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Studies on Histopathological Alterations in the Brain and Gill, of *Cyprinus carpio* Exposed to the Insecticide Afidopyropen

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Abstract

The histopathology of the brain and gill tissues of the fish *Cyprinus carpio* following exposure to afidopyropen was determined by light microscopy. This particular carp species is one of the most prevalent in India. Afidopyropen (0.2 mg/L) was administered to fish for 1, 10, 20, and 30 days as part of an experiment. The tissues in the control group were found healthy. The abnormalities in the brain were Neural Degeneration (ND) Pyknotic Neuron (PN) Necrotic Internal Granule Cells (NIGC) Demyelination (DM). In gill tissues exposed to afidopyropen, hyperplasia, telangiectasia, epithelial separation, and total destruction of lamellae were seen. In the current study, afidopyropen damaged the organs, indicating its potential for harm. There were no sudden histological changes in one day-exposed group of the fishes. It could be due to healthy fish's strong and quick adaptation capacity. The severity of stress and the associated histological changes would likely increase over time as the exposure to afidopyropen continues. However, prolonged exposure to the toxicant extremely affects the fish physiology leading to the form of above said pathological lesions.

Keywords: Afidopyropen, Brain, *Cyprinus carpio*, Gill, Histopathology

1. Introduction

Freshwater sources, including those found in rivers, lakes, and wetlands, are essential for human survival and support a vast array of species. As the world's population grows, so does the need for water-related services. Freshwater ecosystems are under a great deal of stress as a result of anthropogenic activity, pollution, stress, and a changing climate that are disrupting the natural water cycle. One of the most serious environmental issues is the impact of toxic contaminants on aquatic life. The examination of aquatic habitats is crucial for determining the level of environmental impact and the efficacy of restoring actions[1]. Aquatic ecosystems contaminated by industrial effluents and sewage water are characterised by the discharge of a complex mixture of xenobiotics that can change important physiological properties in fish[2]. A complex chain of biological and chemical processes can be triggered by the introduction of contaminants into aquatic environments. To comprehend and foresee the nature of those reactions, one must have a fundamental understanding of ecological principles. Edward outlined a law and gave a clear illustration. Assume that wastewater is being discharged into an estuary by a business. The

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dangerous element mercury is present in the effluent. Algae near the discharge experience a decrease in photosynthetic rates as a result of the mercury in the water[3].

A sensitive approach for determining the influence of toxicants on fish health can be obtained by histopathological examination, which also identifies early illness and damage indicators in cells, tissues, and organs. Numerous researchers have also explored these structural modifications in fish as indicators in various tissues from different species[4]. Thus, when characterising toxicological consequences, histopathology is the gold standard. Nonetheless, it is intrusive, time-consuming, and costly. Additionally, employing biomarkers with a known histological distribution may meet the necessity of localising toxic damage to specific organs or tissues as histological testing is sometimes problematic in human individuals. Therefore, the assessment of the harmful effects of toxic compounds on the entire organism usually incorporates a significant amount of histopathological analysis[4]. When it comes to the quick and accurate identification of both acute and long-term negative effects in fish, histopathological events are also thought to reveal the health status of animals exposed to xenobiotics[5]. A test organism's functional reaction to a toxicant at a sublethal dosage involves tissue alterations, which reveal information about the toxicant nature[6,7]. The number of pollutants found in an organism's tissues is the only direct indicator of how much of a total toxicant is delivered to the biota, which identifies the component most likely to infiltrate and impact the aquatic ecosystem[8]. Pollutants create lesions, and some histopathological disorders have been linked to certain toxicants or mixtures of toxicants. Nearly often, biochemical, physiological, and morphological alterations come before overt toxicity symptoms in afflicted species (such as loss of appetite, loss of homeostasis, discolouration, and death). Any early qualitative or quantitative measurements of such morphological alterations may reveal signs of past or ongoing exposure to chemicals and offer insightful information about the mechanisms of toxicity[9]. Afidopyropen is a recently developed novel insecticide against aphids in the agricultural field. It was initially extracted from *Aspergillus fumigatus* and recently extracted from the fungus *Penicillium coprobium* and used to control sucking insects worldwide[10]. Afidopyropen has a unique mode of action compared to other insecticides. It targets insect chordotonal organs by modulating TRPV channels. It's highly effective at a dose of 50 ppm of 10% afidopyropen[11], but its toxicity to edible fishes in rural areas near crops remains a concern

