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HISTOPATHOLOGICAL ASSESSMENT OF PESTICIDES EXPOSURE ON TILAPIA FISH: IMPLICATIONS FOR HEALTH MONITORING AND ENVIRONMENTAL RISK ASSESSMENT

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Abstract
Histopathological investigations are crucial tools in toxicology research and water pollution monitoring. Using light microscopy, this study assessed the effects of lambda-cyhalothrin on the kidney, liver, and gill tissues of Oreochromis niloticus. In a 28-day period, fish were subjected to sub-lethal amounts of lambda-cyhalothrin (0.24 ppb and 0.36 ppb). Histological analysis showed that, while tissues in the control group remained normal, exposed fish exhibited significant pathological changes. Changes in the gill tissues were noted necrosis, edema, inflammation, epithelial hyperplasia, shorter secondary lamellae, and lamellar fusion. Large sinusoidal gaps contracted, melanomacrophages were present, inflammation, micronuclei cluster development, and tubular epithelial necrosis were all seen in kidney tissues. Large sinusoidal gaps, micronuclei clusters, pyknotic nuclei, and fibrosis were also visible in the liver tissues. The study of biochemical consequences, showed significant abnormalities in lipid metabolism, as seen by increased levels of triglycerides, LDL, VLDL, and cholesterol and decreased HDL. Increased liver enzymes (ALT, AST) and changed protein levels indicated hepatotoxicity, but hyperglycemia and thyroid hormone abnormalities suggested endocrine disturbance. Increased levels of creatinine and urea suggested nephrotoxicity. These results demonstrate the organ and metabolic toxicological effects of lambda-cyhalothrin, highlighting the need for strict environmental regulation and monitoring to reduce ecological concerns in aquatic ecosystems.

INTRODUCTION

Water pollution poses a serious threat to ecosystems due to the accumulation of pesticides and xenobiotics in non-target aquatic organisms, especially fish and predators (Cheng et al., 2020; Liang et al., 2018; Pandey et al., 2019). Many pesticides such as neonicotinoids, carbamates and organophosphates pose a particular threat to non-target species such as fish because of their persistence and accumulation (Mebane et al., 2017; Elezović et al., 1994; Joseph and Raj, 2011; Linde-Arias et al., 2008).

As the most effective substitute for organophosphorus and carbamate pesticides, synthetic pyrethroids have been proved to be dangerous to fish, people, and domestic animals due to changes in a number of metabolic pathways (Amweg et al. 2005, Adhikari et al. 2006). Lambda-cyhalothrin remnants have been

found in the sediments related to agriculture and rainfall. It has been verified that runoff from agricultural, public health, and residential applications includes remaining food. For example, lambda-cyhalothrin concentrations in agricultural watersheds in Stanislaus County, California, ranged from 0.11 to 0.14 parts per billion. Sediments from locations investigated in Imperial, Monterey, Stanislaus, and Placer Counties had lambda-cyhalothrin residues. Starner (2007) reported that residues in sediment varied from 0.003 of to 0.315 µg/g of dry weight. It's possible that the broad application of pesticides on forests and agriculture left harmful substances in the environment. These substances may end up in rivers, streams, and reservoirs of water, where they may have a harmful impact on fish and other marine creatures (John and Prakash, 2003).

Limited investigation has been conducted regarding the histopathological impacts of lambda-cyhalothrin on fish tissues. Gills in fish have direct interaction with the environment. an essential role in maintaining both a stable internal environment and the electrolyte and water equilibrium. It is thought that the main organ where pesticides are stored, biotransformed, and removed is fish liver. The intestinal tract is the first organ to come into touch with food particles poisoned with poisons. These organs have shown to be a reliable indicator of pollution (Hinton and Lauren, 1990).

To find out the histological consequences of the liver, kidney, and gills in tilapia fish exposed to lambda-cyhalothrin over an extended period of time was therefore the goal.

Hazardous material additions to bodies of water alter the aquatic system's biologic, chemical, and physical properties, creating an imbalance in ecology (Yadav et al. 2018a). Aquatic life is seriously harmed by industrial effluents because they contaminate water bodies (Ramona et al. 2001; Gupta et al. 2015). Due to several studies (Censi et al. 2006; Maurya and Malik 2016b, Yadav et al. 2018b), a higher proportion of the pollutants have a capacity to biomagnify and bioaccumulate, which can have a variety of effects and pressures on marine life. Fish and other aquatic animal populations continue to decrease as a result of pollution. Fish are closely reliant on their surroundings, making them more stress-sensitive than numerous additional animal species (Wedemeyer 1996).

Determining the potential use of histopathological alterations as biomarkers for environmental monitoring and detecting environmental stressors depend on an understanding of tissue-level abnormalities. An increasing number of studies have identified histopathological anomalies—such as cellular necrosis, inflammation, and changes in tissue architecture— as indicators of exposure to pollutants in the environment (Khan et al., 2020). Research, for example, has demonstrated a relationship between ecosystem health, pollutant exposure, and particular histopathological indicators, such as alterations in liver enzymes or gill lesions in aquatic species (Smith & Johnson, 2019). Through methodical identification and validation of these biomarkers, scientists can create dependable indicators of the impact of pollution and the quality of the environment. This method aids in the evaluation of the efficacy of pollution mitigation techniques as well as proactive environmental management (Brown et al., 2021).

The gill lamellar epithelium is permeable to dissolved ammonia, carbon dioxide, and oxygen, according to Randall and Daxboeck (1984). The only method by which these compounds can be transferred is by passive diffusion. As such, gill morphological changes may be altered by exposure to lambda-cyhalothrin, and this could have an immediate systemic impact on these exchange systems. Nonetheless, there hasn't been much discussion of the gill histological profile linked to long-term fish contact with the compound lambda and how it affects serum osmoregulation (Rocha and Monteiro 1999). Fish physiological studies have shown that various toxins induce pathological changes, such as neuropathy, hepatic changes, renal damage, and lamellar neuropathy (Ramalingam et al., 2000) for different effects a not lethal effects of lambda-cyhalothrin on Nile tilapia liver, gallbladder and renal neuropathy can be studied. Recent studies highlight the persistence and accumulation of pesticides in water and underscore the need for comprehensive environmental assessments of their effects on fish is highlighted Pesticides such as neonicotinoids, pyrethroids, and organophosphates have been shown to interfere with fish biological processes, including oxidative stress

responses, enzymatic including activity, and overall metabolic health (Brown et al., 2023; Garcia et al., 2024). For example, research shows that organophosphates inhibit acetylcholinesterase, causing neurotoxicity and behavioral changes in cats (Wang et al., 2020).

The study aims to investigates the histopathological alterations in fish organs due to pesticide exposure, correlating these changes with herbicide levels in fish and water. It explores the potential of these alterations as biomarkers for environmental surveillance and assesses ecological risks associated with pesticide contamination in aquatic environments. Emphasizing implications for aquatic ecosystem health and human well-being, the research establishes a foundational histopathological database for tilapia and examines biochemical changes induced by varying pesticide doses.

MATERIALS AND METHODS

Specimen collection and acclimatization

At the Department of Zoology, University of Okara, 28 Nile tilapia fingerlings (10-15cm long, 20-30g) from the Head Baloki fish pond were randomly selected and transferred to the fisheries laboratory. After health checks, they were acclimated in glass tanks with tap water for 10 days. Using a recirculation aerated system, water was changed daily to maintain cleanliness, with oxygen levels at 7.25±0.23mg/L, temperature at 24.5±2.7℃, and pH at 7.46±0.28 throughout the 28-day experiment.

