Tissue-specific Histopathological alterations in *Catla catla* following exposure to Acetamiprid 20% SP

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Abstract:
Nowadays, the worldwide widespread use of pesticides enhances the likelihood of unfavorable toxicity to creatures other than the target pest. Pesticides are a major concern around the world, as they can bioaccumulate into different fish tissues, which will consequently result in serious health concerns for the consumers. In this context, the present study depicts several serious histopathological alterations of vital tissues of freshwater fish *Catla catla* exposed to Acetamiprid 20% SP at a lethal (50 mg/L-1 for 96 hours) and sub-lethal concentration for 1, 4, and 8 days. However, it is strongly suggested that Acetamiprid 20% SP is moderately toxic to *Catla catla.* The fish's evident histological alterations are a high degree of pathological lesions. The examination of the gills indicated fusion, epithelial degradation, vacuolation, hyperplasia, and deformity at the tips of secondary lamellae, and tissue destruction at the epithelial site have all been seen as lesions. Infiltration of leucocytes, atrophy, breaking of muscle fibers, and vacuolation-like changes in the muscles were observed.

Keywords: Acetamiprid 20%SP, *Catla catla*, Toxicity, Bioaccumulation, and Histopathology

Introduction:
The intensive application of pesticides without proper disposal management has led their excess residues to reach the neighboring aquatic ecosystem and its inhabitants mainly fish. In natural water body pesticides get diluted, and therefore to study the silent toxic effect, a Sublethal concentration of Acetamiprid 20%SP (50 mg/L; 1/10th of 96-h LC_{50} value) for the different durations (1, 4, and 8 days) was evaluated through histopathological of tissues of *Catla catla*. Neonicotinoids are highly water-soluble organic insecticides commercialized in over 120 countries (Jeschke *et al.,* 2011). Acetamiprid is the most effective, and best-selling neonicotinoid insecticide on rice paddies and other crops to control plant and leaf-hoppers, aphids, thrips, and insects (Demirci and Güngördü 2020). Long-term exposure to water contaminants, even at low concentrations, can cause morphological, histological, and biochemical alterations in fish tissues, lowering the quality and marketability of the fish (Haredi *et al.,* 2020).
In comparison to nicotine, neonicotinoids are nicotinic receptor stimulants that specifically interact with the insect's nicotinic acetylcholine receptor (nAChR) (Simon-Delso *et al.,* 2015). They are categorized as nAChR competitive modulators by the Insecticide Resistance Action Committee (IRAC) (Malhotra *et al.,* 2021). Because neonicotinoids are rapidly carried into surface water by leaching, percolation, and runoff from agricultural regions, they pose a significant danger to environmental water quality, threatening non-target species and aquatic organisms (Yi *et al.,* 2019; Marins *et al.,* 2021). In aquatic ecosystems with the abundant accumulation of pesticides and related chemicals, fish organs such as the liver, gills, kidney, brain, and muscles are usually the targets of pesticide accumulation leading to the highest injury (Haredi *et al.,* 2020) due to their importance in the quality of fish farming and human consumption. Ghaffar *et al.,* (2020) reported that gill sections from fish treated with neonicotinoid pesticides exhibited secondary lamellae atrophy, lamellar epithelial pillar cell pyknosis, primary and secondary lamellae congestion and degeneration in exposed *Labeo rohita* fish. Time-dependently, acetamiprid affected the histopathological structure of *C. mrigala* gills and liver (Ghayyur *et al.,* 2021).
However, gill and liver tissues were histopathological affected by imidacloprid exposure to *O. niloticus*, and in the kidneys of *O. mossambicus* and *L. rohita* ((Günal *et al.,* 2020; Patel *et al.,* 2016). In fish erythrocytes, the micronucleus (MN) test has been employed as an indicator of environmental mutagenesis. Micronucleus (MN) and erythrocytic nuclear abnormalities (NA) assessment provides data on environmental quality, species health, genotoxicity, and possible risk (Sayed *et al.,*2016). The elevated nuclear abnormalities were reported in *O. niloticus* erythrocytes exposed to deltamethrin insecticide and different concentrations of imidacloprid (Ansoar-Rodríguez *et al.,*2016) silver barb fish exposed to profenofos (Khan *et al.,* 2018).