Histopathology reveals the extent of tissue injury and the kind of tissue modification in the fish that is susceptible to the impacts of the contaminants. However, this intern assists in determining the kind of harmful chemical. The detrimental impact of various contaminants on the fish can thus be better understood through the study of histology. The severity of the damage by a chemical substance can be determined by relating the damage to the fish organs[12]. Data on various organs are obtained by histopathological investigation, which also enables the identification of lesions within certain cell types. The histological examination can be employed by the investigator to find changes in tissues and organs brought on by exposure to toxicants if they have a solid understanding of normal anatomy beforehand. Sublethal (adaptive) alterations can be seen in afflicted cells when a toxicant's concentration is high enough to cause just cellular damage rather than death[13]. The brain is one of the vital organs that functions constantly by the signalling mechanism in organisms. The brain consists of neuronal cells, pyramidal cells, and its substances. The neurons are the basic units of communication in the brain system. Directly or indirectly any interruption or contamination in the environment affects the brain cells of an organism. Previous studies have shown that the exposure of animals to heavy metals severely affects the histology of the brain[7].

Several studies rendered the damages caused by pesticides on the brain of fish. The exposure of the sub-chronic doses of profenofos instigated neurobehavioral, apoptotic, and DNA damage in the fish[14]. Several authors reported that untreated effluent discharged from pulp and paper mills into receiving waters is known to be toxic to some aquatic organisms[15-17]. The presence of edema along with the detachment of the lamellar epithelium is the first sign of pathology in fish and one of the more frequent lesions observed in the gill epithelium of fish exposed to different xenobiotics[18,19].

Manifestation of toxicity in fish including ion transfer, gas exchange, acid-base control, and waste excretion are all performed by the gills of the fish. The gills are the primary organs through which toxicants enter the body of the fish. As a result, they are the first organs to come into contact with environmental pollutants and are sensitive subjects for determining the effects of water toxicants in the surroundings. The gills make up more than 50% of its

surface area and it is not unexpected that they are one of the primary target organs for waterborne contaminants[20,21]. The pathological changes in the fish can be a tool to assess toxicological implications in real-life scenarios. Every symptom or pathological condition represents either a defensive mechanism or a failure of the said mechanism[18]. This study was framed to examine the toxic profile of afidopyropen on the edible fish *Cyprinus carpio* in laboratory conditions. The present work highlights the histopathology of the fish *Cyprinus carpio* exposed to afidopyropen for different durations of days.

2. Materials and Methods

2.1 Test Chemical

Commercial insecticide afidopyropen trade name Sefina 400ml (50g/L DC) volume was purchased from Agrochemical suppliers Dharwad-580003, Karnataka.

2.2 Test Animal

Healthy *Cyprinus carpio* Live specimens of 90 ± 4 g weight and 18 ± 2 cm were collected from the local market and water bodies of Dharwad, fishes were acclimatized for a week in a huge cement tank (4×3×4 feet) at a temperature (25 ± 1 ^{0}C). During this period, a commercial pallet was given and health status was monitored before the test. Healthy fishes were exposed to afidopyropen (0.2 mg/L). Throughout the experimental duration, the oxygen concentration and photoperiods 12 hrs dark and 12 hrs light were maintained in Control and treated groups in the test chamber as per 203 OECD guidelines 2019[22]. The water quality parameters were controlled as follows: Temperature- 25 ± 2 °C. pH- 7.04 ± 0.2. Dissolved oxygen- 7.8 ± 0.8 mg/L. Total hardness- 30.2 ± 3.1 mg as $CaCO^3$/L. Salinity; Nil. Specific gravity- 1.001. Calcium- 17.27 ± 0.92 mg/L. Phosphate- 0.031 ± 0.003 mg/L. Magnesium- 0.77 ± 0.4 mg/L.

2.3 Experimental Design

The experimental fishes were kept in glass aquaria with 20 litres of water in controlled conditions along with aerators and maintained optimum temperatures and fish were split into control and treatment groups in order to evaluate the sublethal impact of afidopyropen on tissues. Fish were subjected for 1, 10, 20, and 30 days to 0.2 mg/l or 1/5th of the LC_{50} i.e., 1.0 mg/L. Fish used in experiments were fed daily. Fish were sacrificed and tissues were removed for histological analysis at the conclusion of each experimental exposure period. Tissue analysis Fish from both treatment and control groups had their tissue (brain and gill) removed and preserved in Bouin's fixatives. For the preparation of tissues for histological research, the Humason (1972) procedure was used[21]. Tissues were dehydrated using various alcohol concentrations, cleaned in xylene, and then embedded in paraffin. A rotary microtome was used to cut the 5µ slices, which were then placed on glass slides. For a general histological examination, sections were deparaffinized in xylene, hydrated in ethanol, and stained with haematoxylin and alcoholic eosin (H and E). Using a light microscope, photomicrographs of stained sections were taken[23].

