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***Research Article** (Open Access)*

HISTOPATHOLOGICAL ALTERATIONS IN LIVER ARCHITECTURE OF C*HANNA PUNCTATUS* UNDER SUB-LETHAL HYBRID PESTICIDE (CHLORPYRIFOS 50% + CYPERMETHRIN 5% EC) STRESS

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ABSTRACT
Sublethal contact with a combined pesticide formulation containing chlorpyrifos 50% and cypermethrin 5% EC can produce substantial histopathological injury in non-target aquatic organisms. The present investigation evaluated pesticide-induced hepatic lesions in the freshwater teleost *Channa punctatus*. Fish were maintained for 14 days at two sublethal concentrations, 0.01025 and 0.0205 mg L^{-1}, representing 1/20 and 1/10, respectively, of the 96-h LC_{50} value of 0.205 mg L^{-1}. Microscopic assessment of hepatic tissue demonstrated a concentration-related pattern of injury characterized by hepatocellular degeneration and vacuolization, loss of normal liver organization, dilation and congestion of the central veins and sinusoids, inflammatory-cell infiltration, and degenerative nuclear changes. The greater severity of lesions at the higher concentration indicated progressive hepatic impairment and physiological stress. The results establish the pronounced hepatotoxic potential of the formulation and support the use of liver histopathology as a sensitive indicator of pesticide toxicity. They also emphasize the ecological threat posed by pesticide-contaminated agricultural runoff and the importance of strong regulatory measures for conserving freshwater biodiversity.

Keywords: *Channa punctatus*, *Chlorpyrifos, Cypermethrin, Histopathology, Hepatotoxicity, Aquatic Toxicology*

INTRODUCTION
Global agriculture was fundamentally transformed by the Green Revolution, during which extensive agrochemical application contributed to remarkable increases in cereal yields. However, the intensive use of synthetic fertilizers and pesticides simultaneously created serious environmental and public-health concerns. The choice of pesticide for a given agricultural application generally depends on the target organism and the compound's mode of action. Insecticides account for approximately 80% of worldwide pesticide use, whereas herbicides and fungicides constitute about 15% and 1.46%, respectively (Farag *et al.,* 2021). Figure 1 presents the principal pesticide categories and their corresponding applications (Al-Khazraji *et al.,* 2020; Rohani, 2023).

Organophosphates and pyrethroids are among the most widely applied synthetic pesticides owing to their effective, broad-spectrum control of numerous agricultural pests (Amin *et al.,* 2021; Ray & Shaju, 2023). Chlorpyrifos, an organophosphate insecticide, exerts neurotoxic effects through irreversible inhibition of acetylcholinesterase, causing acetylcholine to accumulate at cholinergic synapses and thereby disrupting normal neuronal signaling (Wołejko *et al.,* 2022). Cypermethrin (RS)-cyano-(3-phenoxyphenyl)methyl-(1RS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate is a type II synthetic pyrethroid derived from naturally occurring compounds in *Chrysanthemum cinerariifolium*. It prolongs the opening of voltage-gated sodium channels in neuronal membranes, producing sustained membrane depolarization and neurotoxicity. Its high insecticidal efficacy has led to widespread application on wheat, sugarcane, brinjal, okra, cabbage, onion, lettuce, cotton, sunflower, and other crops (Farag *et al.,* 2021).

