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Sublethal λ-Cyhalothrin Exposure Induces Oxidative Stress and Genotoxicity in the Freshwater Fish *Channa punctatus*

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Abstract

λ-Cyhalothrin is a widely used type II synthetic pyrethroid that can pose a significant risk to non-target aquatic organisms. The present study investigated the sublethal effects of λ-Cyhalothrin on oxidative stress and genetic integrity in the freshwater fish *Channa punctatus*. Healthy fish were acclimatised for one month and randomly allocated into five groups (n = 10): negative control, cyclophosphamide-positive control (4 mg/L), and λ-Cyhalothrin low (0.495 µg/L), medium (0.990 µg/L), and high (1.980 µg/L) exposure groups. The selected concentrations represented 1/16th, 1/8th, and 1/4th of the reported 96-h LC_{50} of 7.92 µg/L. Fish were exposed for 7 days under semi-static conditions with daily renewal and re-dosing. Lipid peroxidation, reduced glutathione, and superoxide dismutase were evaluated in blood and liver, while micronucleus and alkaline single-cell gel electrophoresis (comet) assays were performed using peripheral erythrocytes. λ-Cyhalothrin exposure produced concentration-dependent oxidative imbalance, characterised by increased LPO and depletion of GSH and SOD in both blood and liver. Blood LPO increased by 56.96–506.33%, whereas GSH and SOD decreased by 11.11–33.33% and 6.64–26.17%, respectively. Hepatic LPO increased by 70.48–462.86%, accompanied by reductions of up to 32.69% in GSH and 31.90% in SOD. MN frequency increased from 0.065% in controls to 0.24%, 0.46%, and 0.93% at low, medium, and high concentrations, respectively. Similarly, comet assay parameters demonstrated progressive DNA damage, with tail length and olive tail moment increasing by up to 662.08% and 344.30%, respectively. Overall, the findings demonstrate that 7-day sublethal λ-Cyhalothrin exposure induces pronounced oxidative stress and concentration-dependent chromosomal and DNA damage in *C. punctatus*, supporting the use of integrated biochemical and genotoxic biomarkers for assessing pesticide toxicity in freshwater fish.

Keywords: λ-Cyhalothrin; *Channa punctatus*; oxidative stress; genotoxicity; micronucleus assay; comet assay; DNA damage; pesticide toxicity.

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Introduction

Aquatic ecosystems are increasingly vulnerable to pesticide contamination from agricultural runoff, and fish are among the most sensitive non-target organisms used to monitor such pollution (Nwani *et al*., 2010; Al-Ghanim *et al*., 2019). Freshwater fish also bioaccumulate pesticide residues and exhibit biochemical, physiological, and cellular disturbances even under sublethal exposure conditions (Al-Ghanim *et al*., 2019). Among contemporary insecticides, synthetic pyrethroids are widely used and are recognised for marked toxicity to aquatic organisms, particularly fish (Sayeed *et al*., 2003; Fetoui *et al*., 2015). λ-Cyhalothrin, a type II pyrethroid, has been detected in water bodies and has drawn concern because even low concentrations can disrupt normal biochemical and physiological processes in freshwater teleosts (Vieira & Martinez, 2018).

A recurring mechanism of pesticide toxicity in fish is oxidative stress, reflected by elevated reactive oxygen species and damage to lipids, proteins, and antioxidant defence systems (Parvez & Raisuddin, 2005; Fetoui *et al*., 2015; Al-Ghanim *et al*., 2019). In *Channa punctatus*, pyrethroid exposure has consistently been associated with increased lipid peroxidation and altered antioxidant responses, supporting the use of oxidative stress biomarkers in ecotoxicological studies (Sayeed *et al*., 2003).

Prior work has shown that λ-Cyhalothrin and other pyrethroids can significantly elevate lipid peroxidation in fish tissues, indicating membrane damage under toxic stress (Kumar *et al*., 2012; Venturini *et al*., 2018; Vieira & Martinez, 2018). Antioxidant biomarkers such as reduced glutathione and superoxide dismutase also respond sensitively to pesticide exposure, although the direction of change can vary with tissue, concentration, and duration of exposure (Ansari *et al*., 2009; Nwani *et al*., 2010; Venturini *et al*., 2018). Pesticide-induced oxidative imbalance is also closely linked with genetic damage in fish (Ansari *et al*., 2009; Nwani *et al*., 2010; Nwani *et al*., 2013). Blood-based biomarkers are especially useful because erythrocytes in fish provide a convenient system for detecting chromosomal injury and DNA strand breaks *in vivo* (Çavaş & Ergene-Gözükara, 2003; Ali *et al*., 2009; Çavaş, 2011).