Materials and Methods:
The Fish Hatchery Station of Kuchipudi village, Guntur District, Andhra Pradesh, India provided juveniles of *Catla catla* (7 ± 8g, 6 ± 7 cm). Aquaria with dechlorinated tap water (250 L) were used to acclimate the fish for 14 days. The constant aeration was performed using electric air pumps. Stable conditions of water were maintained at 28 °C± 0.25°C, 6.88 ± 0.45 pH, 823 ± 40.1 µS/cm conductivity, 0.045 ± 0.025 mg/L of ammonia concentration. Fish werefed

on a commercial diet (Rice ban). Feeding was postponed by about 24 h before the start of the experiment. The histopathological alterations were evaluated in the gill, liver, kidney, and brain tissues.

Test Chemical: Acetamiprid 20% SP

Acetamiprid 20% SP is an expansive range of neonicotinoid insecticide suggested for the control of sicknesses like Sucking-Type Bugs on Verdant Vegetables, Fruiting Vegetables, Cole Harvests, Citrus Organic products, Pome Natural products, Grapes, Cotton, and Decorative Plants and Blossoms. It contains 20% of dynamic fixing which is comparable to 250g/l of the item. Acetamiprid is an α-chloro-N-heteroaromatic compound. It is a neonicotinoid an insecticide having a place with a chloropyridinyl bunch, and its characteristic item found in Streptomyces canus which is gotten from the normally happening strobilurins. It isn't just utilized in India all through the world, the purposes of acetamiprid are immensely expanded in a couple of years.

Histological Analysis

For histological studies, the gill, liver, kidney, brain, and muscle were first fixed in a solution containing alcohol, formalin, and acetic acid (ALFAC) and then stored in 70% alcohol. The organs were embedded in paraffin, sectioned (5 mm), and the slides were stained with hematoxylin and eosin (HE). The sections were examined by light microscopy and photographed using a digital camera (Takashima and Hibiya, 1995). The presence of histological alterations for each organ was evaluated semi-quantitatively by the degree of tissue change (DTC), which is based on the severity of the lesions.

Results and Discussion:

Gill:

Histological alterations in the gill were seen in the current experiment when *Catla catla* fish were subjected to sublethal and lethal concentrations of acetamiprid 20% SP for 1, 4, and 8 days. In this study's assessment of nuclear abnormalities and histologically negative effects on the gills (Fig. I.A–E), acetamiprid is genotoxic. The gill tissues in control were made up of secondary lamellae and a main filament. Separate capillary channels made up the secondary lamellae. One or two erythrocytes were typically found in each capillary lumen. As the days went on, pathological alterations started to happen; lamellar fusions were seen, along with epithelial cell hypertrophy and hyperplasia. Fish treated with acetamiprid 20% SP had hemorrhagic foci in the pillar cell system.