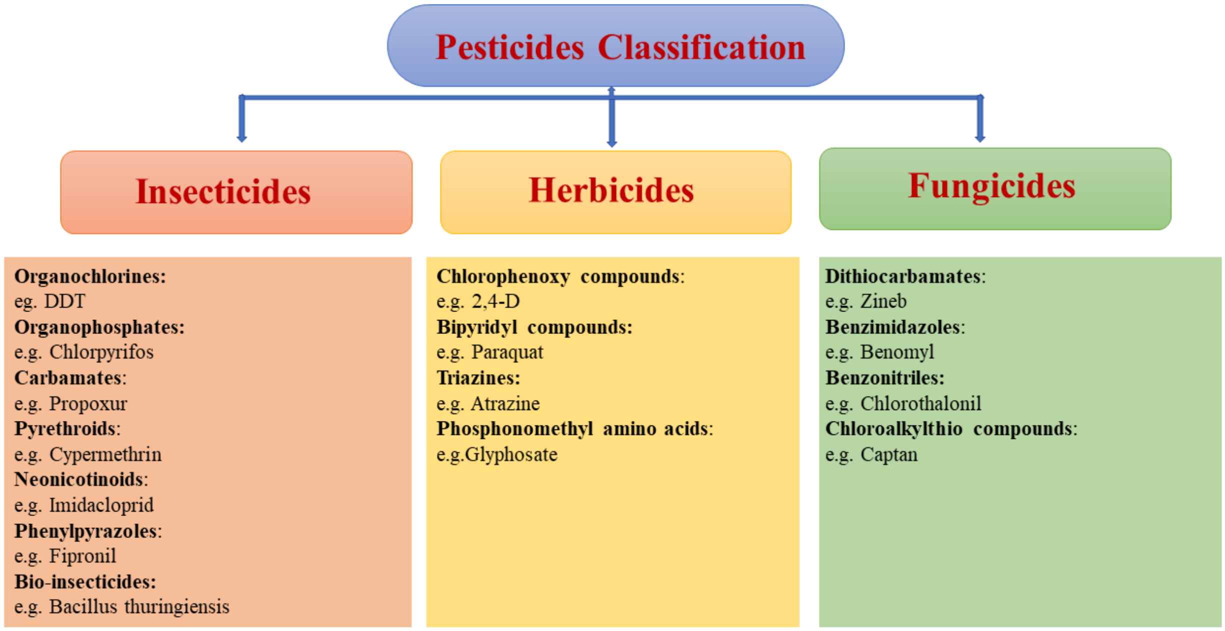

Figure 1. Classification of pesticides

Modern agricultural practice increasingly employs commercial mixtures of chlorpyrifos and cypermethrin to enhance insecticidal performance and limit resistance development. Although such formulations offer practical advantages for pest management, their toxicological consequences for non-target organisms-particularly aquatic species-remain insufficiently characterized. Evidence from earlier investigations indicates that simultaneous exposure to multiple pesticides may generate additive or synergistic interactions and can therefore be more toxic than exposure to individual compounds (Farag *et al.,* 2021; Wołejko *et al.,* 2022).

Fish serve as effective bioindicators of aquatic ecosystem health because xenobiotics are readily bioaccumulated from the surrounding environment, and toxic stress produces quantifiable behavioral, physiological, biochemical, and histopathological responses. Pesticide exposure has been associated with impaired growth, metabolism, immune competence, reproduction, and survival in fish, commonly involving oxidative stress, tissue lesions, and multi-organ dysfunction (Bojarski *et al.,* 2025; Kaviraj & Gupta, 2014; Rohani, 2023). Fish consequently constitute valuable experimental models in aquatic ecotoxicology and environmental risk assessment.

Within ecotoxicological research, histopathological responses are regarded as sensitive biomarkers of toxicant-induced tissue injury and organ impairment. Because the liver performs essential metabolic, detoxification, and xenobiotic-biotransformation functions, it is particularly vulnerable to pesticide-mediated damage. Histological examination provides direct evidence of cellular and tissue-level abnormalities, including hepatocyte degeneration, cytoplasmic vacuolization, vascular congestion, sinusoidal dilation, inflammatory-cell infiltration, and nuclear irregularities. These features establish hepatic histopathology as a reliable approach for assessing pesticide toxicity in fish (Akmal *et al.,* 2024; Kumar *et al.,* 2025; Naseem *et al.,* 2022; Naz *et al.,* 2023; Rohani, 2023).

The spotted snakehead, *Channa punctatus* (Bloch, 1793), is an economically and ecologically important freshwater teleost native to the Indian subcontinent. Its broad distribution, tolerance of laboratory conditions, and sensitivity to contaminants have established it as a model organism for aquatic toxicity