Experimental design

Fish were placed in 40-liter aquariums for a 28-day experiment, receiving commercial feed once daily. Groups with similar fish weights were established. Two treatments based on Lambda-cyhalothrin concentration were administered: Group 2 received 0.24 ppb, Group 3 received 0.36 ppb, and Group 1 served as the control with no exposure. Ethical approval from the University of Okara's Renala Khurd Department of Zoology was obtained before commencing the experiment.

Tissue collection

Fish were collected after being exposed for 28 days, and they were gently dried using a dry cloth. To catch fish, people used fish nets. With a surgical blade, the fish's belly skin was sliced from the ventral side, and the tissues of the liver and gills were extracted. These were then preserved in tubes containing a 10% formalin solution. A random selection of two fish from each group were made, and their tissues were removed for histological analysis.

Histological study

After the fish were taken out of each treatment, their liver, kidneys, and gills were eliminated for histological examination. During the hygienic dissection procedure, the fish's gills, liver, and kidney were removed and preserved in 10% formalin. The histological analysis technique followed the guidelines provided by Spencer et al (2012).

The steps in the histology procedure:

Fixation

In Bouin's fluid the fish samples were fixed. According to *Spencer et al.* (2012) the Bouin's fixative has following components;

- Glacial acetic acid 05.0ml
- Formalin 25.0ml
- Picric acid (saturated aqueous)75.0ml

The process began by combining all of the constituent parts.

Dehydrating and embedding

After collecting samples, they were rinsed in 70% ethanol for 24 hours before being submerged in 80%, 90%, and 95% ethanol for 15 minutes at a time.

Paraffin:

After soaking in xylene for twenty to thirty minutes, the samples were replaced with a solution of soft paraffin and xylene and baked for two hours at sixty degrees Celsius. After that, the samples were baked in light paraffin for two hours at 60 degrees Celsius and in hard paraffin for twenty-four hours.

Embedding, sectioning and mounting:

Once the specimen was acquired, it was chopped with a microtome that measured 2-3 mm. The specimen was placed on the Mayer's albumin-containing slide. The followings are the components of Mayer's albumin as reported by Humason (1962)

- Formalin 0.1ml
- Glycerol 50ml

- Egg albumin 50ml

Staining:
The reagent used for staining was hematoxylin and Eosin.

Hematoxylin:
Following formulation is used for the preparation of hematoxylin stain;

- Hematoxylin (powder) 1.0 gram
- Distilled water 1.0 liter
- Sodium iodate 2.0 gram
- Citric acid 1.0 gram
- Chloral hydrate 50.0 gram
- Potassium alum 50 gram

Procedure for the preparation of hematoxylin stain:
With the aid of slow heating, one litre of water that was distilled contained one gramme of haematoxylin powder. After that, it was given 20.0 grams of sodium iodate and 50.0 grams of potassium alum. To facilitate the full dissolving of the constituents, the mixture was heated. At last, 50 grams of chloral hydrate and 1 gram of citric acid were added to the mixture and dissolved.

Eosin:

- Distilled water 80.0ml
- Eosin powder 1.0gram
- Potassium dichromate 0.5gram
- Picric acid 10.0ml
- Ethyl alcohol 10.0ml
- Picric acid 10.0ml
- Glacial acetic acid (optional) 1 drop

Preparation of eosin stain
One gramme of powdered eosin was found to dissolve in ten milliliters of ethyl alcohol. After that, a concentrated one-tenth of a liter of picric acid was dissolved in the mixture. The mixture was then given 0.5 grams of potassium dichromate, which was added and allowed to dissolve. To minimize the stain's appearance, 80 milliliters of pure water were utilized. A single drop of glacial acetic acid was added at the conclusion of the stain creation process.

Staining procedure:
Xylol was used to de-parafinize the slides before they were stained, and they were then immersed in water. For three minutes, the slides were immersed in ethanol at different concentrations: 100%, 95%, 80%, 70%, and 50%, respectively. After giving the specimen a quick rinse with water, it was stained for one minute with hematoxylin. It was then given a water wash before being immersed in ethanol at 70% and 90% concentrations for three minutes each. After being stained with eosin, the sample was immersed in 95% and 100% ethanol for three minutes each, then xylene for five minutes, and lastly eosin. Following that, a blow dryer was used to air dry it, and it was then submerged in xylene for five minutes. Clove oil was applied last, and Canada balsam was used to fix the slides after they had been wrapped in cover slip.

Microscopy:
For histological examination, the kidney, gills and liver slides from each treatment and control group were examined under a trinocular light microscope. Images were captured straight from the eye piece lens using a camera mounted on a tripod platform.

Blood biochemistry
Chemistry analyser carried out biochemical analysis. After centrifuging the remaining blood samples for 20 minutes at 9400 ×g to extract the plasma from them, the plasma was kept at 4°C. Using bovine serum albumin as a standard, the Waterborg (2009) method was utilised to determine the sample's protein level. The technique described by Khan, Sharma, et al. (2019) was used to measure the glucose. The AST and ALT activity was determined according to the 1957 techniques proposed by Reitman and Frankel. The serum urea and creatinine were measured using kits from Biome Rieux (France). Standard kits were used to measure the levels of serum cholesterol, HDL, and triglycerides (Hassan, El-Khalili, et al., 1995). Following the standard formula supplied by Zaahkouk, Helal, and colleagues (1996), the levels of VLDL and LDL were calculated. The T3, T4, and TSH values were assessed using standard kits that correspond with Hadie, Ghani, et al. (2013) technique.

Determination of parameters of serology
Serum is an important biological parameter of blood which is not blood cell and nor a clotting factor. It is plasma of blood, which does not have fibrinogens.

Serum consists of all antibodies, hormones, antigens, electrolytes, all proteins which are not involved in blood clotting and foreign materials i.e. microorganism and drugs.
The parameters of serology include ALT, AST, cholesterol, urea and creatinine.

Aspartate transaminase (AST)
It is abbreviated as AST also called serum glutamic oxaloacetic trasaminase (SGOT), Aspertate transaminase is an enzyme that is normally occurring in heart cells and liver cells. When heart or liver cells got damaged due to some toxic elements or other harmful action AST is released in blood circulation. Therefore, AST level of blood is increased with liver or heart damaged i.e. due to viral hepatitis. Diagnostic kit was used to measure concentration of AST in blood samples.

Alanine transaminase (ALT)
It is usually found in many tissues, organs especially in liver and fluids of the body such as blood, it is released in serum due to injury in tissues, the concentration of ALT increased particularly due to acute damage to liver cells resulting from toxic hepatitis or viral, objective jaundice and mononucleosis. It is also called glutamic-pyruvic transaminase. For calculation of ALT values diagnostic kit was used manufactured by Dialysis.

Creatinine
As a result of metabolism which occurs in muscles creatinine is formed. In muscles energy is mainly produced from creatine and creatinine is formed by the destruction of creatine. In a day 2% creatinine is produced from creatine. This creatinine is carried to kidneys via blood stream. Creatinine is filtered by kidneys and excreted in urine.
The MICRO-LAB 3000 (Merck, Germany) was used to quantify creatinine quantitatively. Serum samples were used to calculate creatinine using a diagnostic kit made by dialysis (Germany). The Createnine kit contained two types of reagents (R1, R2) and a standard solution (2 mg/dl). 800 µl of R1 and 200 µl of R2 were combined to form 1 ml of the solution that worked. The serum sample was then added in 100 µl. The samples were mixed and examined at 505 nm.