2.4 Ethical Committee

All procedures carried out in this study were done in accordance with the Institutional Animal Ethics Committee's guidelines (IAEC). The animals were used and maintained according to the guidelines imposed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), located in New Delhi, India.

3. Results

3.1 Brain

3.1.1 Control Brain Tissue

The histology of the control fish brain manifested clear undamaged pyramidal cells, Nissl cells and healthy neuronal cells scattered in the regions of the cortex and telencephalon. The traditional histology is more than enough to distinctly label the above-said cells. In the current study, the neuronal cells were distinct and there were no observable alterations.

The neuronal cells were found with a cell body consisting of a nucleus, axon (major branching fibres) and dendrites (numerous smaller branching fibres). The pyramidal cells were intact in structure and were found with sufficient dendritic projections. The neuronal and pyramidal cell density was adequate and was found abundantly throughout the tissue (Figure 1 (A)).

3.1.2 1-Day Treated Fish Brain Tissue

1 day-treated fish brain tissues were examined for any potential damages induced by the toxicant. The cortex and telencephalon part of the brain tissues were found with

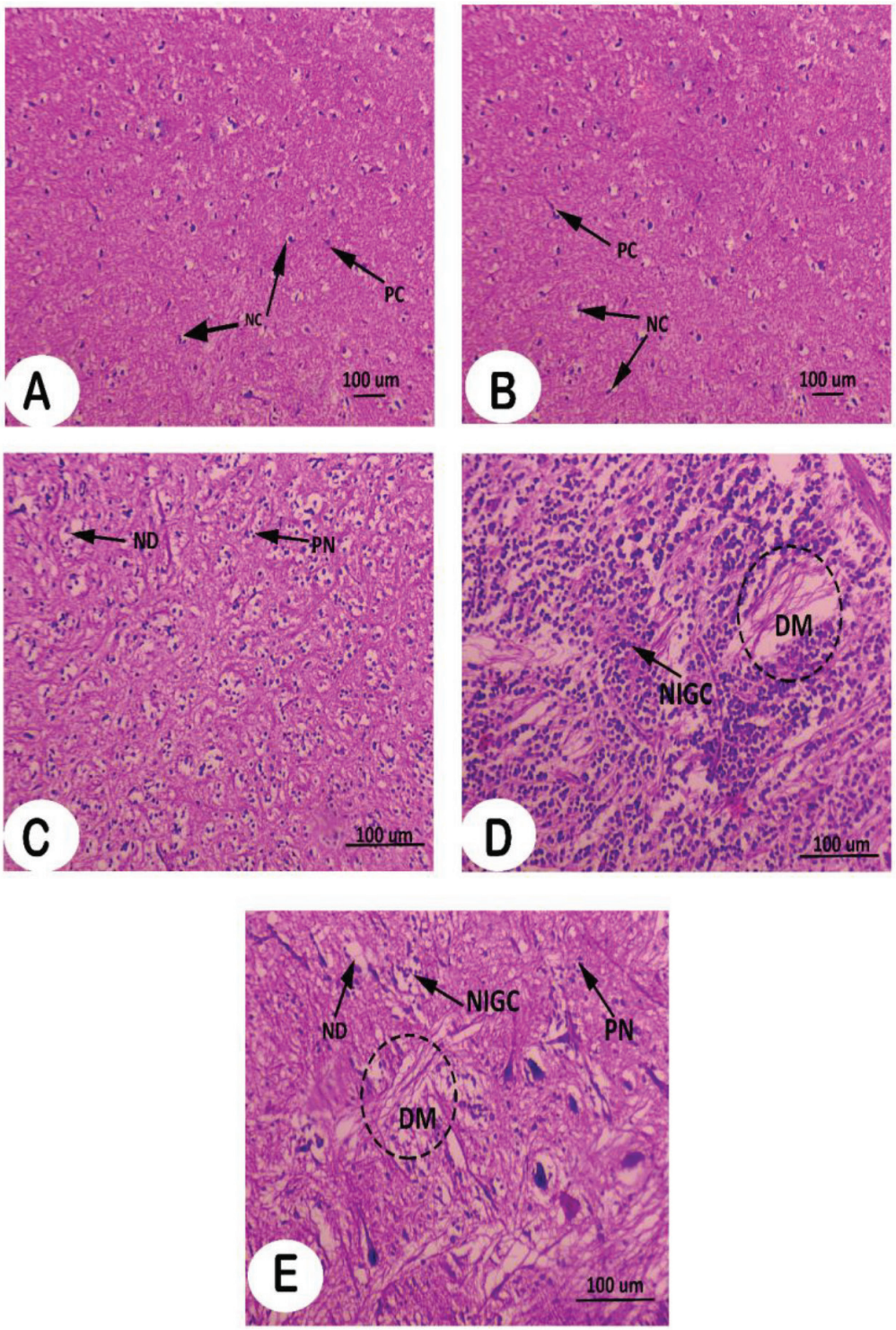


Figure 1. Photomicrograph showing Brain histology of *Cyprinus carpio* exposed to Afidopyropen. **(A)** and **(B)** (4X) showed normal Brain Pyramidal cells (PC) Neural cells (NC); **(C)** with Neural degeneration (ND)Pyknotic neuron (PN); **(D)** Necrotic Internal Granule Cells (NIGC) Demyelination (DM); and **(E)** Neural Degeneration (ND) Pyknotic Neuron (PN) Necrotic Internal Granule Cells (NIGC) Demyelination (DM) (10X).

healthy neuronal cells, pyramidal cells, and Nissl cells. There were no observable damages in the tissue. The dendritic projections were normal. The cortex and telencephalon regions were found with more or less similar tissue architecture compared to control tissues (Figure 1 (B)).

3.1.3 10-Day Treated Fish Brain Tissue

In 10-days exposed fish brain tissues, the neuronal cells were found with appropriate structure but the cells were not intact, instead, the cells were interrupted with loose connections with each other. The 10-day treated group exhibited pyknotic nuclei and neuronal degeneration in some parts of the tissues compared to the control group tissues. However, the overall density of the pyramidal cells was decreased compared to the control group tissues. The dendritic projections were found to be degraded compared to the control group. Nuclear degeneration seems to be in the initial stage as the very few such instances in the brain tissues (Figure 1 (C)).