research. Hepatic lesions in *C. punctatus* have been widely examined to characterize pollutant-induced cellular and structural damage (Kumar *et al.,* 2025; Tabassum *et al.,* 2016).
Although the individual toxicities of chlorpyrifos and cypermethrin have been investigated extensively, comparatively little information is available regarding the histopathological effects of commercial formulations containing both compounds. The growing use of these mixtures, together with their likely entry into freshwater systems through agricultural runoff, creates an urgent need to evaluate their consequences for non-target aquatic organisms (Farag *et al.,* 2021; Wołejko *et al.,* 2022).
Accordingly, this study examined the effects of sublethal exposure to a commercial formulation containing chlorpyrifos 50% and cypermethrin 5% EC on the hepatic histopathology of *C. punctatus*. Adult specimens were exposed for 14 days to concentrations corresponding to 1/20 and 1/10 of the 96-h LC_{50}. Hepatic assessments focused on hepatocyte organization, cytoplasmic vacuolization, vascular congestion, sinusoidal structural changes, inflammatory-cell infiltration, and nuclear degeneration. The resulting evidence is intended to provide baseline information on the formulation's hepatotoxic potential and to support improved biomonitoring and ecological risk assessment of pesticide pollution in freshwater environments.

MATERIALS AND METHODS

Experimental Fish

Healthy specimens of *Channa punctatus* were procured from a local fish market in Ranchi, Jharkhand, India, and transported to the laboratory in oxygenated polyethylene bags. Upon arrival, the fish received a 1–2 min prophylactic bath in potassium permanganate ($KMnO_4$; 3 mg L^{-1}) to eliminate external pathogens and ectoparasites. Prior to experimentation, fish were acclimatized for two weeks in continuously aerated, dechlorinated tap water under a 12 h light : 12 h dark photoperiod. Individuals selected for the study had a mean body length of 10 ± 2 cm and a mean body mass of 18 ± 2 g. Fish condition and the laboratory environment were evaluated daily in accordance with standard guidance for fish toxicity testing (OECD, 2009).

Test Chemical

Turbo 505 EC, a commercial combined pesticide formulation containing chlorpyrifos 50% and cypermethrin 5% EC, served as the experimental toxicant. A stock solution was prepared using distilled water and diluted with dechlorinated tap water immediately before exposure to produce the specified test concentrations. Fresh pesticide solutions were prepared throughout the experiment to maintain stability and uniform distribution (Begum, 2020; OECD, 2009).

Determination of LC_{50} and Dose Selection

The 96-h median lethal concentration (LC_{50}) of the combined pesticide formulation was established through an acute toxicity bioassay performed according to OECD Test Guideline 203 (Fish Acute Toxicity Test). Mortalities were recorded at regular intervals over 96 h, and the LC_{50} was calculated by probit analysis. The estimated 96-h LC_{50} was 0.205 mg L^{-1}. Based on this value, two sublethal concentrations were selected for the 14-day exposure: 0.01025 mg L^{-1} (1/20 LC_{50}) and 0.0205 mg L^{-1} (1/10 LC_{50}) (Begum, 2020; Pandey *et al.,* 2011; Tiwari *et al.,* 2017).
A primary stock solution containing 275 mg L^{-1} of the test substance was prepared using distilled water. Working concentrations were subsequently obtained through serial dilution with dechlorinated water in each 20 L aquarium. Working solutions were prepared immediately before every medium renewal to ensure consistent pesticide exposure throughout the experimental period.

Experimental Design

The study was conducted under controlled conditions in the University Department of Zoology, Ranchi University, Ranchi, India. Following acclimatization, fish were randomly allocated to three treatment groups, each comprising three replicates of five fish per replicate:

- **Group I (Control):** Maintained in pesticide-free, dechlorinated tap water.
- **Group II (Low dose):** Exposed to 0.01025 mg L^{-1} (1/20 of the 96-h LC_{50}).

- **Group III (High dose):** Exposed to 0.0205 mg L^{-1} (1/10 of the 96-h LC_{50}).

Replicates were held in continuously aerated glass aquaria containing 20 L of dechlorinated water. Exposure followed a semi-static renewal protocol in which approximately 80–90% of the test medium was replaced every 24 h with freshly prepared pesticide solution to maintain consistent nominal concentrations (OECD, 2009). Mortality was checked daily, and dead fish were removed immediately to prevent water-quality deterioration. All animal maintenance and handling procedures followed institutional ethical requirements and were approved by the Institutional Ethical Committee of Ranchi University, Ranchi, India.