The micronucleus assay has long been used to detect chromosomal damage in fish erythrocytes, including after λ-Cyhalothrin exposure (Campana *et al*., 1999; Çavaş & Ergene-Gözükara, 2003). Likewise, the comet assay is a sensitive tool for detecting DNA strand breaks and has repeatedly demonstrated concentration- and time-dependent genotoxicity in *Channa punctatus* exposed to pesticides (Ali *et al*., 2008; Nwani *et al*., 2009; Pandey *et al*., 2018). Studies in *Channa punctatus* have shown that combining micronucleus and comet assays provides a stronger assessment of aquatic genotoxicity than relying on a single endpoint alone (Nwani *et al*., 2009; Ali *et al*., 2009; Nwani *et al*., 2013). At the same time, some pesticides produce stronger comet or erythrocytic nuclear abnormality responses than micronucleus induction, which supports using multiple biomarkers in parallel (Ansari *et al*., 2009; Ahmad & Ahmad, 2016). *Channa punctatus* is a suitable sentinel species for such work because it is widely used in freshwater toxicology and responds clearly to pesticide-induced oxidative and genotoxic stress (Sayeed *et al*., 2003; Ali *et al*., 2009; Nwani *et al*., 2013). Earlier studies on this species have documented oxidative stress, lipid peroxidation, antioxidant disruption, and DNA damage after exposure to different classes of pesticides, establishing a strong biomarker foundation for testing λ-Cyhalothrin toxicity (Ali *et al*., 2009; Nwani *et al*., 2010; Nwani *et al*., 2013).

Against this background, the present study evaluates the effects of sublethal λ-Cyhalothrin exposure on oxidative stress and genotoxicity in *Channa punctatus* using lipid peroxidation, reduced glutathione, and superoxide dismutase as biochemical markers, together with micronucleus and comet assays. By integrating these endpoints after short-term exposure, the study is designed to clarify the oxidative and genetic damage potential of λ-Cyhalothrin in an ecologically relevant freshwater fish model.

Materials and Methods

Experimental fish specimens and chemicals

Healthy specimens of the freshwater fish *Channa punctatus* were procured from local fish suppliers and transported to the laboratory under suitable conditions. The fish were selected on the basis of apparent health and uniformity in size and body weight. 29 ± 2.0 g and 14 ± 4.0 cm are the average live weight and length of the specimens. In order to prevent the transmission of dermal infections, 0.05% potassium permanganate ($KMnO_4$) was used to prophylactically treat the fish used in the experiment by dipping them twice in it.

Prior to experimentation, the specimens were acclimatised under laboratory conditions for one month. During acclimatisation, the fish were maintained in dechlorinated/aerated water under controlled environmental conditions and fed boiled eggs, beef liver, and poultry waste. Uneaten feed, faecal matter, and other debris were regularly removed by siphoning to maintain water quality. The physicochemical parameters of the experimental water were monitored using standard methods (APHA, 2005). For the present study, technical-grade λ-Cyhalothrin was obtained from Sigma-Aldrich (St. Louis, MO, USA; CAS: 91465–08-6) and used for preparation of the experimental exposure concentrations. Cyclophosphamide (4 mg/L) was used as the positive control. All other chemicals and reagents used for biochemical, genotoxic and histopathological analyses were of analytical grade.

Experimental design

Following acclimatisation, the fish were randomly allocated into five experimental groups (10 per group): negative control (NC), positive control (PC), low-dose (LD), medium-dose (MD), and high-dose (HD). The NC group was maintained without exposure to λ-cyhalothrin, while the PC group was exposed to 4 mg/L cyclophosphamide. For the λ-Cyhalothrin treatment groups, three sublethal concentrations were selected based on the reported 96-h LC_{50} of 7.92 µg/L for *Channa punctatus* as reported by Kumar *et al*. (2007). The selected concentrations corresponded to 1/16th, 1/8th, and 1/4th of the 96-h LC_{50}, resulting in nominal exposure concentrations of 0.495, 0.990, and 1.980 µg/L for the LD, MD, and HD groups, respectively **(Table 1)**.