Protein
According to Reznick and Packer's (1994) description, Protein oxidation was detected by means of the carbonyl groups' reaction with 2,4-dinitrophenylhydrazine (DNPH) to produce 2,4-dinitrophenylhydrazone. The molar absorption coefficient for aliphatic hydrazones (22,000 M1 cm1) was used to calculate the carbonyl content of the samples, which was determined at 370 nm and expressed as nmol carbonyl/ mg protein. Protein was measured using the Lowry et al., (1951) method, with bovine serum albumin serving as the standard.

T3, T4 and TSH
Following the manufacturer's instructions, the serum levels of T3, T4, and TSH were measured using the ELISA kit (RFCL Limited, India). In less than ten minutes, the O.D. values of the control and sample from experiment were read using a 450 nm filter on a BIO-RAD microplate reader. The plasma concentrations of T3, T4, and TSH were expressed as ng/ml, µg/dl, and µIU/ml, correspondingly.

RESULTS

Histology of kidney for control group
Histology of kidney in control group was showed in figure 1. Eosin and hematoxylin stained of photomicrograph of the kidney show no alternation in the kidney tissue. The histology of the control group (1) showed normal arrangements of tissues and normal distribution of glomerular part of kidney.

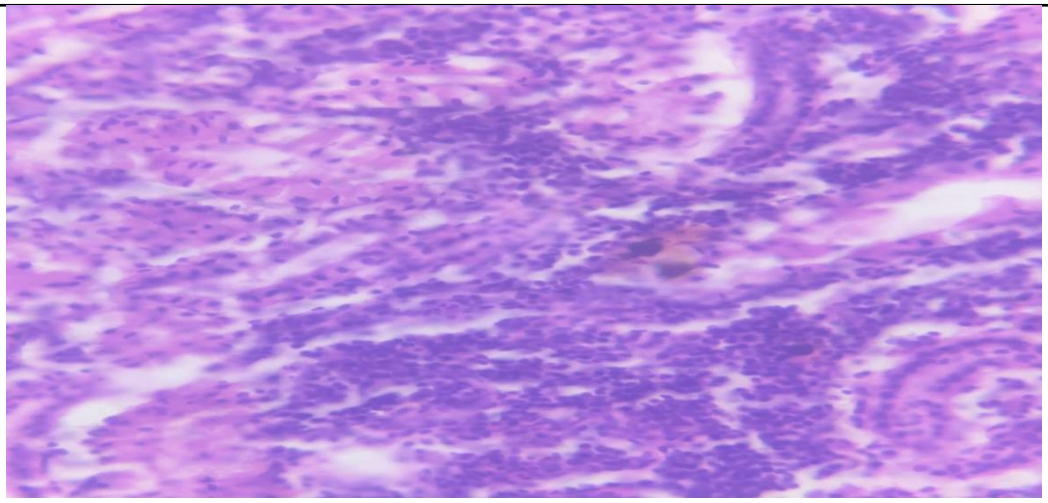

Figure 1: photomicrograph of a kidney with eosin and hematoxylin showed normal arrangements of tissues and normal distribution of glomerular part of kidney.

Histology of kidney for low dose

Histology of kidney after exposure to lambda-cyhalothrin is shown in figure (2). After exposure to lambda-cyhalothrin alternation in kidney tissues were observed in figure (2). The histology of kidney showed different changes in low treated group. Eosin and hematoxylin of a photomicrograph of a kidney reveals irregular Sinusoidal spaces were observed in the treated group for low dose. Necrosis at some places, formation of cluster nuclei, inflammation in tissue and melanomacrophage were seen under microscope. Microscopic image of kidney is shown in (2). Irregular sinusoidal spaces (ISS) functions in the transport of molecules through pores, Three parameters can be used to characterise a sinusoid: its frequency ω (or 2π), its phase θ, and its amplitude A. Abnormalities in function were noted in the group that was treated when any damage caused by poisoning occurred. Necrosis (N), is a kidney element including damage to the kidney tubules cells, which can lead to kidney acute failure at some places, formation of cluster nuclei (CN), failure in identifying a discrete fibroblast cluster might be initiated by limited number of nuclei/cells sequenced.

Figure 4.2: Histological alterations in the kidney's soft tissues at low doses demonstrating differences in the kidney's tissues, such as sinusoidal space (yellow arrow), melanomacrophage (black arrow), tubular necrosis (orange arrow), inflammation (blue arrow), and micronuclei cluster formation (green arrow).

Histology of kidney for high dose

Histology of kidney after exposure to lambda-cyhalothrin with high dose is shown in figure (3). After exposure to lambda-cyhalothrin alternation in kidney tissues were observed in figure (3). The histology of kidney showed different changes in high treated group. Eosin and hematoxylin of a photomicrograph of a kidney reveals irregular Sinusoidal spaces (ISS) were observed in the treated group for high dose. Necrosis at some places, formation of cluster nuclei, inflammation in tissue and melanomacrophase were seen under microscope. Microscopic image of kidney is shown in (3). Irregular sinusoidal spaces (ISS) functions in the transport of molecules through pores. Necrosis (N), is a kidney disorder involving damage to the kidney tubules cells, that can lead to kidney acute failure, formation of cluster nuclei (CN), failure in identifying a discrete fibroblast cluster. The histological alterations found in the fish that were exposed to pesticides and the control group shown in Table 1.

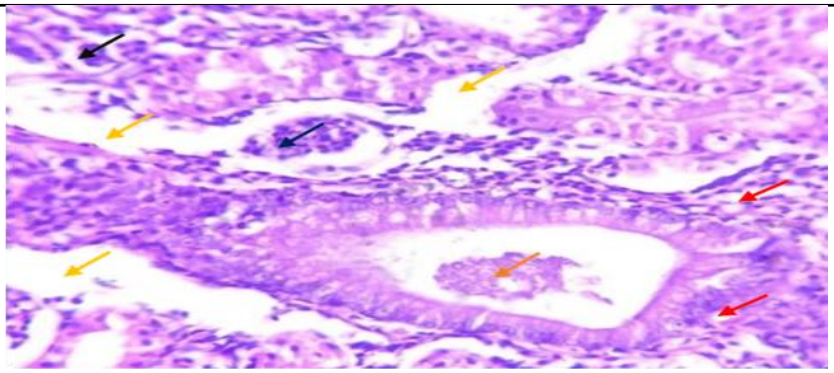

Figure 3: Histological alterations in the kidney's soft tissues at high doses, displaying differences in the kidney's tissues such as melanomacrophase (black arrow), sinusoidal space (yellow arrow), tubular necrosis (orange arrow), and inflammation (blue arrow).

Table 1

Summarized the histopathological impacts in the kidneys of control and lambda-cyhalothrin-exposed Oreochromis niloticus fish

Concentration (ppb) of lambda-cyhalothrin-	tubular necrosis	Inflammation	Micronuclei cluster formation	Sinusoidal space	melanomacrophase
Control	-	-	-	-	-
Low dose 0.24	++	+	+	++	+
High dose 0.36	+++	+++	++	+++	+

None (-), mild (+), moderate (++) and severe (+++).

Histology of liver for control group

The histology of liver after exposure to lambda-cyhalothrin is shown in figure (4). Eosin and hematoxylin stained of photomicrograph of the liver show no alternation in the liver tissue. The histology of control group (4) showed normal arrangements of micronuclei cluster formation and pyknotic nuclei of liver cells.