Water-Quality Analysis

Physicochemical properties of the exposure water were monitored throughout the experiment to verify that conditions remained suitable for fish survival. Dissolved oxygen, pH, temperature, ammonia, nitrate, nitrite, total alkalinity, salinity, total dissolved solids, and electrical conductivity were measured using standard procedures recommended by the American Public Health Association (Baird *et al.,* 2017). All monitored variables remained within acceptable limits during the exposure period (Table 1).

Feeding Regime

Fish were offered a commercial pelleted diet (OPTIMUM, Thailand) *ad libitum* once daily. Residual feed and fecal material were removed daily by siphoning to preserve water quality. In accordance with OECD recommendations, food was withheld for 24 h before pesticide exposure commenced and again before tissue collection to reduce physiological variation associated with feeding and handling.

Sample Collection and Tissue Processing

Livers were excised on day 14, immediately rinsed in physiological saline (0.75% NaCl) to remove adherent blood and mucus, and fixed for 24 h in Bouin's solution. Fixed specimens were dehydrated through a graded ethanol series, cleared, infiltrated with molten paraffin, and embedded in paraffin wax to form tissue blocks. Sections of 5 µm thickness were cut using a rotary microtome and stained with Harris' hematoxylin and eosin (H&E) for light-microscopic assessment. Photomicrographs were acquired using an Olympus CH20i microscope equipped with an image analyzer to compare histomorphological changes among treatment groups (Hossain *et al.,* 2025; Kumar *et al.,* 2025; Stalin *et al.,* 2019).

Statistical Analysis

Experimental observations are expressed as the mean ± standard deviation (SD).

RESULTS

Physicochemical Characteristics of the Test Water

Throughout the experiment, all water-quality parameters remained within ranges considered suitable for maintaining *C. punctatus* (Table 1).

Table 1: Physicochemical parameters of the test water (mean ± SD).

Parameter	Group I	Group II	Group III
DO (ppm)	5.4 ± 1.5	5.8 ± 1.48	5.4 ± 0.55
Ammonia (mg/L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Nitrate (mg/L)	0.0 ± 0.0	1.0 ± 2.24	0.0 ± 0.0
Nitrite (mg/L)	0.0 ± 0.0	0.0 ± 0.0	0.02 ± 0.04
Total alkalinity (mg/L)	120 ± 5.02	142.0 ± 45.08	129.0 ± 46.42
TDS (ppm)	239 ± 7.85	139.4 ± 20.86	119.6 ± 18.7
Temperature (°C)	30.5 ± 1.0	25.54 ± 0.46	25.36 ± 0.24
Salt (%)	0.02 ± 0.01	0.01 ± 0.0	0.01 ± 0.0
Ph	6.9 ± 0.5	6.64 ± 0.37	6.86 ± 0.05
EC (µS/cm)	479 ± 16	277.4 ± 38.38	356.0 ± 35.04

Effect of the Combined Pesticide on Hepatic Histopathology

Sublethal chlorpyrifos–cypermethrin exposure for 14 days produced clear, concentration-dependent histopathological injury in the liver of *C. punctatus*.

Group I (Control)

Control livers retained normal tissue organization. Polygonal hepatocytes (H) formed the hepatic parenchyma and displayed homogeneous cytoplasm, centrally positioned rounded nuclei (N), and conspicuous nucleoli (Nu). Irregularly distributed sinusoids (S) separated the hepatocyte cords, with Kupffer cells (KC) located along their internal lining. The central vein (CV) and its endothelial lining (E) were intact (H&E; ×400; Figure 2).