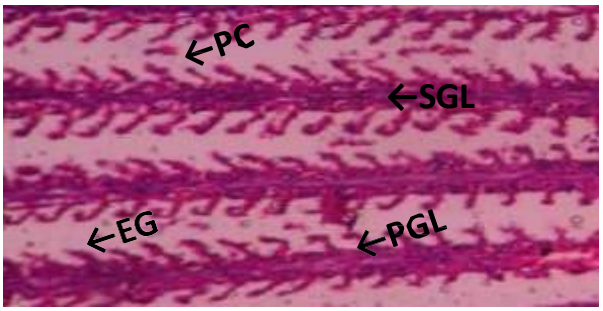

Fig. I. A. Control

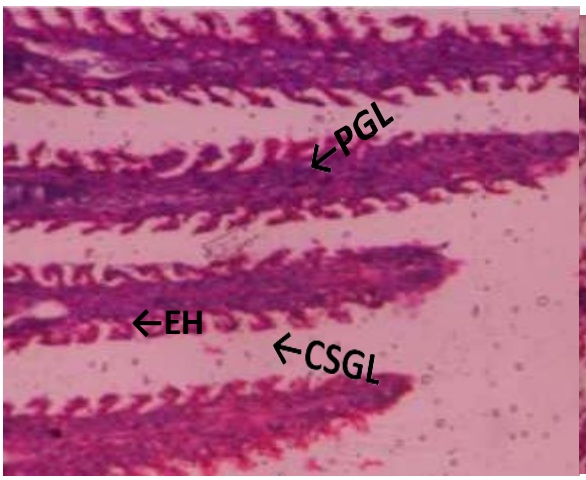

Fig. I. B. 1-daySublethal

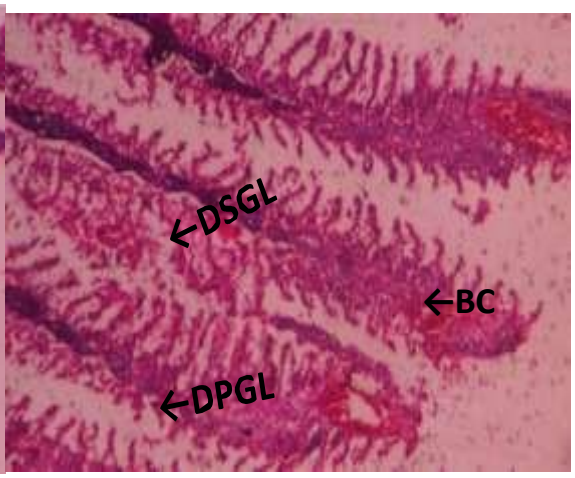

Fig. I. C. 1-dayLethal

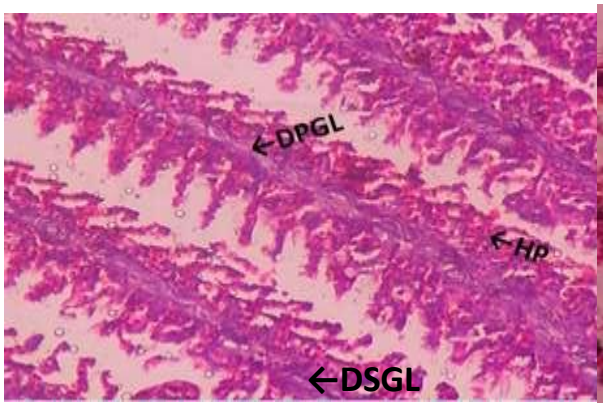

Fig. I.D. 4-days Sublethal

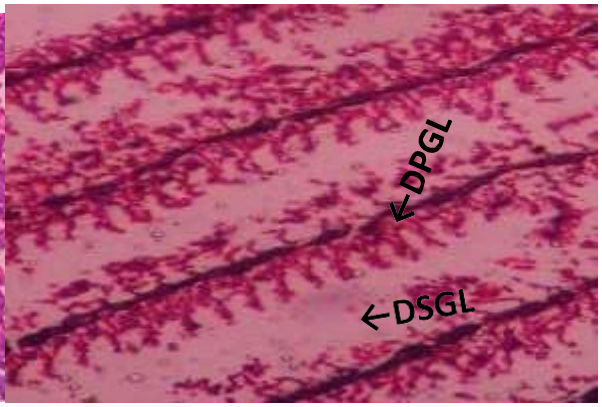

Fig. I. E. 8-days Sublethal

Liver:

In the current study, *Catla catla* fish were exposed to sublethal and lethal concentrations of acetamiprid 20% SP for 1, 4, and 8 days. Histological changes in the liver were observed. In control fish livers, hepatocytes contained uniform cytoplasm, a large, spherical nucleus with a single nucleolus, and varying amounts of dispersed and peripheral heterochromatin (Fig. II.A–E). Hepatocytes were discovered in sinusoids, which are cord-like blood capillary structures. On the luminal surface of the sinusoid endothelium, Kupffer cells were found. The liver is a sizable, crucial organ found in all fish. Hepatocyte cytoplasmic vacuolation, a pathological change that entirely obscured the cellular structure, was found.