3.1.4 20-Day Treated Fish Brain Tissue

In the 20-day exposed fish brain tissues, the pathological alterations were accelerated compared to the 10-day treated fish groups. The neuronal cells were scattered and there was a destruction in the cell's architecture. However, the damage seemed severe compared to the 10-day treated fish group. The tissue was also found with acute necrotic internal granule cells. The demyelination was also seen along with necrotic granule cells in the tissues. The damage that occurred in the 20-day group was extreme compared to the control groups. The pyramidal cells and Nissl cells were damaged and found with lesser density compared to the control group (Figure 1 (D)).

3.1.5 30-Day Treated Fish Brain Tissue

The 30-day treated group of the fish brain was analysed for histological changes. The tissues were found with a continued level of damage compared to the 20-day treated group. The neuronal degeneration was prominent with demyelination and pyknotic nuclei. In this group, the neuronal degeneration was characterised by decreased axon cells and dendritic projections. Demyelination was found severely attributed to tissue degeneration compared to the 20-day treated group. However, the presence of pyknotic nuclei suggests the damage caused to the brain in the 30-day exposed fish group. The tissue was severely affected by the toxicant compared to the control group fish tissues. The Nissl cells and pyramidal cells were reduced in density compared to the control group. Nonetheless, the 30-day treated group was majorly attributed to the demyelination compared to the control group fish tissue (Figure 1 (E)).

3.2 Gill

3.2.1 Control Group Fish Gill Tissue

The control group fish gill tissues were found with normal histological structures. The gills were intact with primary gill lamellae, secondary gill lamellae, and chloride cells. The primary and secondary gill filaments were provided with a rich blood supply and were found distinctly arranged one by one without any interruption. The cells of the secondary gill lamellae are supported by pillar cells with appropriate cell structures. There was a clear demarcation between the primary and secondary lamellae. The secondary gill lamellae were arranged in a row attached to the primary gill lamellae which indicates the normal structure of the gill tissues (Figure 2 (A)).