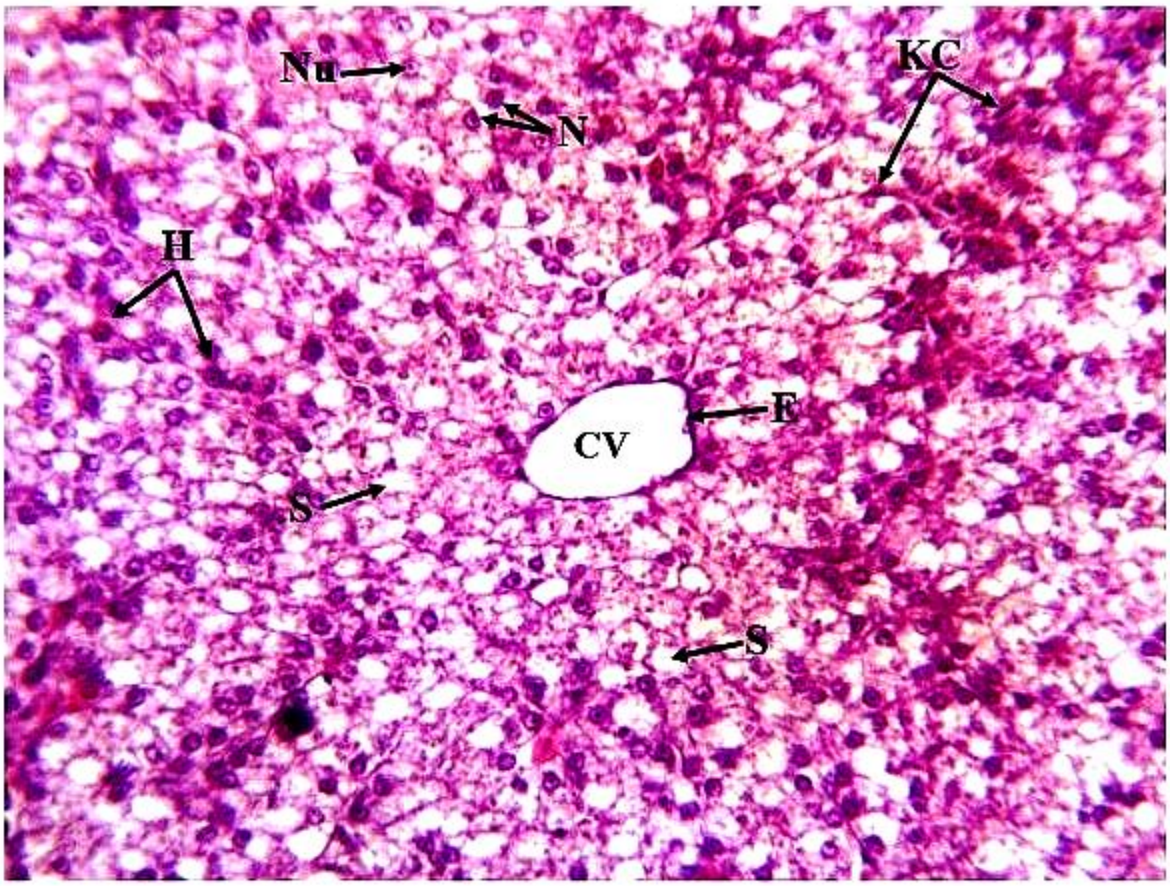

Figure 2: Transverse liver section from untreated Channa punctatus showing preserved hepatic organization. The parenchyma comprises polygonal hepatocytes (H) with uniform cytoplasm, centrally placed rounded nuclei (N), and distinct nucleoli (Nu). Sinusoids (S) occur irregularly between hepatic cords, Kupffer cells (KC) line the sinusoidal surface, and the central vein (CV) retains an intact endothelium (E). H&E staining; ×400.

Group II (1/20 of the 96-h LC_{50})

Exposure to 1/20 of the 96-h LC_{50} produced lesions of moderate severity. The central vein(CV) exhibited slight dilation and deformation together with degeneration of its endothelial lining (dE). A loss of hepatocyte population, dilation of sinusoidal space and Fatty degeneration of hepatocytes (Fd) resulted in loosening of hepatic parenchyma with syncytial cell like appearance (fH) The regular polygonal morphology and chord- like appearance of hepatocytes were disrupted. Most of the hepatocytes exhibited moderate to severe level of cytoplasmic as well as nuclear degeneration (dN,An) and pronounced cytoplasmic vacuolization (V). Sinusoids showed severe dilation and congestion (cS) giving an appearance of multiple regions of focal haemorrhage with Infiltration of leucocytes (L). Mild Kupffer (KC) hyperplasia was observed. (H&E; ×400; Figure 3).

