Eosin Stain (HE), X400  
HC:Hepatic Cells HCC : Hepatic cell cords LGG: Lipid and Glycogen Granules

FIG. B LIVER of *Cyprinus carpio* exposed for 10 days to sublethal concentration of Deltramethin 11% EC  
Haematoxylin/Eosin Stain (HE),X400AHC:Atrophy in Hepatic Cells

## Kidney – General Structure (Plate-3A)

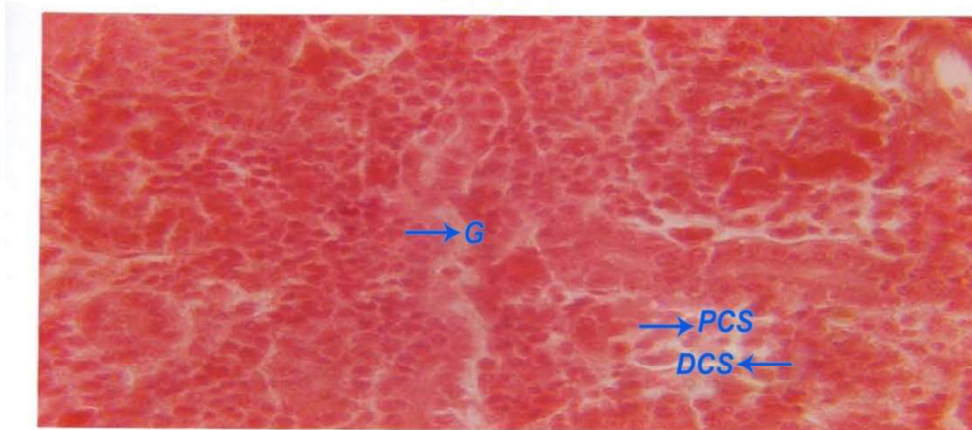
The kidney of the fish is hematopoietic, reticulo-endothelial endocrine and excretory functional components. It is a retroperitoneal organ. It has an anterior part as the hemopoietic nature of function and posterior aminotellic excretory functional nature of the part. It has papillae, finally all connected to a sac like structure the urinary bladder. Physiologically the fish kidney structure anatomically suited for high filtration rate, reabsorption of salts in the anterior part (proximal) and dilution of urine in posterior part (distal) as of convoluted tubules. The nephron as such has renal corpuscle, proximal convoluted tubule first and second segments, columnar cells – brush border with large spherical nucleus at the base, as well as less dense brush border of columnar cells with oval shape nucleolus, mainly originated from cuboidal cells. This leads into distal convoluted tubule of lower columnar cells, oval basally located nuclei. Finally it ends in collecting tubular part that open into urinary bladder.

The degeneration as well as atrophy is observed as per Plate-3B, in the parts of the kidney renal tubules, Glomerulus and the whole tissue pathologically damaged to have more spaces, lacking the internal coordinative function of absorption, secretion and excretion.



# PLATE-3

## FIG-A



## FIG-B

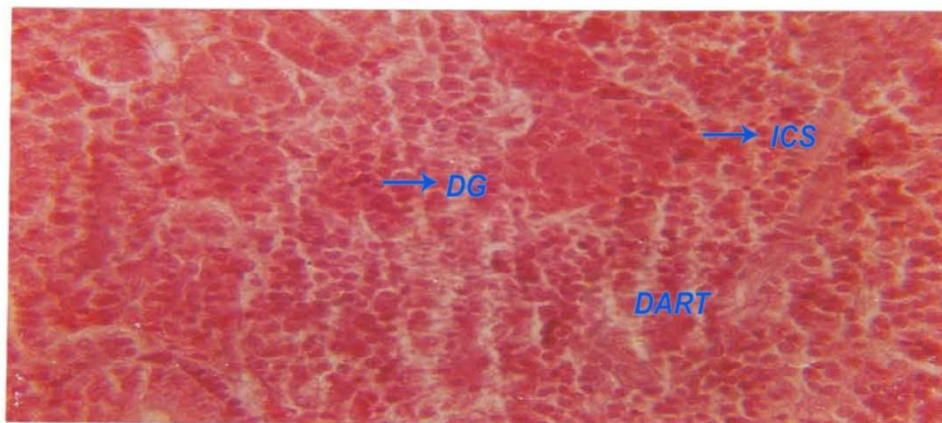


FIG. A Normal Kidney Structure in *Cyprinus carpio* Haematoxylin / Eosin Stain (HE), X400  
 PCS:Proximal Convoluted Segment DCS:Distal Convoluted Segment G:Glomerulus

FIG. B Kidney of *Cyprinus carpio* exposed for 10 days to sublethal concentration of Deltramethin 11% EC  
 Haematoxylin/Eosin Stain (HE), X 400 DART : Degeneration and Atrophy in Renal Tubules DG :  
 Degeneration in Glomerulus ICS : Intercellular Spaces formation giving mesh like appearance



## DISCUSSION

### Gill

Sana Ullah *et al.* (2019) in their review work mentioned about the reports of histopathological effects of the tissues/organs in the fish. They mentioned about the outlines of communique by Ullah *et al.*, (2019), Parlak (2018), Liu *et al.*, (2018). Guirdiola *et al.*, (2014), Al-Ghanbousi *et al.*, (2012), Kan *et al.*, (2012) and Yildirim *et al.*, (2006).