The experiment was conducted for 7 consecutive days under semi-static conditions. The exposure medium was renewed at 24-h intervals, following which freshly prepared λ-Cyhalothrin solutions were added to restore the respective nominal concentrations. A similar water-renewal procedure was followed for the control and positive-control groups. Throughout the exposure period, the fish were monitored regularly for mortality and visible behavioural or morphological alterations. At the end of the 7-day exposure period, fish were sampled for subsequent analyses. Oxidative stress parameters, namely, lipid peroxidation, reduced glutathione, and superoxide dismutase, were evaluated in the fish liver and blood. In addition, damage to genetic material was evaluated using the micronucleus and comet assays performed on the fish blood.

Table 1: Experimental groups, treatments, and exposure concentrations used in *Channa punctatus* during the 7-day λ-Cyhalothrin exposure study.

Group	Treatment	Dosage/Concentration
NC	**Vehicle only**	**0 µg/L λ-cyhalothrin**
PC	**Cyclophosphamide**	**4 mg/L**
LD	**λ-Cyhalothrin**	**0.495 µg/L**
MD	**λ-Cyhalothrin**	**0.990 µg/L**
HD	**λ-Cyhalothrin**	**1.980 µg/L**

Lipid peroxidation assay (LPO)

LPO was estimated by measuring the concentration of malondialdehyde (MDA), a major end product of lipid peroxidation, according to the method of Buege and Aust (1978)**.** Briefly, fish samples were homogenised in an appropriate ice-cold buffer, and the homogenates were centrifuged to obtain the supernatant. An aliquot of 1 mL of the supernatant was mixed thoroughly with 2 mL of TCA–TBA–HCl reagent, containing 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid (TBA), and 0.25 N hydrochloric acid (HCl)**.** The reaction mixture was heated in a boiling water bath for 15 min to allow the formation of the pink-coloured TBA–MDA complex. After cooling to room temperature, the precipitated material was removed by centrifugation at 1000 × g for 10 min**.** The absorbance of the resulting supernatant was measured spectrophotometrically at 535 nm against an appropriate reagent blank.

Reduced glutathione assay (GSH)

The GSH content of the tissue samples was estimated according to the method described by Beutler *et al.* (1963). The method is based on the reaction of the sulfhydryl group of GSH with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman's reagent), resulting in the formation of the yellow-coloured chromophore 5-thio-2-nitrobenzoic acid (TNB), which is measured spectrophotometrically at 412 nm. Briefly, fish samples were homogenised in an appropriate ice-cold medium, and the homogenates were processed for the removal of proteins using a suitable protein-precipitating solution. Following centrifugation, the clear supernatant was collected for GSH estimation. An aliquot of the deproteinized sample was mixed with 0.3 M phosphate buffer (pH 8.0) and DTNB reagent. The reaction mixture was allowed to develop colour, and the absorbance was measured at 412 nm against an appropriate reagent blank using a UV–visible spectrophotometer. The GSH concentration was determined using a standard curve prepared with known concentrations of reduced glutathione and expressed relative to the protein content of the respective tissue samples. The DTNB-based reaction and measurement at 412 nm are consistent with the established Beutler approach and its subsequent applications to tissue samples.

Superoxide dismutase assay (SOD)

SOD activity was determined according to the pyrogallol autoxidation method of Marklund and Marklund (1974). From the raw homogenate, clear supernatant was collected for the enzyme estimation. The reaction mixture consisted of 50 mM Tris–cacodylic acid buffer (pH 8.20) containing 1 mM diethylenetriaminepentaacetic acid (DTPA). An aliquot of the tissue supernatant was added to the reaction mixture, followed by the addition of freshly prepared 0.2 mM pyrogallol to initiate the reaction. The increase in absorbance resulting from pyrogallol autoxidation was recorded at 420 nm at regular intervals. The rate of pyrogallol autoxidation in the absence of the sample was used as the reference, and the inhibition of autoxidation produced by the tissue extract was used to estimate SOD activity.

Micronucleus assay (MN)

The MN assay was performed using peripheral blood erythrocytes to assess chromosomal/genetic damage following λ-Cyhalothrin exposure. The method was adopted from Das & Nanda (1986) with minor modifications. Immediately after blood collection, a thin smear was prepared by placing a drop of freshly collected blood onto a clean, grease-free microscopic slide. The smears were fixed in absolute methanol for 10 min and subsequently allowed to air-dry at room temperature. The fixed smears were then stained with 6% Giemsa prepared in Sorensen phosphate buffer (pH 6.9) for 20 min. After staining, the slides were rinsed gently, air-dried, and examined under a light microscope using a 100× oil-immersion objective. This general approach is consistent with established micronucleus procedures used for *Channa punctatus*, including protocols in which peripheral blood smears are fixed in methanol, stained with Giemsa, and scored at 100× magnification.

