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HISTOPATHOLOGICAL ALTERATIONS OF THE GILL, LIVER AND KIDNEY OF THE FISH *CATLA CATLA* (HAMILTON) EXPOSED TO DICHLORVOS 76% EC (NUVAN)

SUNDARA RAO G.[1], P. RAMA KRISHNA PRASAD[1], G. SAMBASIVA RAO[1] AND N. GOPALA RAO[2]

Department of Zoology and Aquaculture, Acharya Nagarjuna University,
Nagarjunanagar 522 510, A.P., India

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Abstract –The fish, *Catla catla* exposed to an organophosphate Dichlorvos 76% EC (Nuvan) in the laboratory for 10 days in sublethal concentration as $1/10^{th}$ of 96h LC_{50} value (1.445 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 effected 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.

INTRODUCTION

The contamination of aquatic bodies by many pollutants is a phenomenon that is happening and is a global problem, since the subdivision hydrosphere of the planet earth is the highest component apart from atmosphere and lithosphere. The water component of marine, esturine and freshwater is in the process of continuous pollutants defilement act because all pollutants are not toxicants but all toxicants are pollutants and one among them is none other than the chemicals the pesticides. The large scale contamination by them was noticed and cautioned by Tang *et al*., 2021; Kelly Mac Namura, 2019 and Zeshan and Praveen, 2020 for global safely. In India, because of green revolution (second time), blue as well as white revolutions that are promoted time to time enhanced not only these pesticides production but also their use. Yadav and Dutta, 2019; Priyadarshini and Kumar Anamika, 2019; Srivastava *et al*., 2018; Somayyah *et al*., 2017; Monali and Roy, 2017; and Indira Devi, 2017 explained the use and alarming levels of pesticide load in the aquatic environment that were earlier reported.

In the aquatic environment, these toxicant chemicals of pesticides are harmful and effect the non-target organisms which according to the review articles by Sana Ullah *et al*., (2019); Prusty *et al*., (2015); Ullah and Zorriezahara (2015); Murthy *et al.,* (2013) for all pesticides including the synthetic pyrethroids, Sunanda *et al*. (2016) for chlorpyrifos and Chandrasekhara Rao *et al*., (2017) for Dichlorvos (the present tested chemical). In the review articles, the effects that are mentioned also include a study of histopathology and their effects.

The above articles also contain and recognized the effect as 'biomarkers' that serve the indices of the toxicant action according to Kaviraja and Gupta (2014) as well as by Sana Ullah *et al*. (2019).

The indices study of biomarker (Yanchavo *et al.,* 2015) of histopathology, as organs are made of tissues which intern by cells only. The fundamental units and then the biochemical interactions make the living thing to have their sustenance and all physiological aspects. If by any reason, the organs of the organisms, fish gills, liver and kidney are damaged due to the toxic effect of the toxicant pesticides such lesions when studied serve definitely the key of the toxicants at its extreme action.

Such histopathological studies of the toxicant pesticides were reported in the above mentioned review articles as well as by individual studies by Naqash Khan *et al*., (2021); Dhivya and Devadason, (2021); Shehezad *et al*., (2021); Padmini *et al*., (2021); Rachele *et al*., (2020); Kadam *et al*., (2019); Arumugan Stalin *et al*., (2019); Sengi and Isisag, (2019); Mohammad *et al*., (2019); Greesma *et al*., (2019); Suchitha *et al*., (2018); Japamalai, (2017); Bhat *et al*., (2016); Nannu *et al*., (2015); Nidhi Srivastava, (2014); Kaya *et al*., (2013); Manju Singyh and Santosh Kumar, (2013); Tamil Selvi and Ilavazhana, (2012) and Yogita Devi and Abha Misra, 2012.

However, not much work is the present studies focused on toxicant Dichlorvos (76% EC of Nuvan) was carried to the fish *Catla catla*, by taking 1/10th of LC_{50} value of 96h of continuous flow through system and this for the first time such study is attempted.

Hence, in the present study, the fish *Catla catla* was taken, which is a food of fish by many, palatable one of the majority of the human beings among the freshwater fish and is tested for the histopathological studies of the three organs gills, liver and kidney, (the entry point, metabolic point and exit point of the toxicant).