Similarly, Saumya Biswas (2019), too, in their review article, made a mention of the work, by Staicu (2007) and Srivastava *et al.*, (2010) also. All the reports mentioned above two articles pertain to deltamethrin induced changes that are observed histopathological manifestations of the chemicals in different organs of the fish.

Ullah *et al.*, (2019) in the silver fish, due to the exposure of Deltamethrin as a toxicant, liver reported to have a damage as 'congestion, increased sinusoidal spaces and enlargement as well as the necrosis of cells, disorientation of the cells which got inflammation, fibrosis, shrinkage, hemosiderosis while in gills, a disruption of gill arch, atrophy of lamella and necrosis. Similar such observations are also evident in the present study. Even the studies of Mohammad *et al.*, (2016), due to toxic action of deltamethrin in fish, Nile tilapia, similar such changes of Ullah *et al.*, (2019) and the present study also were reiterated. Apart from reported changes in liver too, confirms the present study observations.

Parlak (2018) while working on the fish, *Danio rerio*, reported that deltamethrin had a profound impact on during development stages of the organs, more so in gills and liver that resulted several contortings in the stages of development. The same aspects were reported by Liu *et al.*, (2018) in the same fish resulting lesions in the fish tissues/organs while in development, that were observed histopathological nature. Cunhar *et al.*, (2018) in the fish *Colosoma macropomum* observed damage of the gills, while due to toxic action of the deltamethrin.

Guirdiola *et al.* (2014) due to the toxic effect of deltamethrin in the fish *Sparus aurata*, the largest and highly metabolized organ the liver had reported structural changes. Al-Ghanbousi *et al.*, (2012) in the fish *Aphanius dispar* reported to respiratory effect which were due to architectural damage of the gills. The histopathological changes, as fusion of the secondary lamellae, damage of the squamous epithelium lining of the gill and in the cytoplasm of cells appearance of vacuoles and also peeling, in pathological terms termed as 'desquamation'. The same alterations are observed even in the present study.

Kan *et al.* (2012) in the fish *Oreochromis niloticus*, reported the changes at the tissue of the liver due to the poisonous action of deltamethrin. The pathological terms necrosis, pycnosis and hypertrophy and also in gills, the epithelial lining lifting, hypertrophy too, in mucous cells as well as hyperplasia and even the present study is no exception. Diana *et al.*, (2007), in their study report of deltamethrin to the fish *Carassius auratus* after exposure at 2 µg/L observed 'hyperemia' fusion of the secondary lamellae, rupture of the epithelial layer, atrophy and proliferation of the chloride cells, hyperemia which can be correlated as similar observation of the present study.

In the same fish Yildirim *et al.*, (2006), observed that deltamethrin resulted changes in the liver and gills. The terminology they used in the former organ as 'hydropic degeneration' and later fusion of lamellae of secondary gill arches, and used two aspects as hyperemia' (excess of blood) and 'telangiectasis (aging effects). The above effects as such changes and terminology aptly, also suits even in the present study.

Cenziget *et al.*, (2016a and b) in the present studied fish and with the same toxicant reported necrosis, aneurysm, lifting of linings, oedema and hyperplasia which all holds good, even for the present study also.

Cenzig and Unlu (2006b), in the fish *Gambusia affinis* mosquito fish, exposure to deltamethrin at 0.025 µg/L and 0.5 µg/L concentration for 10, 20 and 30 days reported the changes in the liver, as hepatic lesions such as hypertrophy of cells, Kupffer cells increase, focal necrosis apart from circulation disturbance of blood, fatty degeneration due to nuclear pyconosis and narrowing of sinusoids which all support the present studied fish using the same toxicant. This is also supported by the study of Yeldirim *et al.*, (2006) *Oreochromis niloticus*. The three important chemicals of the type II synthetic pyrethroids, deltamethrin, cypermethrin and fenvalerate but they differ in their toxic effects to the fish, Even we turn to focus our attention on the other two examples also apart from the toxicant tested the following reports are worthy to mention.