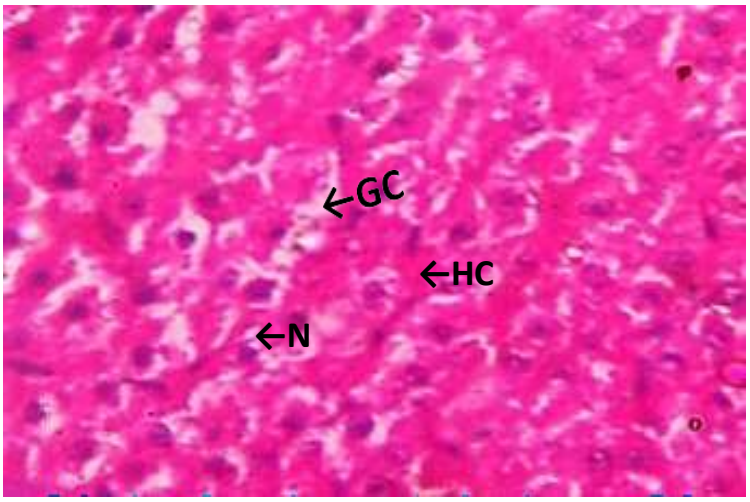

Fig: II. A. Control

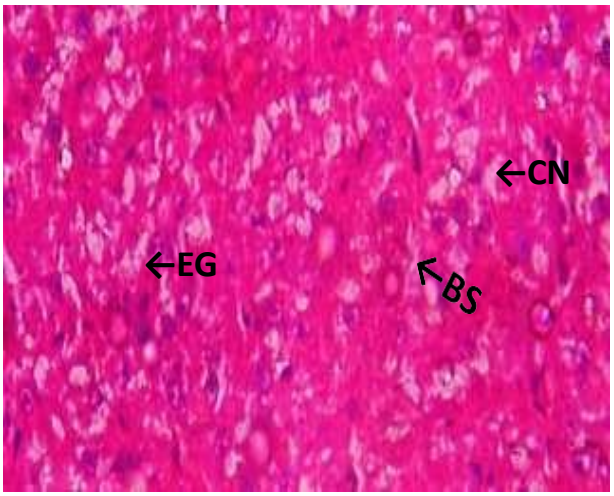

Fig: II. B. 1-day Sublethal

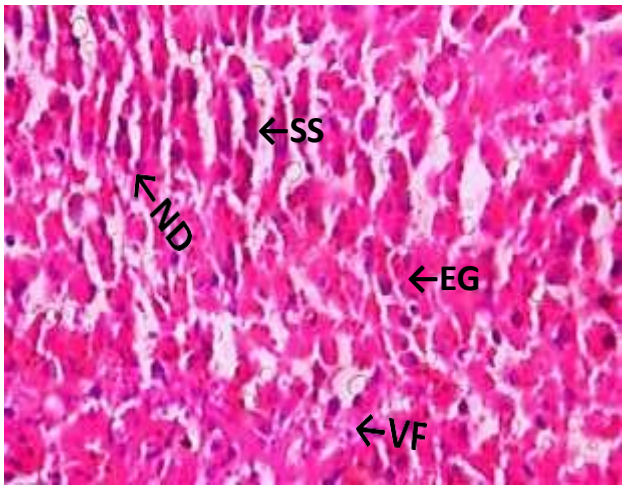

Fig: II. C. 1-day Lethal

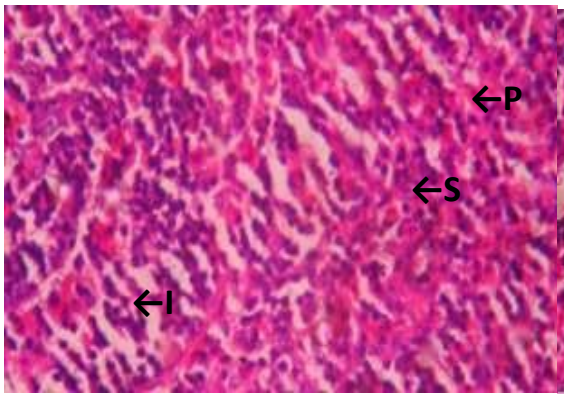

Fig: II. D.4-days Sublethal

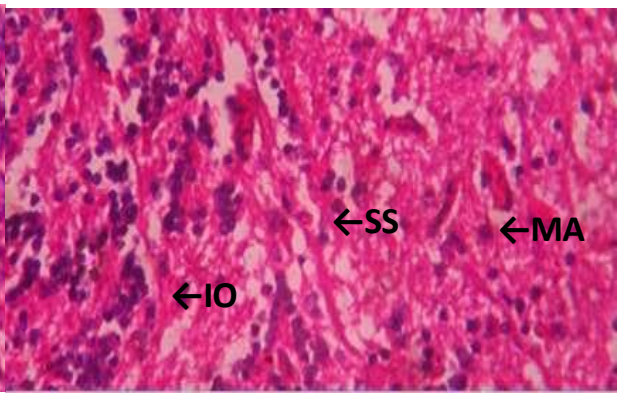

Fig: II. E.8-days Sublethal

Kidney:

Present study Changes in the kidney's histopathology after exposure to Acetamiprid 20%SP for 1, 4, and 8 days, as well as sublethal and lethal concentrations of 1/10th of LC50 for 96 hours Animals used in experiments had their hepatic cords and renal tubular cells destroyed after receiving intramuscular injections of bovine serum albumin. These findings support the impact of pesticides on the Indian main carp, *Catla catla*, as indicated by kidney tissue. Necrosis, degenerative changes in hemopoietic tissue, swelling of renal tubules, and hypertrophy were seen in the kidney. Renal damage ruptured in the glomeruli and diminished renal tubules and their lumen. The glomeruli and renal tubules of fish exposed to Acetamiprid 20%SP were severely damaged resulting in the tubules gradually disappearing and occasionally being replaced by interstitial lymphoid tissue (Fig. III. A-E).