MATERIALS AND METHODS

Fresh water fish common carp, *Catla catla* was first acclimatized to the laboratory conditions for 10 days. 50 numbers of the fish are exposed to 76% EC (Nuvan) Dichlorvos for 10 days by taking into consideration of LC_{50} value of 96 hours (1/10th of the 96h LC_{50}) value 1.445 µg/l as per the APHA guidelines (1998, 2005 and 2012).

The fish are randomly selected for histopathological observations after 10 days of exposure. The exposed fish tissues viz., the gill, liver and kidney tissues are separated and also not exposed fish to the toxicant as well, which serve as control.

The method for fixation and processing of Humason (1972) is followed wherein the physiological saline solution of 85% NaCl was only used for rinsing and also for cleaning. After fixing in Bouins solutions for 48 hrs, then processed through graded series of different alcohols, cleared in xylen. The tissues/organs are embedded in paraffin wax.

According to Humason (1972) thickness of 6 µm sections were made to cut and stained by Ehlrich haematoxylin/Eosin dissolved in 70% alcohol and mounted on Canada balsam. The sections were observed in digital microscope (Intel ply – Q x 3 at 200 x magnification). By the Q x 3 Computer Intel Pentium attached microscope of 400x lens (made in China) the changes that manifested in the respective tissues are examined and photographed.

OBSERVATIONS AND RESULTS

Gill: Normal structure as the control (Plate VI.1)

The fish, *Catla catla*, the teleost group of the fish which is one among the major carp have four pairs of gills made of cartilage the principle respiring organ through which it filters the waters including the contaminated one apart from food the plankton. Each gill apart from paired, has double rows of the gill arches termed primary lamellae and from the base of it radiate the transvers rows of double the secondary lamellae and the number of it is more. The entire gill arches are covered by operculum and the primary lamellae are well protected by the epidermis as a thick covering and in its mucus cells are more in number whose function is to secret the protein mucin. Below to this external of covering, lymphoidal tissue whose arrangement is specific. The lamellar arch of the gill containing the primary lamellae part has mucoidal epithelium which contains predominantly chloride cells for transportation of respiratory gases as well as for secretion of ions. The whole, component as a compound tissue of several layers, squamous epithelium, epidermal cells, cuboidal cells and finally columnar cells. The secondary lamellae play the role in respiration where it provides the facility of diffusion. The secondary lamellae are inturn have microvilli for adsorptive processes and as such provides many functions. Besides all, there are several goblet cells whose function is secretory.

Pathological manifestations observed in the gills

In the fish exposed to sublethal concentration of the gills of the fish *Catla catla*, overall fusion of the secondary gill laminae, whose prime function is to provide greater surface area of diffusion of inspiring oxygen gas and expiring carbondioxide is drastically reduced (Fig. 2 & 3 of Plate 1).

In the histopathological observations prominent feature of the epithelial linning of the secondary lamellae is lifted which means seperated from the tissue of compound nature which lacks functional continuum in the process of functioning. In certain

regions/portion hyperplasia (Enlargement of the organ) is also noticed. When the place legended as 'C' (Fig. 3) hyperplastic curling of the secondary lamellae is observed which prevents the functional, coordination. The dialation of the capillaries, pathological term as 'telangiectasia'is also noticed in the gill tissue ('T' – Fig.3) and such abnormal nature of the structure the secretion and diffusive functions are curtailed. Shortening of the secondary lamellae, cartilaginous core disruption (damage) and Atrophy (inundation of the tissue) are also noticed in the pathological observations which make the gill had an impact on the processes of respiration which is its prime function, apart from its other ones including structural coordination and cohesion.

The oxygen consumption of fish is affected if any and the same can be taken into cognigence as the tissue damage of the gill which impairs metabolism due to lack of oxygen and the fish was likely to be retardation of the growth. In aquacultural practices, the carp *Catla catla* is one of the candidate species, if the growth is impaired there is a economical loss in the profitable venture of the aquaculture.

General Histology of Liver (Plate 2)

The entire surface of the liver is covered by a serous membrane (squamous Epithelium) derived from endodermal derivative. It is permeated by the contective tissue extend internally as thick cords called by name hepatic cords (Mesodermal derivative). It has many fibers reticular, of both yellow and white and as a whole the entire thing in the forms of a sinusoid (lobular nature) permeated by canalculi which carry bile Juice at the central part which chemical contains glycolipid granules of larger quantities.