Monir *et al.* (2015) in the fish *Pangasianodon hypophthalmus* due to cypermethrin toxic action induced certain changes in the liver and gills; Ullah *et al.*, (2015) in the fish *Tor putitora* gills and liver using cypermethrin, Singh *et al.*, (2015) in the fish *Chrias butracus* using  $\lambda$ -cyhalothrin which induced changes in the gills and kidney.

Manjula Vani and Veeraiah (2014) in the fish *Cirrhinus mrigala* exposed to Cypermethrin of 10% only, observed alterations of degeneration, bulging of the tips of the primary gill lamellae, resulting club shaped and even observed necrotic changes in the epithelial cells of the secondary lamellae, all the of them were due to the action of the toxicant. They opined also commercial formulations having other ingredients (90%) are also contribute toxic action, which even of the present study can be of similar nature. Velisek *et al.*, (2009) in the fish *Onchorynchus mykiss* using bifenthrin, Marigounder *et al* (2009), in the fish *Cyprinus carpio* using bifenthrin and Singh and Singh (2008) in the fish *Heteropneustes fossils* using Cypermethrin too reported such changes due to the toxicants. All the above studies had a profound effect on the fish tissues/organs wherein the toxicants cause such changes. Even the studies of the other toxicants of the same type of synthetic pyrethroids, fenvalerate and cypermethrin support the present study.

Anitha Sussan and (2012) too reported in the fish *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* using fenvalerate technical grade and 20% EC as toxicants reported the histopathological changes in the liver tissues//organ. Blood cells deposition due to hyperemia was the notable change as in the present study, they mentioned as their observation similar to the present study.

According to the study report of Sakr *et al.* (2005) in the air breathing fish *Clarias gariepinus* exposed to fenvalerate, Yacobu (2002) using same as toxicant in the fish *Ctenopharyngodon idella* both opined in the similar line of above.

Anitha Susan (2012) studied at sub-lethal concentration of fenvalerate (0.0006 mg/L) exposure in the fish *Cirrhinus mrigala*. The observed changes in gill as a damage of the epithelial layer and separated from the primary lamellae and bulging of the later is more compared to the secondary lamellae. Tilak *et al.* (2001) reported changes in the gill of the fish *Ctenopharyngodon idella* exposed to technical grade fenvalerate and 20% EC. The observations as progressive degeneration bulging of the tips of primary gill lamellae, club shaped secondary lamellae and also necrotic changes, in the compound epithelial tissue of the gill. Vee-raiah (2001) reported in the fish *Cirrhinus mrigala* exposed to the Cypermethrin, it resulted the same changes as above in the gill.

## Liver - Discussion

Karin *et al.* (2016) reported in the fish *Hypothalmichthys moltrix* after acute exposure of deltamethrin, the present studied toxicant and observed necrosis, hypertrophy of hepatocytes vacuolization, nuclear atrophy as well as pyknosis and even the narrowing of the blood vessels.

Sayeder *et al.* (2007) in the fish *Oreochromis niloticus*, Staicu *et al.* (2007) in the fish *Carassius auratus* (exposed to 1, 2, 3, 7 days) both using deltamethrin as toxicant made the point clear that hepatotoxicity only was resulted.

Andem *et al.* (2016) reported in the fish *Oreochromis niloticus* exposed to Cypermethrin in the African clarified mud catfish, *Clarias gariepinus* fingerlings, reported necrosis haemorrhage and hyperplasia in the hepatic cells.

Sree Vani and Veeraiah (2014) reported on the effect of Cypermethrin in the fish *Cirrhinus mrigala*. The histopathological changes which coincides with the present study includes: (1) degeneration of cytoplasm volume which had an impact on nuclear volume (2) Atrophy (3) Vacuoles appearance (4) necrosis, and (5) disappearance of hepatocytic cell wall and disposition of hepatic cords. Velumurugan (2009) reported histopathological changes of cypermethrin in the fish, *Clarias gariepinus* liver apart from gills and kidneys.

Velisek (2006) reported on the rainbow trout, *Oncorlynchus mykiss* exposed to cypermethrin resulted changes in the liver cells mainly focused their attention of the appearance of the vacuoles that disturbs

many biochemical functions of the organ. Similar such thing is observed in the present study. Metabolic conversion of Glycolytic cycle, Glycolysis and Gluconeogenesis all are important in the liver, which when damaged the survival of the fish is going to be limited.