3.2.2 1-Day Treated Fish Gill Tissue

One day treated group of fish gill tissues was found with normal histological structures without any interruptions. The primary lamellae were found with a rich supply of blood and facilitated the flow towards secondary gill lamellae. The secondary gill lamellae were intact and arranged in succession attached to the primary gill lamellae. The blood supply was supported by distinct pillar cells. The chloride cells were also found at the base of the secondary lamellae supporting the exchange of Cl^- ions in the gills. There were no major changes compared to the control group, and the gill tissue seemed normal, similar to the control tissue (Figure 2 (B)).

3.2.3 10-Day Treated Fish Gill Tissue

The 10 days treated fish gill tissue was found with mild alterations compared to control tissues. The primary lamellae were normal in structure but the secondary lamellae were disturbed. The secondary lamellar tissues were damaged and there was mild destruction of secondary lamellae in some parts of the tissues. However, the gill appeared normal except for damaged areas of the tissue. Another pathological observation was telangiectasia, characterised by the club-shaped structures on the secondary lamellae. The condition of telangiectasia persisted in the secondary lamellae compared to the control group tissue. The blood supply was normal to

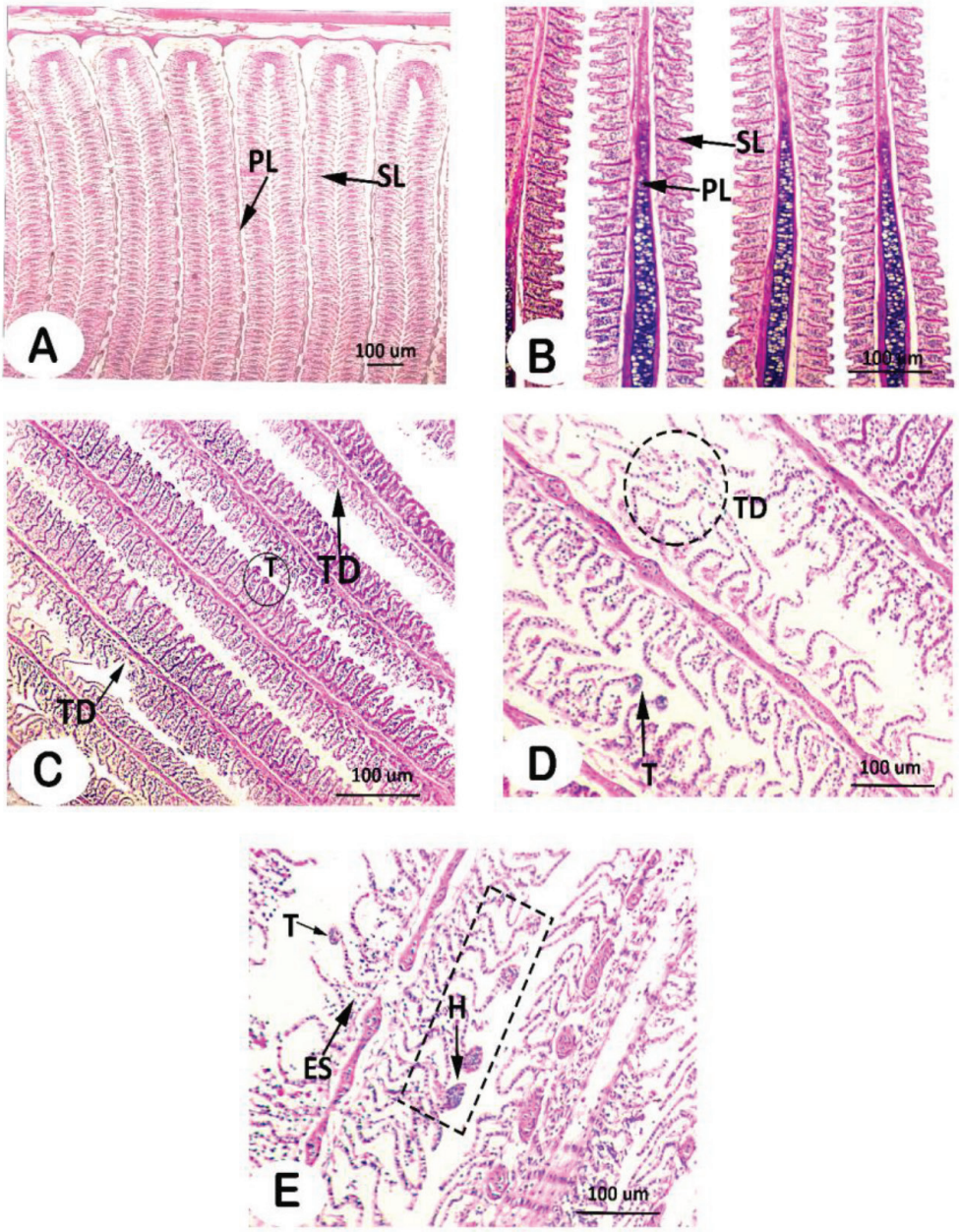


Figure 2. Photomicrograph showing Gill histology of *Cyprinus carpio* exposed to Afidopyropen. **(A)** (4X) and **(B)** showed normal Brain Primary gill Lamellae (PL) Secondary gill Lamellae (SL); **(C)** with Telangiectasia (T) Total Destruction of the lamellae (TD); **(D)** Telangiectasia (T) Total Destruction of the lamellae (TD); and **(E)** Telangiectasia (T) Hyperplasia (H) Epithelial Separation (ES) (10X).

both primary and secondary lamellae along with pillar cells (Figure 2 (C)).

3.2.4 20-Day Treated Fish Gill Tissue

In the 20-day treated fish group, the gill tissue was found with a moderately damaged gill structure. The primary lamellae were found normal with blood supply. The secondary lamellae were structurally altered compared to the control group tissues. The intact arrangement of the secondary lamellae was highly altered and the instances of telangiectasia were also more compared to the control group. The pillar cells of the secondary lamellae were changed and subjected to chemical exposure. It also

seemed to have a poor blood supply compared to the control group (Figure 2 (D)).

3.2.5 30-Day Treated Fish Gill Tissue

The fish gill tissues of the 30-day exposed groups were found with a relatively high frequency of damage compared to the control group. The primary gill lamellae were found with a moderate supply of blood and the same was supplied to secondary lamellae. The secondary lamellae were highly altered and exhibited pathological conditions such as telangiectasia, hypertrophy, epithelial separation, and secondary gill tissue destruction.