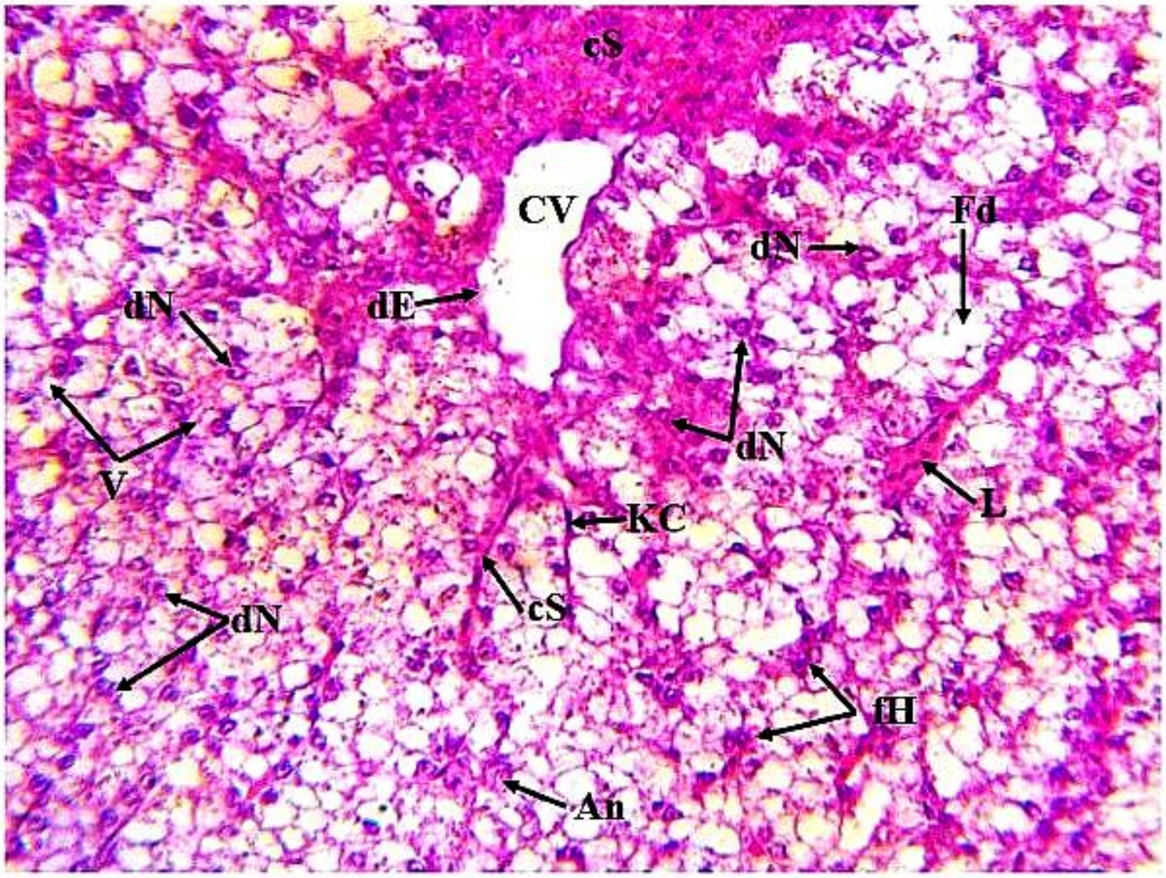

Figure 3: Transverse liver section from Channa punctatus after 14 d of exposure to 1/20 of the 96-h LC_{50} of the chlorpyrifos-cypermethrin formulation. Mild dilation and distortion in central vein (CV) with degeneration of endothelial lining (dE) at some places. Fatty degeneration of hepatocytes (Fd) Loss of membrane integrity led to presence of fused hepatocytes (fH), Degeneration in nuclei (dN) was evident in form of shrinkage, peripheralization, condensation and loss of nuclear material anuclear hepatocytes (An). Hepatocytic cytoplasm showed severe vacuolization (V). Sinusoids with severe dilation and congestion (cS), Infiltration with leucocytes (L). Hyperplasia of Kupffer cells (KC) were marked as compared to control group liver sections. H&E staining; ×400.

Group III (1/10 of the 96-h LC_{50})

Severe hepatic lesions developed in fish exposed to 1/10 of the 96-h LC_{50}. The central vein (CV) was markedly dilated and congested, its endothelium was disrupted (dE), and substantial leukocyte infiltration (L) was evident. Although the parenchyma remained relatively compact, hepatocytes had lost their regular arrangement and exhibited widespread cytoplasmic and nuclear degeneration (dN). Aggregation and shrinkage of nuclei (AH) occurred frequently, producing a multinucleated-like appearance. Lymphocytes were prominent throughout the hepatic parenchyma, and the sinusoidal spaces showed mild congestion and dilation (cS). Kupffer cells (KC) showed mild hyperplasia, collectively indicating progressive pesticide-induced hepatic injury (H&E; ×400; Figure 4).