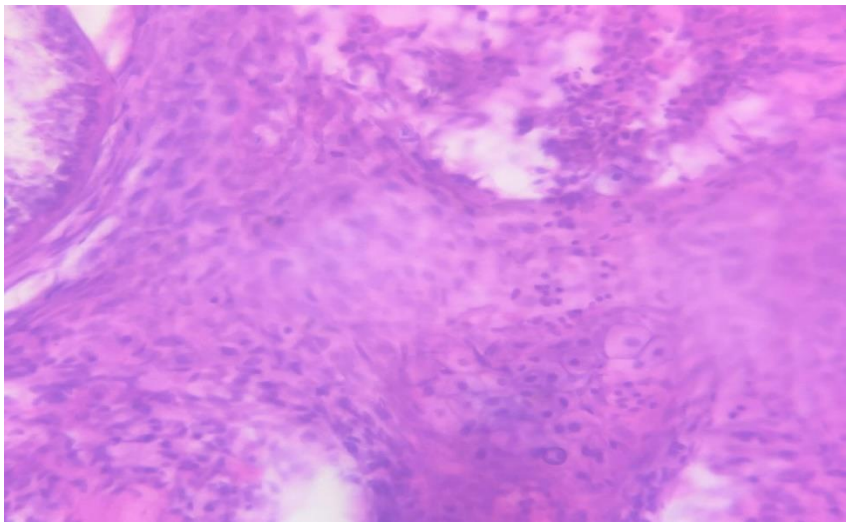

Figure 4: Photomicrograph of a liver with eosin and hematoxylin showing normal arrangements of micronuclei cluster formation and pyknotic nuclei of liver cells.

Liver histology for low dose

After exposure to lambda-cyhalothrin, alterations in liver tissues were seen in figure (5). The histology of liver showed different changes in low dose of lambda-cyhalothrin treated groups. Necrosis (N) in hepatocytes, is the cell death, cluster nuclei formation (CN) are fatty, change could result from several things, such as: (1) greater mobilisation of lipids from peripheral storage, (2) incapacity of the liver cells to secrete fat due to faulty or insufficient lipid transport, micronuclei cluster formation and pyknotic nuclei, large sinusoidal space, fibrosis, micronuclei and cluster formation.

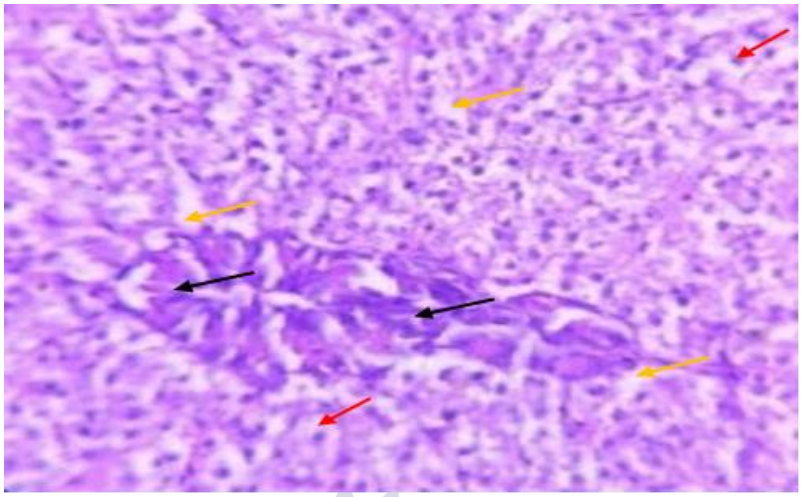

Figure 5: *Demonstrating histological alterations in the liver's soft tissues in the group that received the least amount of treatment; changes include the production of micronuclei clusters and pyknotic nuclei (red arrows), a wide sinusoidal gap (yellow arrows), fibrosis (black arrows), and micronuclei clusters (green arrows).*

Liver histology for high dose

After exposure to lambda-cyhalothrin, alterations in liver tissues were seen in figure (6). The histology of liver showed different changes in high lambda-cyhalothrin dose treated groups. Micronuclei cluster formation, pyknotic nuclei, large sinusoidal space, and micronuclei cluster formation were exposed. Also due to necrosis cell death occur in liver also function abnormally. The histological changes were seen in the pesticides exposed and control fish are shown in Table 2.

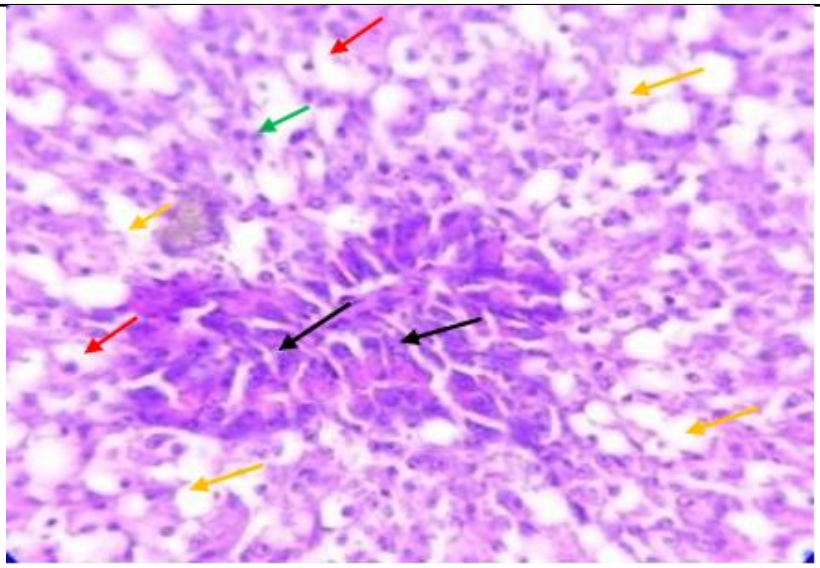

Figure 6: Showing histological changes in soft tissues of liver in high treated group, alterations in liver tissues as micronuclei cluster formation and pyknotic nuclei (red arrow), large sinusoidal space (yellow arrow), fibrosis (black arrow), micronuclei cluster formation (green arrow)

Table 2

Summarized the histopathological impacts in the liver of control and lambda-cyhalothrin-exposed Oreochromis niloticus fish

Concentration (ppb)	Fibrosis	Pyknotic nuclei	Micronuclei cluster Formation	Sinusoidal space
Control	-	-	-	-
Low dose 0.24	+	+	+	++
High dose 0.36	++	++	++	+++

None (-), mild (+), moderate (++) and severe (+++).

Histology of gills for control group

Histology of gills after exposure to Atorvastatin is shown in figure (7). Eosin and hematoxylin stained of photomicrograph of the gills show no alternation in the gills tissue. The histology of control group showed normal arrangements of primary and secondary lamella.