PLATE-1

Histopathological Alterations in the gill of The Fish Catla catla Exposed to dichlorvos 76% EC

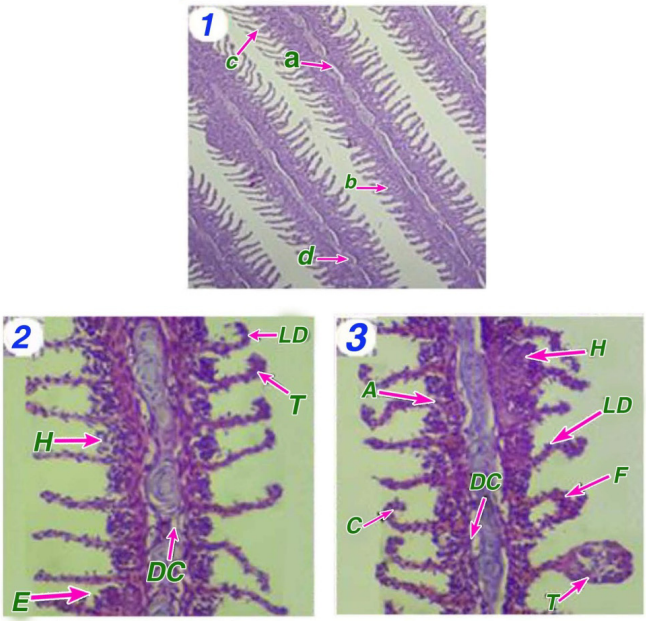

FIG -1 : Control-normal structure

(a) primary lamellae (b) secondary lamellae
(c) lamellar epithelial cells (d) pillar cell

FIG - (2&3) : Exposed

(F) fusion of secondary gill lamellae
(E) epithelial uplifting of secondary gill lamellae
(H) hyperplasia of gill epithelium (C) curling of secondary gill lamellae
(T) telangiectasia (DC) disruption of cartilaginous core
(LD) lamellar disorganization and (A) lamellar atrophy

Liver reddish colour with polygonal shape with a prominent spherical nucleus which by location centrally positioned. The chromatin part of the tissue is at the periphery and nucleolus is prominent because of its role in synthesis of RNA. The whole tissue/organ has both rough as well as smooth endoplasmic reticulam (RER and SER) with acinor cells that serve in the secretory as well as detoxification aspects respectively. With numerous metochondria for energy synthesis the liver performs several functions for which energy is needed and is the largest metabolic gland.

Pathological observations

Mild necrosis, blood is congested infiltration of the whole tissue that interfere in functional continuum hepatocytes showed pyknotic/pyknosis aspects that disturb the functional cohesion, cascade type of biochemical reactions are also impaired and in the serve pathological condition phagocytes accumulation vacuoles appearance and hypertrophy of the entire tissue is observed.

General Histology of Kidney (Plate 3)

Fish kidney, aminotellic in secretory function, conservation of silts aglomerular in its function apart from haemopoietic nature. It has reticulo-endothelial endocrine as well as excretory roles to perform. It has glomerula filteration as well as salts conservation of reabsorption in 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 glomalular 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 gap between loops of the glomalar 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 cubodal epithelium, densely arranged. The segment of the RT is having microvilli for absorption whereas they are absent in the segment I (Brush border).

PLATE-2

Histopathological Alterations in the Liver of The Fish Catla catla Exposed to dichlorvos 76% EC