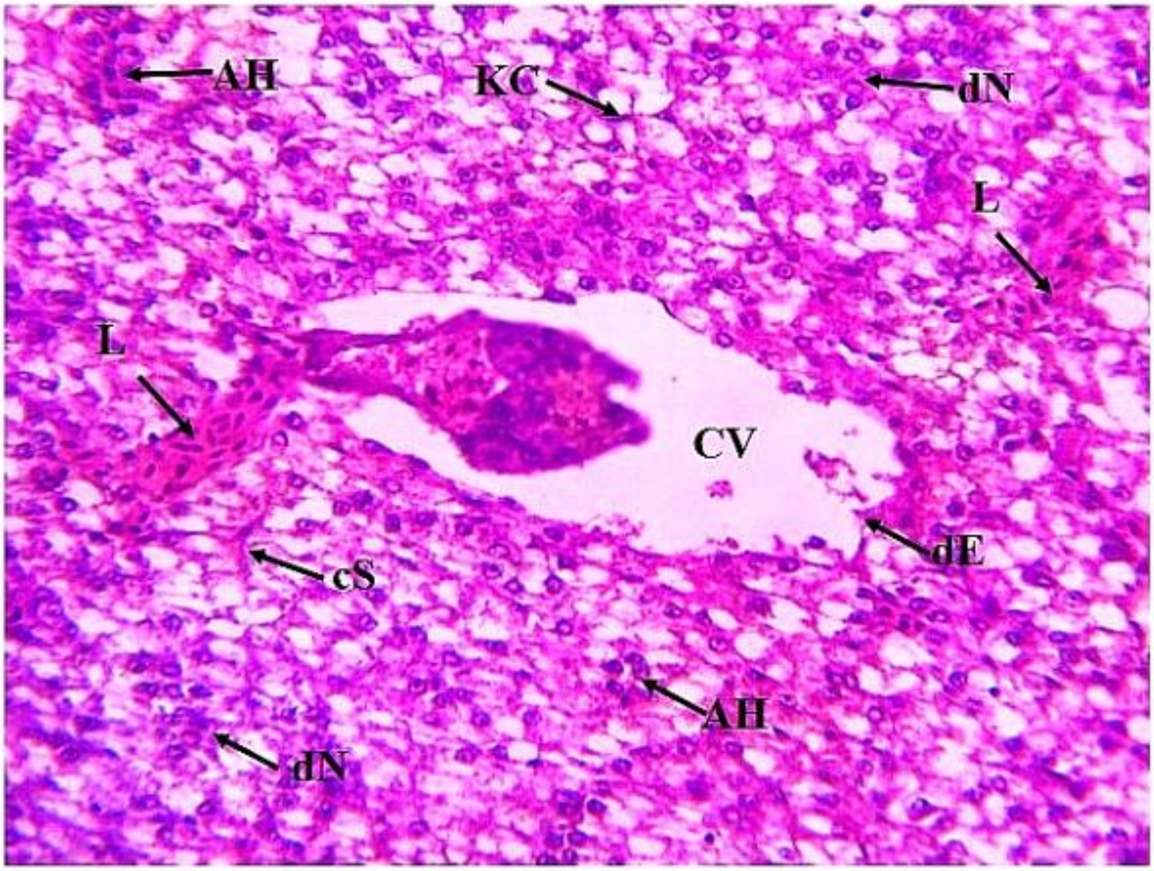

Figure 4: Transverse liver section from Channa punctatus after 14 d of exposure to 1/10 of the 96-h LC_{50} of the chlorpyrifos-cypermethrin formulation. Severe lesions comprising pronounced dilation and congestion of the central vein (CV), endothelial degeneration (dE), extensive leukocytic infiltration (L), irregular hepatocyte organization, severe cytoplasmic and nuclear degeneration (dN), nuclear aggregation and shrinkage (AH), lymphocytic infiltration of the parenchyma, congestion and dilation of the sinusoidal spaces (cS), along with hyperplasia of Kupffer cell (KC). Were marked as compared to control group liver sections. H&E staining; ×400.