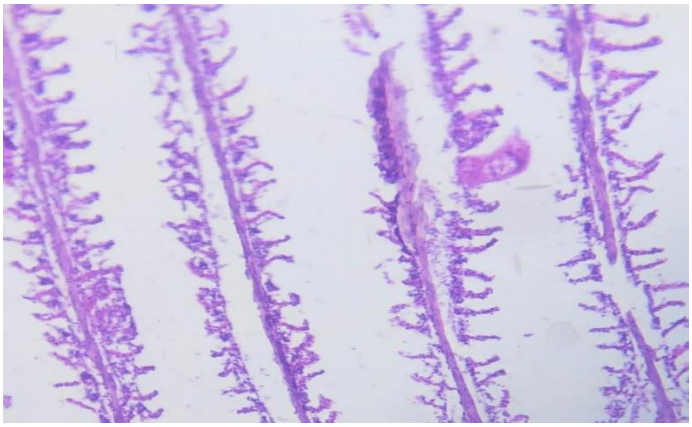

Figure 7: photomicrograph of a gills with eosin and hematoxylin showing no changes in the gill tissues due to the primary and secondary gill lamellae

Histology of gills for low dose

Histology of gills after exposure to lambda-cyhalothrin is shown in figure (8). After exposure to lambda-cyhalothrin, alterations in gills were observed (8). The histology of gills showed different changes in low treated groups like distorted primary lamella, inflammation, edema marginal channel, hyperplasia of epithelial cell, fusion of primary and secondary lamella are gill filaments of bony fish function as gas exchange, iron regulation, acid-base balance, ammonia excretion, hormone production. Hyperplasia of epithelial cell (HEC) is collections of tube-shaped assemblies or clefts lined by a cuboidal to columnar epithelium function

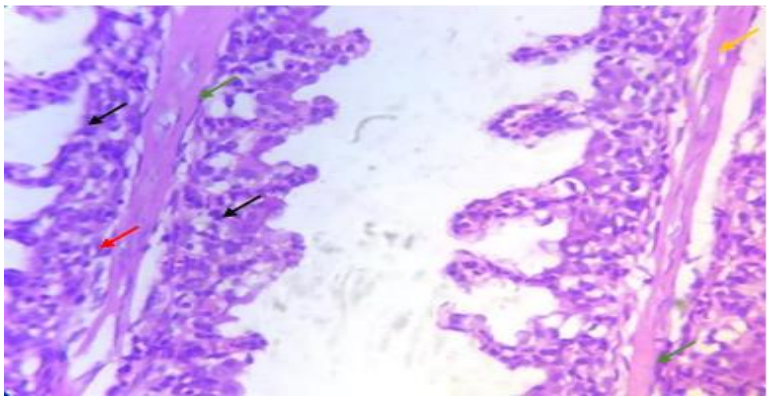

Figure 8: Histological changes in gill tissues showing alterations in gill tissues as fusion of primary and secondary gill lamellae (green arrow), inflammation (black arrow), edema (yellow arrow) and hyper plasia (red arrow)

Histology of gills for high dose

Histology of gills after exposure to lambda-cyhalothrin is shown in figure (9). After exposure to lambda-cyhalothrin, alterations in high dose treated group gills were observed (9). The histology of gills showed different changes in high treated groups like distorted primary lamella, inflammation, edema marginal channel, hyperplasia of epithelial cell, fusion of primary and secondary lamella are gill filaments of bony fish function as gas exchange, ammonia excretion, ion-regulation, acid-base balance and hormone production. Hyperplasia of epithelial cell (HEC) is collections of tube-shaped assemblies or clefts lined by a cuboidal to columnar epithelium function. Fish that were exposed to pesticides showed histological alterations, as did control fish are shown in Table 3.

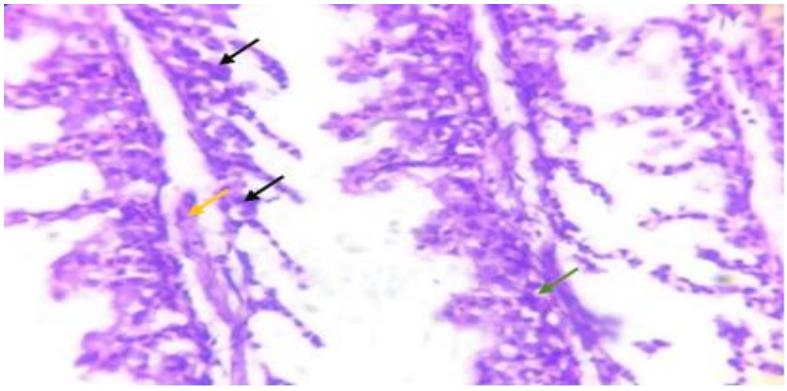

Figure 9: showing histological changes in gill tissues showing alterations in gill as fusion of primary and secondary gill lamellae (green arrow), inflammation (black arrow), edema (yellow arrow) and hyper plasia (red arrow)

Table 3

Summarized the histopathological impacts in the Gills of control and lambda-cyhalothrin-exposed Oreochromis niloticus fish

Concentration (ppb)	Inflammation	Fusion of primary and secondary plasia Gills lamella	edema	Hyper
Control	-	-	-	Low dose 0.24
+	+	++		
High dose 0.36	++	++	++	+++

None (-), mild (+), moderate (++) and severe (+++).

Biochemical analysis

Table 4

Comparison between control group and treated (low and high) groups

Parameters	Control	Low (0.024mg/L)	High (0.036mg\L)
	Mean ±SD	Mean ±SD	Mean ±SD
Cholesterols (mg/dl)	348.1±2.47	377.7±2.56	395.5±3.00
Triglycerides(mg/dl)	502.7±1.77	642.5±2.05	767.8±0.85
HDL (mg/dl)	88.74±2.02	99.87±1.06	119.7±1.90
LDL (mg/dl)	134.2±1.65	152.8±2.01	184.2±1.76
VLDL (mg/dl)	127.5±2.40	139.8±1.35	160.4±1.45
ALT (U/L)	31.68±1.35	40.90±1.51	50.04±2.03
AST (U/L)	98.57±1.45	131.2±2.05	153.7±1.45
Total Protein (g\dl)	17.60±1.86	14.94±2.03	10.93±1.53
Albumin (g\dl)	6.31±1.79	6.32±2.17	5.36±2.04
Globulin (g\dl)	9.90±1.08	7.68±1.18	6.38±1.27
Sugar (mg/dl)	62.93±2.61	107.2±1.72	135.9±2.31
Serum Urea (mg/dl)	4.46±0.86	5.25±1.10	6.50±1.10
Creatinine (mg/dl)	4.54±2.10	5.36±1.95	6.30±2.13
Serum BUN (mg/dl)	25.47±2.15	32.30±2.10	41.23±1.51
TSH (miU\L)	0.41±8.08	0.55±0.04	0.93±0.06
T3 (nmol\L)	2.24±0.81	2.14±0.96	2.05±0.96
T4 (nmol\L)	96.12±1.02	83.60±1.10	75.60±1.10

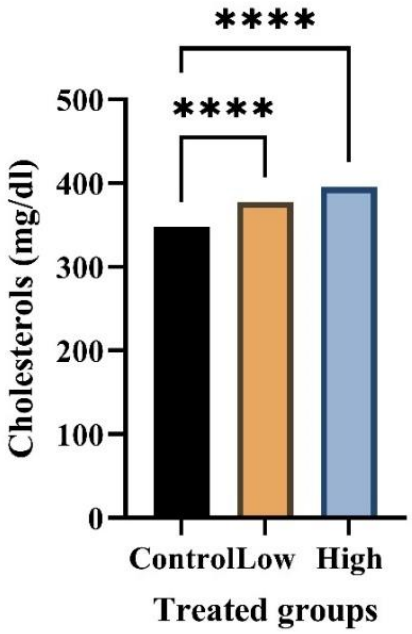

Figure 10(a): Lambda-cyhalothrin significantly alter the Cholestrol of N. *tilapia*