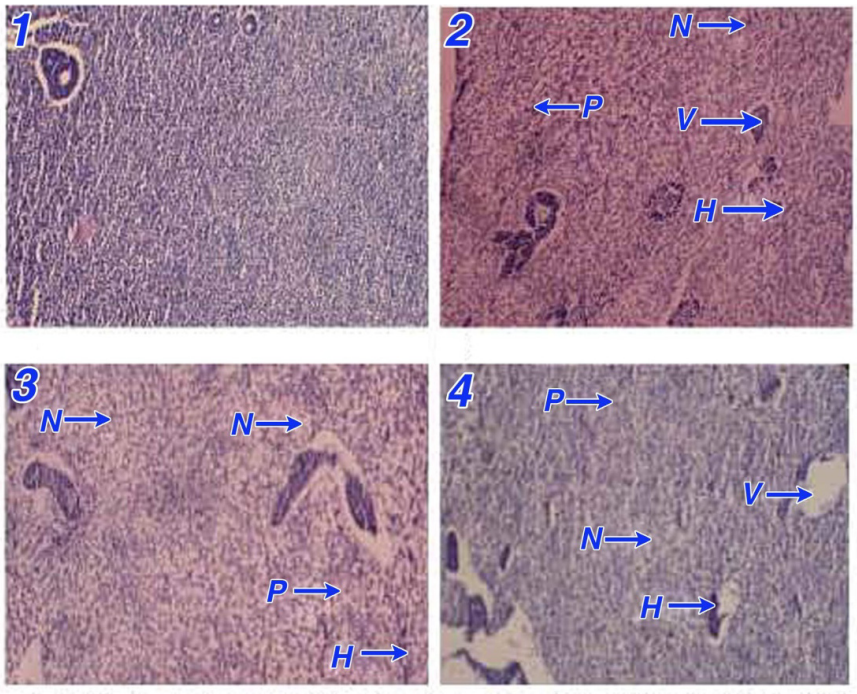

Fig. (1) Control-normal structure
(2,3&4) Exposed (N) necrosis (P) pyknosis (V) vacuolation and (H) hemorrhage.

Pathology of the Kidney – observed (Plate 3)

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

DISCUSSION

Gill

Naquash Khan *et al*. (2021) in the fish *Ctenopharyngodon idella* studied the gill of the fish and reported during the risk assessment of the toxicant serving as 'biomarker' indices of the toxic action that prevailed, in the two different concentration of sublethal for of '5' different days of exposure (as 1st, 3rd, 6th day, 9th day, 12th day and 15th day). The chemical was used for mosquito control had an impact on the tissues/organs that had drastic impact on the normal functioning. Similarly, in the fish *Oreochromis niloticus* due to exposure to lead nitrate and in sublethal concentration (0.32 mg/L)

PLATE-3

Histopathological Alterations in the Kidney of the Fish Catla catla Exposed to dichlorvos 76% EC

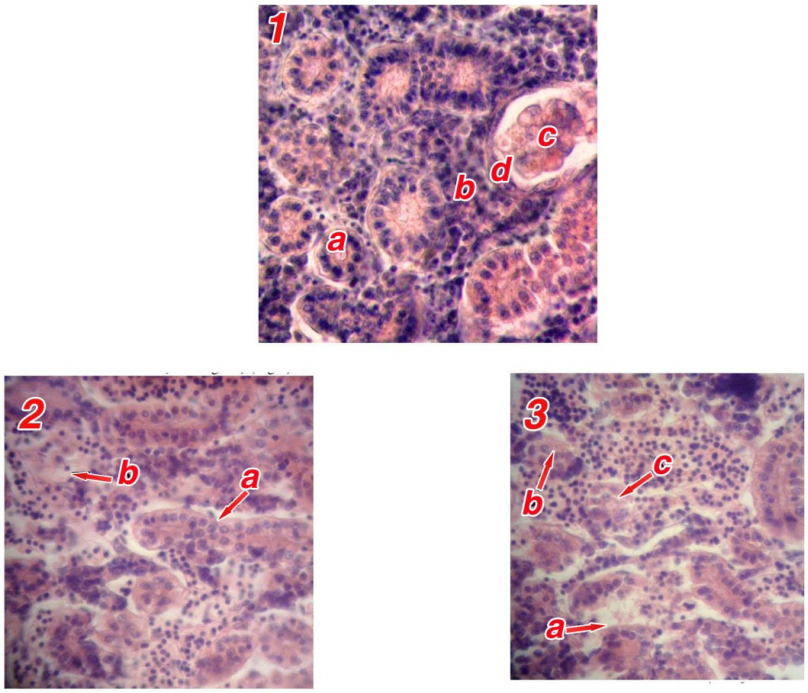

Fig. (1) Control-normal structure
(a) Renal tubules (b) Hematopoietic tissue
(c) glomerulus (d) Bowman's capsule.

Fig. 2. Exposed
(a) Tubular shrinkage
(b) Reduction of the Interstitial haematopoietic tissue

Fig. 3. **Exposed**
(a) Reduction of the interstitial haematopoietic tissue
(b) Tubular shrinkage
(c) degeneration in the epithelial cells of renal tubule

for 21 days. The observations of the present study the hyperplacia of the lamellae fusion of both primary and secondary, the decease of the space, curling and shortening too apart from aneurysm (excessive localized swelling) was reported by Dhivya and Devadason (2021).