DISCUSSION

The present findings demonstrate that sublethal exposure to the chlorpyrifos–cypermethrin formulation produced substantial, concentration-dependent hepatic histopathology in *Channa punctatus*. Increasing the pesticide concentration intensified hepatocyte degeneration, cytoplasmic vacuolization, vascular congestion, endothelial injury, inflammatory infiltration, and nuclear abnormalities, confirming the hepatotoxicity of this commercial mixture.

Comparable hepatic responses to organophosphate and pyrethroid exposure have been reported in several fish species. Rohani (2023) identified hepatic vacuolation, necrosis, cellular swelling, hepatocyte degeneration, hemorrhage, pyknosis, and fatty change as characteristic effects of pesticide exposure, while Haque et al. (2018) described a similar hepatotoxic pattern. In *Oreochromis niloticus*, Majumder and Kaviraj (2021) observed concentration-dependent liver damage following cypermethrin treatment: lower doses caused hepatocyte vacuolation and distortion of the nuclear membrane, whereas higher doses produced extensive vacuolation and pyknosis. Kaur and Mishra (2020) reported abnormal nuclei, nuclear hypertrophy, necrosis, tissue-space formation, and vacuolation in chlorpyrifos–cypermethrin-exposed *C.*

punctatus, findings closely aligned with those recorded in the present study. Kumar et al. (2025) likewise documented dose-related increases in pyknosis, vacuolization, and cytoplasmic degeneration in *C. punctatus* following cypermethrin and malathion exposure.

Agbohessi et al. (2023) proposed that high toxicant burdens impose stress on hepatic vasculature in *Clarias gariepinus*, resulting in sinusoidal obstruction and congestion, vessel rupture and hemorrhage, or compensatory dilation that facilitates circulation. Those authors associated hepatocellular vacuolation with intracellular lipid accumulation, reflecting metabolic disturbance and an imbalance between the production and release of cellular substances. This interpretation is consistent with the vascular and vacuolar changes observed in the current experiment.

Additional evidence supports the present observations. Cypermethrin exposure in *Schizothorax esocinus* caused loss of hepatic cell boundaries, accumulation of bile pigment, necrosis, leukocyte infiltration, and pyknosis (Akhtar *et al.,* 2020). Fiaz et al. (2017) recorded vascular congestion, cell-membrane dissolution, pyknosis, necrosis, and vacuolation in exposed *Labeo rohita*. Such nuclear degeneration, contraction, and aggregation are indicative of severe cellular stress and progression toward cell death. Stalin et al. (2019) further demonstrated that chlorpyrifos induces vacuolation, blood congestion, and hepatocellular necrosis, with damage becoming more pronounced as exposure duration increases. Although their study evaluated duration rather than concentration, the progressive response is compatible with the dose-related injury detected in the present investigation.

Collectively, these results reinforce the value of hepatic histopathology as a sensitive indicator of pesticide pollution in aquatic systems. Rohani (2023) emphasized that histopathological assessment can reveal toxicant effects, facilitate aquatic-contamination surveillance, and indicate the severity of pesticide injury. Majumder and Kaviraj (2021) similarly concluded that tissue-level evaluation can assist in defining ecologically safe pesticide concentrations and managing natural water resources. Liver-index results reported by Agbohessi et al. (2023) revealed moderate tissue alterations in pesticide-exposed fish, further demonstrating the utility of this approach for toxicity assessment.

CONCLUSION

This study demonstrates that sublethal exposure to a commercial chlorpyrifos–cypermethrin formulation induces significant, concentration-dependent hepatic histopathological alterations in *Channa punctatus*. The observed lesions-including hepatocyte degeneration, cytoplasmic vacuolization, vascular congestion, inflammatory infiltration, and nuclear abnormalities-confirm the hepatotoxic potential of this widely used pesticide mixture at environmentally relevant sublethal concentrations. These findings underscore the importance of hepatic histopathology as a sensitive biomarker for monitoring pesticide contamination in freshwater ecosystems and highlight the need for regulation of combined pesticide formulations to protect non-target aquatic organisms.

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