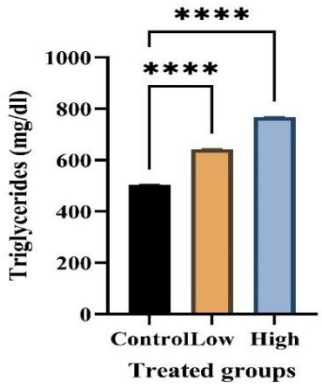

Figure 10(b): Lambda-cyhalothrin significantly alter the Triglycerides of N. *tilapia*

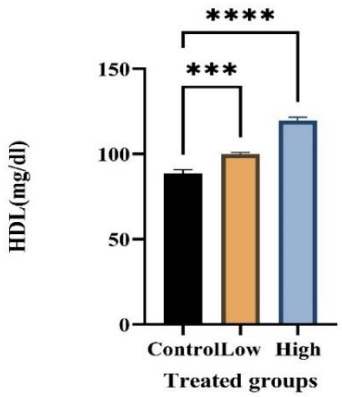

Figure 10(c): Lambda-cyhalothrin significantly alter the HDL of N. *tilapia*

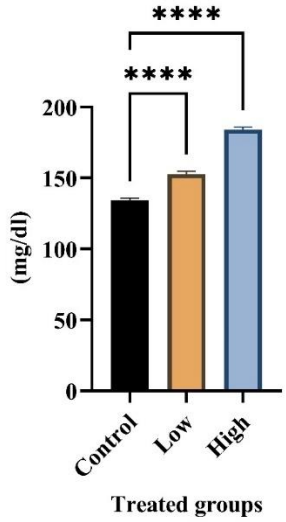

Figure 10(d): Lambda-cyhalothrin significantly alter the LDL of N. *tilapia*

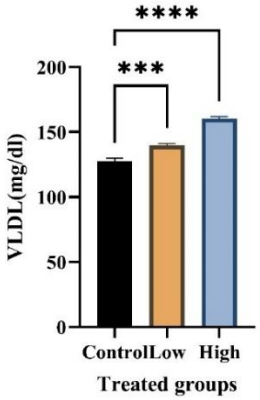

Figure 10(e): Lambda-cyhalothrin significantly alter the VLDL of N. *tilapia*

Figure 10: (a) demonstrating differences in the following: (a) cholesterol; (b) triglycerides; (c) HDL; (d) LDL; (e) VLDL variances among the groups. Each value is expressed as mean ± standard deviation (SD) at the 0.05% percent significance level.
* = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = not significant

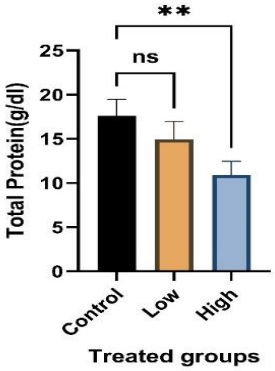

Figure 11(a): Lambda-cyhalothrin significantly alter the level of total protein in N. *tilapia*

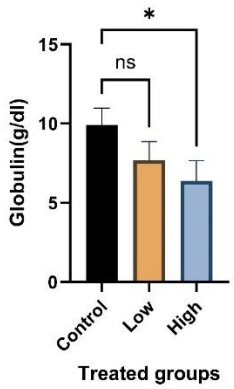

Figure 11(b): Lambda-cyhalothrin significantly alter the level of Globulin in N. *tilapia*

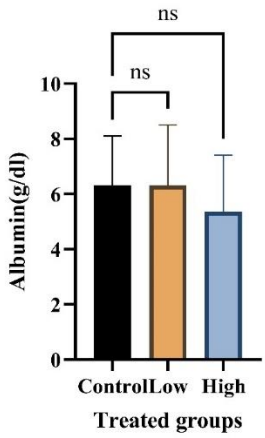

Figure 11(c): Lambda-cyhalothrin significantly alter the level of Albumin in N. *tilapia*

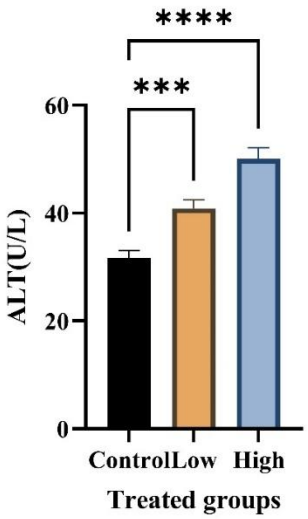

Figure 11(d): Comparison liver enzyme(ALT) of N.*tilapia* treated with Lambda-cyhalothrin

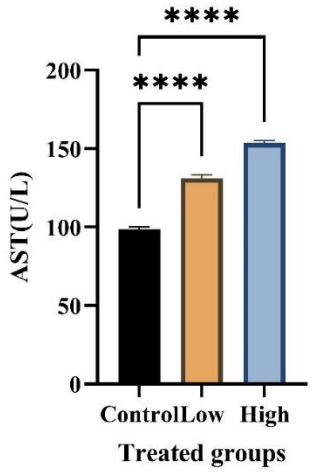

Figure 11(e): Comparison liver enzyme (AST) of N. *tilapia* treated with Lambda-cyhalothrin

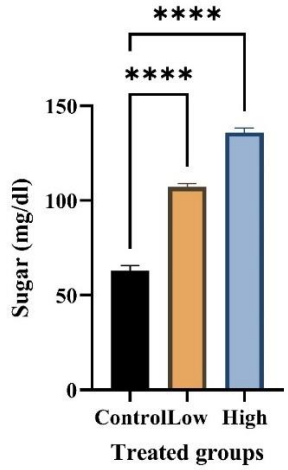

Figure 10(f): Lambda-cyhalothrin significantly alter the level of sugar in N. *tilapia*

Figure 11: (a) showing differences in total proteins between the groups (b) globulin, (c) albumin (d) ALT (e) AST and (f) blood glucose are the examples of the differences between the groups. Each value is expressed as mean ± standard deviation (SD) at the 0.05 percent significance level.
* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant

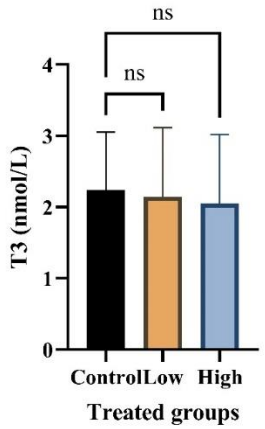

Figure 12(a): Lambda-cyhalothrin significantly alter the T3 in N. *tilapia*

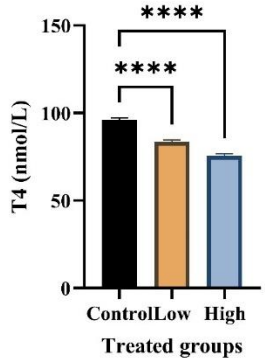

Figure 12(b): Lambda-cyhalothrin significantly alter the T4 in N. *tilapia*

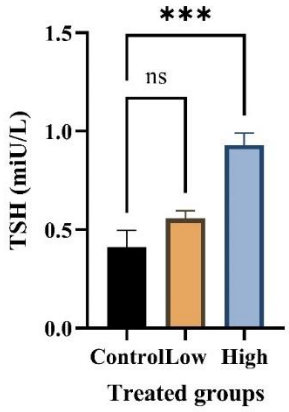

Figure 12(c): Lambda-cyhalothrin significantly alter the TSH in N. *tilapia*

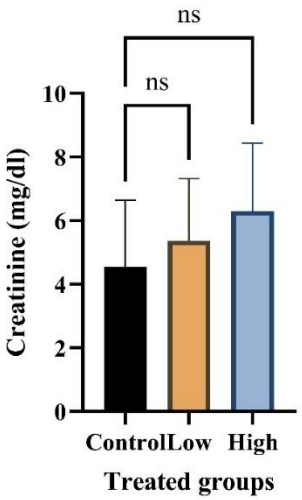

Figure 12(d): Lambda-cyhalothrin significantly alter the TSH in N. *tilapia*

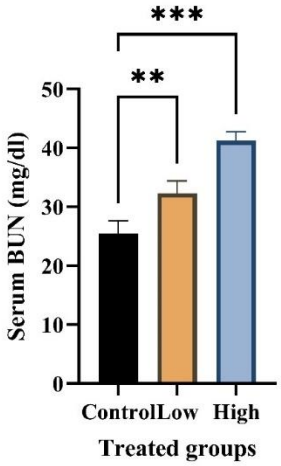

Figure 12(e): Lambda-cyhalothrin significantly alter the BUN in N. *tilapia*

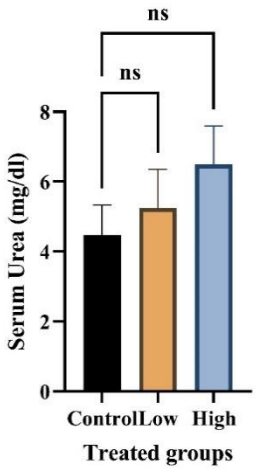

Figure 12(e): Lambda-cyhalothrin significantly alter the BUN in N. *tilapia*

Figure 12: (a) demonstrating differences in T3 level; (b) demonstrating differences in T4 value; (c) demonstrating differences in TSH level; (d) demonstrating differences in urea; (e) demonstrating differences in creatinine; and (f) demonstrating differences in BUN (blood urea nitrogen) value among the groups. Each value is expressed as mean ± standard deviation (SD) at the 0.05 percent significance level.

Not significant = ns, * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$

Discussion

Pesticides and other natural pollutants can get into people's bodies through their food, water, and air. These substances may interfere with endocrine functions, which could lead to cancer, reproductive issues, and other consequences. Prater, M. R., et al (2002). Pyrethroid herbicides are recognised as extremely poisonous to fish and other aquatic invertebrates, despite the fact that they rarely pose a threat to mammals (Kumar et al., 2008). According to earlier studies conducted by some authors, lambda-cyhalothrin killed and immobilised D. magna and other aquatic creatures (Hasenbein et al., 2016), Even so, these endpoints might not be sensitive enough to identify subtle alterations in the cladoceran organism that could affect the aqueous habitats' ecological connections. The study's findings illustrated the effects of varying lambda-cyhalothin concentrations on the sensitive kidney, liver, and gills of N. tilapia (Rehman et al., 2024; Khuram et al., 2024; Asif et al., 2024).

For toxicological research and water pollution monitoring, histopathological analyses of various fish tissues subjected to pollution are helpful methods. Fish subjected to varying pesticide concentrations exhibit tissue changes, which are a functional response of the organism and reveal details about the type of toxin. Histology can teach us about the state and function of organs. Reduced growth, survival, and physical fitness, decreased reproductive results, and increased susceptibility to pathogenic agents are all consequences of organ tissue damage and injury. The liver tissue produces ROS, or reactive oxygen species, that are created during the detoxification of pesticides. These ROS can react with essential macromolecules like lipid, carbohydrate, protein, and nucleic acid, causing oxidative harm to aquatic organisms Üner, N., et al., (2006). Damage from ROS to natural and fundamental cellular components is frequently thought to be an important cause of underlying histological diseases Sepici-Dinçel, A., et al., (2009).

Fish exposed with various pesticides may have pathological modifications in their kidney, liver, gills, and spleen, which can impact their homeostasis and lead to physiological disorders in fish. This study documented the histopathological changes in several tilapia fish organs caused by pesticides and other waterborne pollutants Abdullah, A., et al., (2008).

Histological changes are excellent indicators of the effects that different toxicants have on an organism. Important organs such as fish gills, kidney and liver may experience changes in morphology and physiology due to exposure to toxicants (Hamed and Osman, 2017). Fish's osmoregulatory and respiratory systems depend on their gills. The control fish's gills showed no histological alterations (Basharat et al., 2024).

The results shown here need multiple adjustments. Following exposure to 0.24 and 0.36 parts per billion of lambda-cyhalothrin, gill tissues showed changes, including hyper plasia, oedema, and the fusion of both primary and secondary gill lamellae. Respiratory discomfort is among the first signs and symptoms of pesticide exposure (McDonald, 1983). Additional studies demonstrated hemorrhage at the major lamellae, epithelium hypertrophy and hyperplasia, neighboring secondary lamellae fusing, epithelium lifting up, and secondary lamellar necrosis and desquamation (Cengiz and Unlu, 2002, 2003).

According to Erkmen et al. (2000), Fish exposed to cyphenothrin will exhibit clumps of lamellae, necrosis and degeneration of secondary lamellae, oedema, lifting of the epithelial layer from the gill lamellae, and shortening of the secondary lamellae.

The gill lamellae of guppy Poecilia reticulate treated with chlorpyrifos in earlier studies on Nile tilapia showed shorter gill lamellae, fusion, total lamella destruction, increased vacuolation, and an uneven appearance (De Silva & Samayawardhena, 2002). Zeta cypermethrin treatment of L. reticulatus resulted in necrosis, exudation, hyperplasia, displacement of the

epithelium layer from the gill lamellae, and shortening of the secondary lamellae, according to Caliskan et al. (2003).

Cengiz and Unlu (2006) reported observing epidermal hypertrophy, desquamation, tissue necrosis, epithelial lifting, oedema, aneurism, dilatation of the large lamellae capillaries, and lamellar fusion in the gills of Gambusia affinis treated to deltamethrin. Gills are commonly used as models for studies on the effects on the environment and are regarded as trustworthy indicators of the purity of the water. Considering that the gills serve as the primary route for the introduction of chemicals like pesticides The test fish's gills underwent a histological examination, which clearly showed serious cellular defects areas of inflammation, lesions, cancer, necrosis, pigment, and inclusion bodies. In contrast to the test fish under control, fish exposed to concentrations of 0.36 ppb had a noticeable departure from normal coloration and behavioral responses. The gills are vital organs that carry out several functions like as respiration, ammonia excretion, and ion and water exchange (osmoregulation). Gills are quite close to the external aquatic environment, making them a prime target for contaminants and changes in water quality. The study found that the test fish's gills underwent notable histopathological alterations as the test chemical dosage increased.

The control fish's kidney showed no histological alterations. After fish were displayed to 0.24 ppb and 0.36 ppb lambda-cyhalothrin, histological changes in treated kidney groups demonstrated deficiencies in kidney tissues as inflammation, micronuclei cluster formation, tubular necrosis, sinusoidal space, and melanomacrophase. Renal disorders may be helpful indicators of environmental contamination in fish since the kidneys absorb a significant amount of post-branchial blood (Ortiz et al., 2003). According to Dhanapakiam and Premlatha (1994), Cyprinus carpio renal cells exposed to malathion and sevin displayed hypertrophy of the cells, changes in the nuclear structure, the formation of vacuoles, necrosis, and degradation of renal components. Labeo rohita-affected cells exposed to hexachlorocyclohexane exhibited necrotic changes at the nucleus and tubule dilatation, which were suggestive of karyolysis and karyohexis, according to Dass and Mukherjee (2000).