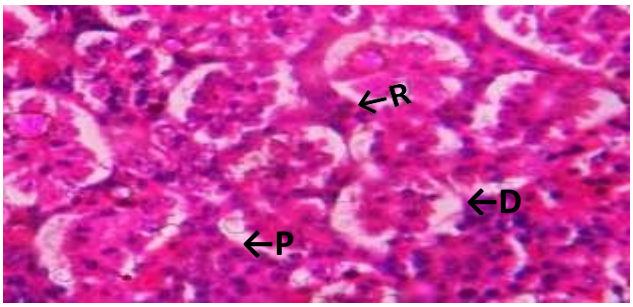

Fig. III. A. Control

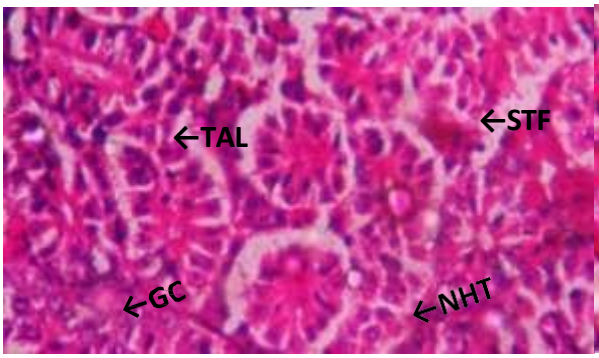

Fig: III B. 1-day Sublethal

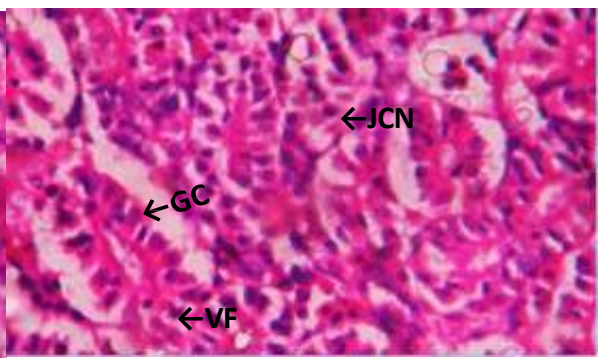

Fig: III. C.1-Day Lethal

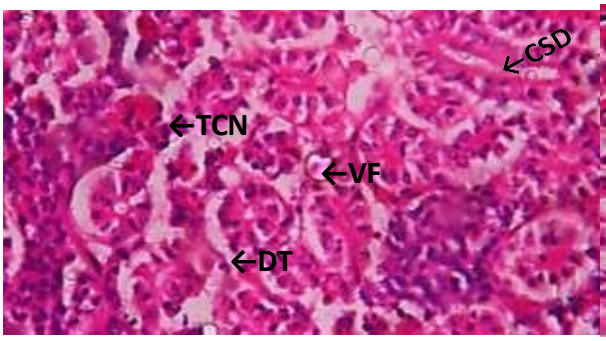

Fig: III. D. 4 days Sublethal

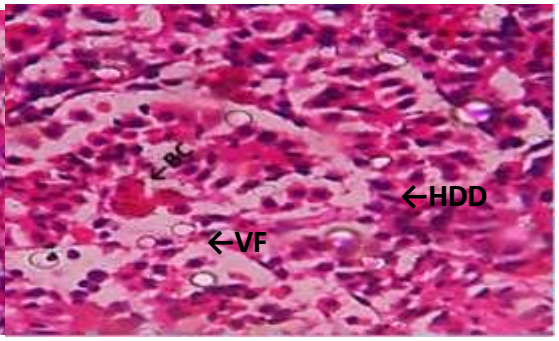

Fig: III. E. 8 days Sublethal

Brain:

Present study Histopathological changes in brain tissue of freshwater fish *Catla catla* exposed to Acetamiprid 20% SP in the lethal sublethal concentration include edema, necrosis, atrophy, pyknosis, swelling of axon, vascular degeneration, severe damage in brain cells and broken neuron bundles, degenerated dorsal olfactory area, degenerated ventral olfactory area and blood streaks were observed (Fig. IV. A-E). Histopathological changes in the brain include vacuolation of the brain parenchyma and moderate swelling of the cerebrum's pyramidal cells. Other pathological changes observed in the brains of exposed fish include atrophy, necrosis, dissolution of nissel bodies, axon swelling, and vacuolization of the nerve fibers' myelin sheath.