The tissue was shown with an altered arrangement of secondary lamellar. The epithelial separation in the secondary gill lamellae was frequent in nature followed by other pathological conditions. It was evident that the primary and secondary gill lamellae were both affected severely compared to the control group (Figure 2 (E)). The gills play an important role in separating the internal system from that of external media in fish. It is also one of the organs which are directly connected to the surrounding environment. In the present study, the control group gill was found with a normal arrangement of the primary and secondary lamellae supported by chloride and pillar cells. The treated groups showed marked alterations with respect to the days of exposure. In 10 and 20-day treated groups the pathological lesions were moderate compared to the control but in the 30-day treated group, the damages enhanced markedly.

4. Discussion

The present study revealed several pathological changes in the brain, and gill, of the fish *Cyprinus carpio* exposed to afidopyropen. The brain is one of the vital organs of the body that controls almost all functions of the body. There is restricted literature available on the traditional histology of the brain of fish. However, the present study attempted to reveal the pathological lesions caused by the pesticide in the fish brain.

In the current study, the brain histology of the control fish showed normal brain structure with neuronal cells, pyramidal cells, and Nissl cells lying in the cortex and telencephalon region of the brain. The histology of 1 day day-treated group was also found with a similar normal structure without any sign of alterations. The 10-day treated group manifested mild pathological conditions such as pyknotic nuclei and neuronal degeneration (Figure 1 (C)). In 20-day and 30-day treated groups the pathological conditions were drastically increased compared to the control group. The pathological conditions including destructed neuronal cell structure, acute necrotic internal granule cells, demyelination, necrotic granule cells, decreased density of pyramidal and Nissl cells, and pyknotic nuclei were successively increased from 20 days to 30 days exposed fish groups.

The occurrence of relatively high damage in these two groups renders the long-term effect of afidopyropen on the brain tissues of the fish. The pathological lesions were attributed to the toxic effect of afidopyropen. The occurrence of necrosis and infiltration of glial cells could be a sign of initial toxicity in the fish. Gliosis condition in the cerebrum region of the fish brain was observed upon exposure to Endosulfan in a previous study[24].