Shehzad *et al*. (2021) reported in the fish *Cirrhinus mrigala* due to exposure of three pesticides for 10, 20 and 30 days exposure duration, where gill was drastically damaged, the entry point of the toxicant. Most of the observations were similar of the present study. Padmini *et al*. (2021) reported in the fish *Channa gachuva* due to the present studied toxicant Dichlorvos in sublethal concentration for 30 days, Juan *et al*. (2021) in the fish *Solea senegalentis* due to Malathion too had an impact of the gill architectural damage. Rachale *et al*. (2020) in the sea water fish, *Thallassoma pavu* to 96h exposure only (a short term) of chlorpyrifos as toxicant, scattering of globlet cells apart from the severe damage of the tissue as in the present study reported which impair the respiration, the important physiological process. Kadam and Patl (2019) in the fish *Channa gachua* due to exposure of Dichlorvos in lethal concentration (Duration of the experiment was 96h only) and similar changes as alterations were reported as in the present study.

Arumugan Stalin *et al*. (2019) in the fish *Channa punctata* reported gill morphological changes of alterations due to the exposure of the toxicant chlorpyrifos for 10, 20 and 30 days of duration in sublethal concentration. The prominent of the impediments that caused in the transportation of ions molecules of the lifting of gill epithelium which even was observed in the present only.

Sezgi and Sema (2019) reported in the Zebra fish *Danio rerio* for 4 days exposure in 1, 2 and 4 mg/L concentration of fonofos an organophosphate and observed for any alterations as effect. The epithelial lifting of both sides of secondary lamellae apart from curling of it, hyperplacia and fusion of the both the lamellae which are even observed in the present study were resulted. The pathological condition of desquamation (shedding of layers) was also observed due to its effect.

Due to exposure of an organophosphate, the diazonin in three different fish exposed to six different concentration some of the alterations that were reported are in agreement of the present study and the organs were not to have the normal function (Md. Islam *et al*. 2019).

In the fish *Oreochromis mossambicus* (Peters) due to Quinalphos, an organophosphate after 5 days exposure only in 0056 and 0.14 μg/L (LC_{50} value of 96h was 0.56 μg/L) by Greeshma *et al*. (2019) studied and reported. Viewing the study of a 'biomarker' of the present case also and the results of curved as well as shortening of the secondary lamellae, apart from Edema and Haemorrhage were also reported as alterations.

In the same above fish due to the another organophosphate, Monocrotophos exposure at 0.00045 ppm concentration for 3 periods of exposure (10, 20 and 30 days) Sucitha *et al*. (2018) reported the alterations of the gills. Atrophy was reported as erosion of epithelial linning (the squaenous epithelium) apart from swelling and fusion of both the lamellae (the primary and secondary).

Japamalai (2017) in the fish *Labeo rohita* due to the exposure of the present studied toxicant Dichlorvos 76% EC, a different terminology was used bulging of the primary gill lamellae tips, curling of the secondary lamellae (gill filaments) in their report, the grandular Epithelium consisting of columnar (pillar) cells lost their nucleus resulting the fusion of the secondary lamellae all were reported.

Chandrasekhar Rao *et al*. (2017) in the review article of Dichlorvos on the effects of fish as histopathological nature mentioned the earlier reports of different organs of gills, liver and kidney. Bhat and Bhat (2016) in the fish *Labeo rohita* fish intestine, Kumar (2016) in the fish *Channa punctata*, Kumar and Gautam (2014) in the same fish, Srivastava *et al*. (2014) in the fish *Cirrhinus mrigala*, Sukhiritha and Usharani (2013) in the fish *Danio rerio*, Velumurugan *et al*. (2009) in the *Cirrhinus mrigala*, Sukla *et al*. (2005) and Benarji and Rajendranath (1991) in the fish *Clarias batracus.*

Nguyen *et al*. (2015) reported an important aspect how Dichlorvos effect the carbohydrate metabolism paving the way of the disruption of the epithelium layers to regenerate and the atrophy of the lamella lacks the process as a result the synthesis of glycoproteins and glycolipids was not there, hence the damage.

Yanchova *et al*. (2015) in their review article mentioned that the lesions of the respiratory structure of the gills of the fish performing both respiratory as well as excretory processes get effected due to even subtle changes.

Mandeep Kaur and Rajendra Jindal (2016) in the fish *Ctenopharyngodon idella* due to chlorpyrifos, Sadia Sharmin *et al*. (2016) in the fish *Cyprinus carpio* due to Malathion exposure, Hasan Kaya *et al* (2014 &

2013) in the same fish due to phosalone exposure, Nidhi Srivastava *et al*. (2014) in the fish *Cirrhinus mrigala* exposed to Nuvan and Velumurugan (2009) in the same fish were also earlier reported which showed alterations in the gills.