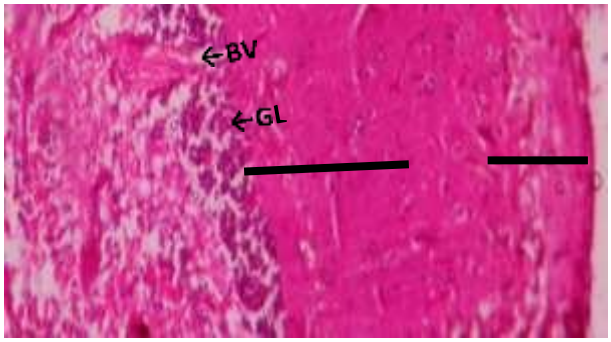

Fig. IV. A. Control

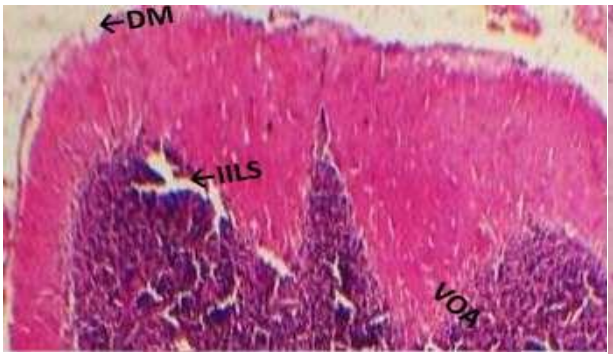

Fig: IV. B. 1-day Sublethal

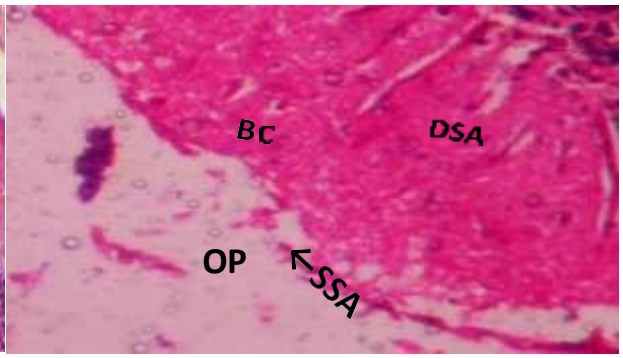

Fig: IV. C.1-Day Lethal

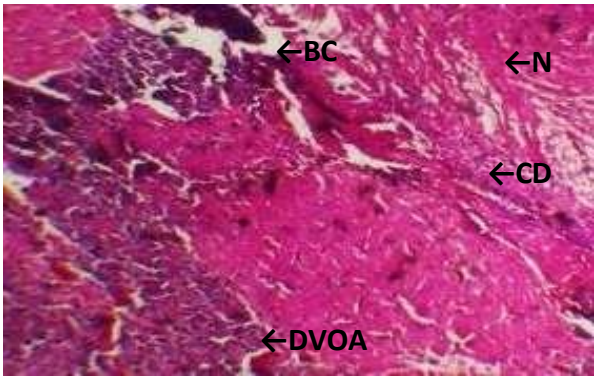

Fig: IV. D. 4 days Sub-lethal

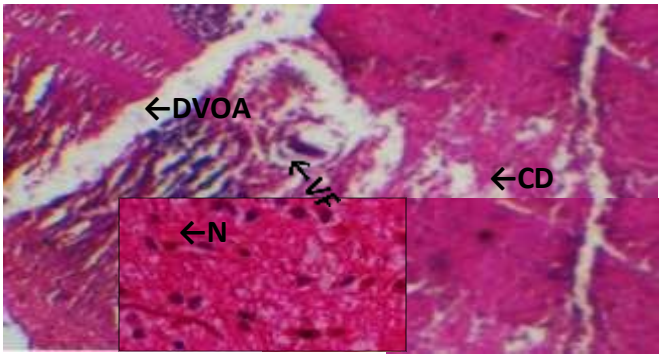

Fig: IV. E. 8 days Sub-lethal

Light micrographs of the gill, liver, kidney and brain of *Catla catla* showing the effect of acetamiprid 20%SP exposure. Where, **A:** Control group **B:** Sublethal of 1 day group **C:** Lethal of 1 day group, **D:** Sublethal of 4 days group, and **E:** Sublethal of 8 days group.

Lalitha V (2022) investigate the sublethal & lethal concentration histological changes observed in the gill including lamellar aneurysm, curling of secondary lamellae, shortening of the secondary lamellae, hypertrophy of epithelial cells, a fusion of secondary lamellae, deformation of the cartilage core, blood congestion, collapsed secondary lamellae, excessive mucus secretion, disorganization of the secondary lamellae, hemorrhage at primary and secondary lamellae, gill lamellar necrosis were observed. Toxicological effect of the metals on tissues chiefly muscles and gills. Several Abnormalities in gills and muscle tissues were observed (Nazish Shah, and Ahsan Khan, *et al.,* 2020).

Ch Anitha (2022) observe the secondary lamellae frequently fused along their whole length. In addition, with a collapsed pillar cell system, sub-epithelial cell edema, along the interlamellar epithelium and genotoxic as assessed by nuclear abnormalities and histological adverse effects on gills. Histological changes such as lesions, necrosis, curling of secondary gill lamellae, hyperplasia, and clubbed tips of secondary gill lamellae were observed in gill tissue (Juliet Selvarani Arulraj, *et al*.,2019). The aqueous extract *Azardiracta indica* has a significant effect on the respiratory organ of the gill of*, Labeo rohita* (S. Geetha, *et al.,* 2019). The showing group histopathological changes in the gill tissues, such as shrunken and narrow secondary gill lamellae slight to reasonable penetration of seditious Cells in Primary and Secondary Gill lamella. (S. Geetha, *et al.,* 2019).