In a previous study Das[25], recorded massive cerebral tissue degeneration in *Labeo rohita* upon Endosulfan exposure. However, there were no other signs of toxicity observed in the fish in his studies. In the present study, the severe necrosis of neuronal cells, pyknotic nuclei, and demyelination was attributed to exposure to afidopyropen insecticide. In the previous study, similar severe necrosis was observed in the parenchymal tissue of *labeo rohita*. It was concluded that the chemical was toxic in nature and indicated neurotoxic effects in the fish[26]. The behaviour of the fish mainly depends on the brain activity compelled by neuronal processors. The brain contains massive connections of neurons that highly contribute to the rapid discissions required for behavioural reactions. The pathological lesions such as congestion and necrosis in the brain region are considered to be the result of pesticide exposure[27]. Present results were concordant with[28], where the histology of the brain tissues of the fish *Channa punctata* exposed to propiconazole manifested increased congestion, vacuolisation, generalised spongiosis and necrosis. In my study, similar results were obtained and can be interpreted as the result of afidopyropen

The gills are the prime organ of respiration, any change in the surrounding environment alters the morphology of the gill[29]. Pathological conditions such as hyperplasia, altered pillar cells, epithelial separation and oedema are considered potential toxicity indicators in the fish gill. In a previous study, the exposure of the *Catla catla* fingerlings to pesticides and heavy metals exhibited pathological lesions that are extended to record mortality also[30]. In some

cases, such as agricultural field runoff and integrated fish farming, the residues of the pesticides were found to cause DNA damage and oxidative stress in the gill cells of the fish *Cnesterodon decemmaculatus*[31]. The epithelial separation might be due to damage to pillar cells resulting in collapsed vascular integrity. This led to abundant blood rushing towards the lamellar epithelium pushing it to separate outwards[32]. The pathological condition telangiectasia can be described as the condition of club-shaped bulging of secondary lamellae and is believed to be the result of disintegrated pillar cells in the secondary lamellae. It persists in various forms in different species of fish. In Nile tilapia, it appeared as bulged portion in the middle of the secondary lamellae. While in *Cyprinus carpio* it appeared more towards the tip of the secondary lamellae[33].

In the previous study, it was found that the condition was persistent in the high-dose pesticide-treated group and considered to be a potential threat to the gill structure and function. The results of the present study are in parallel with the studies of Neglur *et al*,[34]. However, in his study, the insecticide concentration was altered and the exposure day was kept constant while in the present study, the exposure concentration was kept constant and the days of exposure were increased to study the effect. It is clear that the concentration of the pesticide and the duration of exposure both contribute to damage to the organs in the fish.

Hyperplasia is one of the lesions found in the gills in the present study. The increase in the mass of cells or multiplication of cells resulting not from cell proliferation is considered as hyperplasia. Sometimes the cells also accumulate in a particular area making it difficult to differentiate it from the condition hypertrophy. In the current study, it was found that the cells of the gills accumulated at the tip of the secondary lamellar area and are prominent. This type of hyperplasia is suspected to cause decreased lamellar space and further, it contributes to reduced respiration rate in the fish[34].

In a recent study, the scanning electron microscopy results contemplated that hyperplasia as one of the potential lesions in the gills that reduces the surface area leading to lamellar fusion[35]. The epithelial lifting or separation is considered to be a defensive mechanism as the separation increases the distance between the cells of secondary lamellae and the toxicant in the media[16]. In consideration of the above-mentioned histological conditions, it was proposed that these pathological responses are considered a defensive mechanism against the pollution caused by various reasons[36].

In the present study, the increasing pathological conditions with the increase in the exposure days can be considered as the long-term effect of the afidopyropen on the fish *Cyprinus carpio*. However, there might be a chance of adaptations in short-term exposure as observed in 1 day-exposed group in the current study. There were no sudden histological changes in 1 day-exposed group of the fishes. It could be due to the strong and quick adaptation capacity of the healthy fish. However, prolonged exposure to the toxicant extremely affects the fish physiology leading to the form of above said pathological lesions.

5. Conclusion

The histopathological assessment of the fish was observed in the brain and gill of *Cyprinus carpio* on exposure to sublethal concentration of afidopyropen. By the observations pertaining to the histopathological responses of freshwater fish, *Cyprinus carpio*. The current investigation came to the conclusion that afidopyropen was extremely hazardous to the fish *Cyprinus carpio,* causing brain and respiratory toxicity via altering the cellular shape of the gill tissue. This damage increased over time of exposure. Hence, there appeared an irrecoverable loss to the biochemical integrity of the cell due to afidopyropen stress.

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