The above all studies are based on the sublethal concentrations of static renewal only and the literature when reviewed the work on *Catla catla*, pathological attentions are not studied earlier. This is the first time report based on $1/10^{th}$ of LC_{50} value of continuous flow through system (CFTS) and even the results are much in agreement of the other studies only the study also proved the same. The gill architectural damage leads to cessation of all biochemical process and the over metabolism is impaired.

Liver and Kidney – Discussion

Naqush Khan *et al*. (2021) reported on the risk assessment of the insecticides ecologically in the fish *Ctenopharyngodon idella*, using the chlorpyrifos as a toxicant considering the study as Biomarker the liver metabolically active organ and kidney the exit point after detoxification when damaged pathologically the functioning of the respective organs will not be normal.

Dhivya and Devadason (2021) due to the metal toxicity in the fish *Oreochromis niloticus* reported hypertrophy, vacuoles appearance in the cytosol, necrosis, the boundaries of the hepatic cells were not clearly demarcated and finally the nuclei of the hepacytes had pyknotic aspect.

Shehzad *et al*. (2021), made a study of comparison chlorfenopyr, Dimethoate and Acetamiprid of the organophosphates in the fish *Cirrhinus mrigala* under long term exposure. They reported that in the liver, the effect was duration dependant, necrosis, sinusoids enlargement vacuolation and hypertrophy similar as in the present study.

Arumugam Stalin *et al*. (2019) in the snake head murrel due to chlorpyrifos intoxification, the liver was reported that the hepatocytes had necrosis and lamina porpria damaged apart from fibrosis.

Md. Islam *et al*. (2019) in the fish *Channa punctatus*, *Heteroneustes fossils* and *Anabas testidinus* after exposure to diazonin exposure resulted vacuoles in the sinusoids of the liver and glomular part of the kidney damaged and the toxic stress resulted severe alterations in the metabolic as well as excretory organ.

In *Oreochromis niloticus* fish while exposing to the synthetic pyrethroid cyhalothrin, Juliet Selvarani *et al*. (2019) observed the changes in the tissues of gill and liver that were reported. 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.

Yanchova *et al*. (2015) in their review article recognized the histopathological alterations in the organs/tissues as biomarker study and mentioned some of the earlier reports that made liver and kidney had a architectural damage due to the pesticides exposure. Some of the changes even in the present were reported as of the reported studies.

Nannu *et al*. (2015) using Kinalux an organophosphate reported liver and kidney damage in the fish Nile tilapia. The degeneration of the hepatocytes, rupture of the capillaries and Cannelculi resulting hemorrhage and RBC cells were not metabolized. In the kidney, the convoluted tubules got disintegrated, vacuoles in the cytosol, lumen and epithelium.

Satyapaul *et al*. (2014) studied and reported in the fish *Channa punctatus* only kidney due to the exposure of folidol. The appearance of the vacuoles, hypertrophy apart from pykuosis was reported and majority of the results are in agreement.

Yogita Devi and Abha Mishra (2013) in the same above fish, due to the exposure of the chlorpyrifos reported that liver had an alterations such as necrosis, hypertrophy, dialation of the sinusoids apart from pyknosis and karyokinesis.

Velumurugan *et al*. (2009) in the fish *Cirrhinus mrigala* due to the exposure of the present studied toxicant had a severe impact on the liver, swelling or bulging of the cells, congestion degenerative vacuoles, Nucleus disintegration and nucleus bulging were reported. As a result of the above changes the metabolic organ do not function normally.

The following studies were reported earlier by taking the synthetic pyrethroids as toxicants.

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

Andem *et al*. (2016) on the fish Oreochromis niloticus exposed to cypermethrin in the African claried mud catfish, *Clarias gariepinus* fingerlings,

reported necrosis haemorrhage and hyperplacia in the hepatic cells.

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 observations of similar nature 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 Bownans capsule had necrosis and a lot of atrophy in the tissue. Such observations even support the results of the present study.

Sree Vani and Veeraiah (2014) reported on the effect of cypermethrinin 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.

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

Rajini *et al*. (2015) in the fish *Danio rerio* 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.

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.

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 76% 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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