The liver is the part of the body that undergoes the biggest morphological alterations in fish exposed to pesticides. As the organ most directly involved with detoxification and biotransformation, the liver is also one of the most impacted by waterborne toxins due to its location, anatomy, and blood supply. The liver of the control fish demonstrated no histopathological changes. The results of this study showed several alterations in the liver, such as fibrosis, broad sinusoidal gaps, necrosis, and changes in the liver tissues, such as the creation of pyknotic nuclei and micronuclei clusters.

These changes could be associated to the direct detrimental the impacts of contaminants on tissues because the liver is the centre of purification of various contaminants and materials. Soufyet et al., (2007) the primary organ involved in detoxification is the liver (Dutta et al., 1993). Hepatic alterations may serve as useful markers of prior the exposure to environmental stressors. According to Gill et al. (1990), a freshwater fish called Punctius conchonius developed aberrant liver behaves as a result of repeated exposure to sub-lethal pesticide concentrations. These deficiencies included hepatocyte fatty degeneration, hypertrophy, nuclear pycnosis, vacuole formation, and karyolysis.

In another study, Cengiz et al. (2001) noted various abnormalities related to the liver, such as degeneration, hypertrophy, haemorrhage, enlargement of the sinusoidal structures, nucleus in pycnosis, vacuole formation of cell cytoplasm, and infiltration of monochrome lymphocytes. There have been reports of Corydoras paleatus, vacuole formation, atrophy, and localised necrosis as indications of being exposed to methyl parathion (Fanta et al., 2003).

The liver of G. affinis treated with deltamethrin was examined by Cengiz and Unlu (2006), who found that there was fatty degeneration due localised necrosis, nucleus in pycnosis, hepatocyte hypertrophy, which enlarged kupffer cells, disturbance of the circulatory system, and narrowing of the sinusoids. Numerous pathogenic changes have been observed in fish exposed to different pesticides. Similar alterations were noted in the liver of Catlacatla subjected to chlorpyrifos by Tilak et al. (2005).

Pathological alterations included hepatocyte cytoplasm degradation, atrophy, vacuole

development, blood vessel rupture, necrosis, and loss of hepatocyte cell membrane arrangement. The size of the hepatic cords is discovered to have diminished, and the nucleus became pyknotic. It is known that xenobiotics, as well as pesticides and other toxicants and metabolites that pose serious health risks to N. *tilapia* kidney, liver and placental tissues, were adversely affected by lambda-cyhalothrin levels non-lethal controls on how to to all histological examinations.

This study, along with other research, suggests that severe physiological issues caused by histological abnormalities in the kidney, liver, and gills may lead to the death of fish. Our investigation revealed that tilapia exposed to pesticides experienced significant metabolic alterations, which likely contributed to physiological stress or even harm. Elevated levels of cholesterol, triglycerides, LDL and VLDL indicate impaired lipid metabolism, which is a common phenomenon in aquatic animals exposed to pesticides Similar changes by El-Sayed et al., (2020) and Ali et al., (2018) found in the fat of Oreochromis niloticus. (2018), who linked these changes to oxidative stress and liver dysfunction. These findings suggest that pesticides disrupt lipid homeostasis, leading to lipid accumulation in the blood, leading to oxidative damage and liver dysfunction

The observed decrease in HDL levels is particularly concerning given HDL's role in reverse cholesterol transport. This decline corresponds with the results of Kaya et al. (2019), who found that Cyprinus carpio exposed to pyrethroids had lower HDL levels. While there is little information on atherosclerosis in aquatic species, these decreases can suggest poorer cholesterol clearance and a higher risk of cardiovascular problems in fish. This emphasises how aquatic organisms may have long-term cardiovascular adverse effects from exposure to pesticides. Antibiotic-treated fish also significantly increased levels of ALT and AST, liver enzymes that are markers of hepatocellular injury.

Ahmad et al., (2017) reported similar results in chlorpyrifos-treated fish, with significantly elevated liver enzymes due to oxidative stress and membrane disruption. These enzymes are reliable indicators of liver damage, and numerous studies across different fish species and pesticide types consistently report elevated levels, underscoring the hepatotoxicity of these chemicals.

Additionally, our study found reduced levels of albumin, globulin, and total protein, suggesting both impaired protein synthesis and liver dysfunction. These results are in line with those of Rahman et al. (2019), who reported a comparable reduction in Catla catla protein levels after exposure to malathion. Since these proteins are manufactured by the liver, the observed losses suggest that fish exposed to pesticides have severe hepatic stress and diminished synthesising capacity (Iftikhar et al., 2024, Sattar et al., 2024; Bilal[a,b], 2021).

The elevated blood sugar levels seen in this study could be a stress response, possibly caused by elevated cortisol levels, as suggested by Ramesh et al., (2018). Stress-induced hyperglycemia has been documented in a variety of ecologically exposed fish species, and studies of the ability of pesticides to disrupt neuronal tissues indicate body responds normally to toxic stress is further confirmed such as Gupta et al., (2021), Cats exposed to organophosphates experienced similar hormonal abnormalities, including changes in thyroid hormones (T3, T4, and TSH). These hormones are important for metabolism, and their dysfunction predisposes cats to chronic metabolic disorders.

Pesticides also significantly affected renal function, as indicated by significant increases in urea and creatinine levels. This result is consistent with Ali et al. (2020), which showed nephrotoxic effects of pesticides in fish, leading to decreased glomerular filtration, and subsequent renal damage Elevated BUN and creatinine levels suggest possible renal function impairment, from direct exposure to nephrotoxic pesticides or from inappropriate systemic oxidative - This can happen through stress

The biochemical changes observed in the present study corroborate earlier findings and highlight the impact of pesticides on fish health. These findings emphasize the importance of strict regulation and continuous environmental monitoring to reduce the negative effects of pesticides on aquatic ecosystems Recurring theme occur again in various studies confirm that aquatic life is vulnerable to environmental pollutants and environmental susceptibility to environmental exposure caused by pesticides Child.

Conclusion

This study provides an in-depth analysis of the histopathological and biochemical effects of pesticide

exposure on the tissue integrity and physiological state of Nile tilapia (Oreochromis niloticus), highlighting significant adverse effects. Research has shown that pesticides that include lambda-cyhalothrin can cause significant injury to vital organs like the kidneys, liver, and gills. The documented histological abnormalities, which included fibrosis, inflammation, sinusoidal gaps, edema, pyknotic nuclei, tubular necrosis, micronuclei cluster formation, and hyperplasia, were directly linked to higher pesticide concentrations and extended periods of exposure. Notable abnormalities in the biochemical processes of lipid metabolism, liver function, glucose regulation, thyroid activity, and renal function were found by the investigation. The raised levels of cholesterol, LDL, VLDL, and triglycerides along with the decreasing HDL suggest a breakdown in lipid homeostasis. While a decrease in total protein and albumin indicates compromised liver function, elevations of ALT and AST point to significant hepatic damage. Significant renal failure is indicated by elevated creatinine, BUN, and urea levels; on the other hand, endocrine disturbance is suggested by an increase in glucose and altered thyroid hormones (T3, T4, and TSH). All of these findings demonstrate the severe physiological stress and cellular damage that pesticide exposure causes, underscoring the grave risk that it poses to fish health and the greater aquatic ecosystem.

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