The effect of fungicides on the gill was epithelial Hyperplasia with the lamellar union, epithelial hypertrophy edema, general necrosis, and degeneration of primary and secondary Gill lamella (Choudhury N, 2018). Along with these pathogenic changes, karyolysis was frequently present in hepatocyte nuclei. Since it takes part in procedures like the biotransformation and excretion of xenobiotics, the liver can be seen as a target organ and one of considerable relevance to fish (Lalitha V, 2022). Therefore, the liver can be studied in environmental monitoring due to its high sensitivity to contaminants. Thus, alterations in its structure can be significant in the evaluation of the health of fish (Ch Anitha, 2022). The histological study appears very delicate parameters and is critical in determining cellular vagaries that might be in the aim organ liver. So, for these reasons, the hepatic cells are injured.

Rajeswari *et al.,* (2020) Observed Necrosis of hepatocytes with enlarged sinusoids in freshwater fish *Ctenopharyngodon idella* exposed to cyhalothrin acute and chronic levels were observed. Effect of Arsenic on freshwater catfish, *Mystus vittatus* observe the cytoplasm and nuclear degradation; cellular degradation, and damaged hepatocytes Prakash and Verma (2020). Anitha A *et al.,* (2015) Observed cytoplasm with nuclear degradation; cellular degradation and damaged hepatocytes were observed in fish *Labeo rohita* exposed to Pyraclostrobin. In the nephron of bony fish, the glomerulus, tubules, and collecting duct are all present. Renal corpuscles and renal tubules make up the nephron. Bowman's capsule enclosed a cluster of capillaries (glomerulus) in the renal corpuscles. On the inner side of the glomerulus, endothelial cells covered capillary gaps in a microscopic image. Mesangial cells filled the gap between capillaries (Lalitha V,2022). Kidney damage in the form of hyaline degeneration is characterized by swollen tubular cells, nuclear membranes, shrinking tubular lumen, the appearance of hyaline objects in some tubular lumen, and pink colour in hematoxylin–eosin staining. In myxosporean infection, Kidney tissue damages, such as Glomerular destruction, haemorrhagic and leucocytic infiltration are the main histopathological effect (Ch Anitha, 2022). According to V. Tamizhazhagan *et al.,* (2016) disintegrated neural cells, severe necrosis, damaged brain cells, and neural bundles in fish *Ophiophagus punctatus* exposed to malathion. Neural necrosis and edema in a Purkinje layer of cerebellar regions of the brain in fish brown trout treated with cadmium. Histopathological changes in brain tissue of *Catla catla* exposed to A, HM, and A+ HM included edema, necrosis, atrophy, pyknosis, axon swelling, vacuolar degeneration, severe damage in brain cells and broken neural bundles, degenerated dorsal olfactory area, degenerated ventral olfactory area, and blood streaks (Lalitha 2022). Among the most prominent of these tissue changes that were repeated in the treatments were degeneration, vacuole degeneration, necrosis, haemorrhage, congestion, and edema as well as disintegration between different brain tissues. In the fish *Cyprinus carpio* exposed to lethal concentrations of an organophosphate insecticide portae, it caused mild damage to the fish's brain one-day Further exposure for four days caused apparent damage (Lakshmaiah, 2017).

Conclusion:

Conclusion of the current study: Acetamiprid 20%SP is extremely toxic and significantly affects *Catla catla* fish tissues when used at lethal and sublethal dosages. The acetamiprid 20%SP (a lethal dosage (LC_{50}) of 50 mg/L) was administered to the freshwater fish *Catla catla* at varied concentrations, and the gill, liver, brain, and kidney tissues underwent significant histological changes as a result. Thus, the current study contributes to a better knowledge of the effects of various pesticides on aquatic ecosystems and fish health and the need for close monitoring of their use among locals and communities.

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