## Discussion – Kidney

Stain *et al.* (2007) in the fish *Carassius auratus*, Yieldrin *et al.* (2006) in the fish *Oreochromis niloticus*, Cenzig (2006b) in the fish *Cyprinus carpio* exposure to deltamethrin reported changes aimed on the histopathological nature of effects in the kidney. The above studies reported lesion, epithelial cells of the squamous epithelium of the renal tubules, nuclei of differential staining (the term-pycnotic nuclei) in the haemopoietic tissue, which is supposed to produce erythrocytes, capillaries which are important for filtering of excretory wastes, glomeruli degeneration, the appearance of vacuoles in the cytoplasm, hypertrophy of renal cells and lumen of the tubule is narrowed. All the three above studies were similar in lines and coincide with the present study.

Even with the studies using Fenvalerate and lambda – cyhalothrin (in the concentrations of 1.5-3.0 ppb and also 0.3 & 0.6 ppb respectively) in the fish *Cirrhinus mrigala* in sub-lethal concentration. Velumurugan *et al.* (2007) reported in the similar lines and added an extra observation that at the point of filtration Bowman capsule and glomerulus, the space which was expanded rendering the process not to be normal. Such things can also be viewed due to the severe damage caused by the toxicant in the kidney of the present studied fish also.

Such observations were also reported by Anitha Susan *et al.* (2012) in the three fish *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* kidney tissues. The kidney degeneration was due to the toxicant action as a result biochemically the excretion process of the fish, ultimately will be affected.

Neelima *et al.* (2015) in the fish and Manjula Vani and Veeraiah (2014) reported using cypermethrin as toxicant in the fish *Cirrhinus mrigala* and *Cyprinus carpio* respectively. The changes of degenerative nature are noted and also support the present work. Such similar observations were also reported by Velumurugan *et al.* (2009) using Cypermethrin as toxicant in the fish *Clarias gariepinus* (Burchell, 1822). The damage of the blood tissue, border of the Bowman's capsule had necrosis and a lot of atrophy in the tissue. Such observations even support, further. The present studied fish tissue using the deltamethrin as the toxicant.

Prasanth (2011) in the fish *Cirrhinus mrigala* exposed to the toxicant cypermethrin and kidney damage in histopathological aspect was observed and reported.

In the sections of microphotographs, the author observed the cuboidal epithelium resulted due to vacuoles appearance degenerative cytoplasm, nuclear divisions were resulted such that the lymphatic cells profusely increased, along with macrophages. Such things can be visualized even in the kidney that was damaged by the toxic action even in the present study.

It is the nature of the toxicant, to which groups/category it belongs the irrespective of the fish tissues viz., gill, the entry point, liver highest metabolic site and kidney the exit point when get damaged and result will be on the very survival of the organism.

Liliana cristina soare *et al.* (2019) in their aspect of observation that the stress induced by the pesticides in the environment firmly opined that the main point of exchange of ions with the medium, when got damaged permeability will not be there and the oxygen uptake got substantially reduced. The chloride cell proliferation result in excess salt imbalance and such changes will make the gill tissue not to function normally.

Rajini *et al.* (2015) in the fish *Danio rario* upon exposure to pesticides in combination of Chlorpyrifos (50%) and Cypermethrin 5% EC [one is an organophosphate and other the synthetic pyrethroid] reported changes in fish tissues. The study is a different one, wherein the lesions that were observed in the tissues of gills, liver, kidney apart from spinal cord for different days of exposure of 7, 14, 21 and 28 days viewed via recorder of observation of lesions by mass spectroscopy and chromatography. Such advanced studies pinpoint the focus of the exact position of the lesion that occurred. Histopathological changes in the fish tissues, that were reported confirms the things were possible by a record of such things, noticed, separately. But the changes that were reported mostly coincide with the present study.

With the present studied fish *Cyprinus carpio* but using different toxicant carboxin – thiram (fungicide), the gill tissue damage was reported by Hamad *et al.* (2015). The secondary gill lamellae shrunk while fusing, aneurysm, clubbing and hyperplasia of gills and damage of the liver sinusoids. Similar changes that are observed in the present study were also mentioned.

In the fish *Oreochromis niloticus* while exposing to the synthetic pyrethroid cyhalothrin, Juliet Selvarani *et al.* (2019) reported the observed changes in the tissues of gill and liver. The toxicant is another example of the type II synthetic pyrethroid. The gill lesion, necrosis curling of secondary gill lamellae, and in liver irregular shape of the nucleus, aggregation, hepatolysis and malformation of the tissue were reported. Most of the changes are also observed even in the present study.

## CONCLUSION

The tissue damage by changes that are observed by microscope and all architectural alterations are the signs of the toxic action. They serve as a tool of biomarker study to infer the indices of the toxicant in the pollution load. When we focus our attention on the 11% EC, as a commercial formulation, the ingredients have to be viewed seriously in checking the quality control. The effects are on the vital organs gill, liver and kidney and the possibility of the survival is limited and hence might be the causative factor of toxicity.

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