For each fish, 1000 erythrocytes were examined for the presence of micronuclei. Micronuclei were identified according to the following criteria: (i) the micronucleus was clearly separated from the main nucleus and showed no connection with it; (ii) it exhibited staining intensity and colour similar to that of the main nucleus; and (iii) its size was less than approximately one-third of the diameter/area of the main nucleus. Only intact, clearly distinguishable erythrocytes were considered for scoring. These criteria are consistent with commonly applied criteria for identifying micronuclei in fish erythrocytes.

The frequency of micronucleated erythrocytes was calculated using the following formula:
MN frequency (%) = Number of micronucleated erythrocytes/Total number of erythrocytes scored × 100

Thus, scoring 1000 erythrocytes per fish provided the basis for determining the percentage of micronucleated erythrocytes in each experimental group. The slides were preferably coded before microscopic examination so that scoring was performed without knowledge of the treatment group, thereby reducing observer bias. Such blinding/coding is recommended for micronucleus scoring procedures.

Alkaline single-cell gel electrophoresis (SCGE)/comet assay
The alkaline single-cell gel electrophoresis (SCGE)/comet assay was performed following the method described by Singh *et al*. (1988) with appropriate modifications. Briefly, clean frosted microscopic slides were coated with a base layer of 1% normal-melting-point agarose and allowed to solidify. Immediately after blood collection, the blood samples were suspended in chilled, calcium- and magnesium-free phosphate-buffered saline (PBS). Cell viability was assessed using the trypan blue exclusion method, and samples showing acceptable cell viability (> 95%) were used for subsequent comet assay analysis.

For slide preparation, an aliquot of the cell suspension was mixed with 0.5% low-melting-point agarose and spread over the precoated slide to form the second agarose layer. A final layer of low-melting-point agarose was then applied over the cell-containing layer. The slides were allowed to solidify under chilled conditions. Following solidification, the slides were immersed in freshly prepared cold lysis solution containing 2.5 M NaCl, 100 mM Na_2EDTA and 10 mM Tris (pH 10.0), supplemented with 10% DMSO and 1% Triton X-100, and kept at 4°C overnight to lyse the cellular and nuclear membranes and expose the nucleoids.

After lysis, the slides were placed in a horizontal electrophoresis chamber containing freshly prepared alkaline electrophoresis buffer (300 mM NaOH and 1 mM Na_2EDTA; pH >13) and incubated at 4°C for 20 min to allow DNA unwinding and expression of alkali-labile sites and DNA strand breaks. Electrophoresis was subsequently carried out under alkaline conditions at 4°C for 20 min at 15 V and approximately 300 mA. Following electrophoresis, the slides were gently neutralised with 0.4 M Tris buffer (pH 7.5) and stained with ethidium bromide (20 µg/mL).

For each specimen, two slides were prepared, and randomly selected comet images were examined under a fluorescence microscope equipped with an appropriate filter system. DNA damage was quantified using an image-analysis system, and tail length and olive tail moment were used as the principal parameters for quantifying DNA strand damage. The DNA damage parameters were evaluated by the image-analysis software (***Tritek CometScore version 2.0.0.0.38***) based on the proportion of total cellular DNA migrating into the comet tail. A known DNA-damaging treatment was included as a positive control for assay validation, where applicable. All slides were coded prior to microscopic scoring to minimise observer bias.

Total protein estimation
The total protein content of the tissue samples was estimated by the Bradford method using Coomassie Brilliant Blue G-250 as described by Bradford (1976). Briefly, tissue homogenates were prepared in an appropriate ice-cold buffer and centrifuged to obtain the clear supernatant. An aliquot of the supernatant was mixed with Bradford reagent and allowed to stand at room temperature for colour development. The binding of Coomassie Brilliant Blue G-250 to proteins resulted in a shift in the absorption maximum, producing a blue-coloured complex. The absorbance of the reaction mixture was measured spectrophotometrically at 595 nm against an appropriate reagent blank. A standard calibration curve was prepared using known concentrations of bovine serum albumin (BSA), and the protein concentration of each sample was determined from the corresponding standard curve. The results were expressed as mg protein/mL of tissue homogenate. Protein concentration was subsequently used for normalisation of the biochemical and antioxidant enzyme activities and was expressed according to the requirements of the respective assays.

Statistical analysis
The experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using Graphpad Prism (8.0.1). Differences among the experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test to determine differences between individual groups. The assumptions of normality and homogeneity of variance were assessed before performing parametric analyses. Differences were considered statistically significant at $p < 0.05$. The results were presented using appropriate tables and graphical representations.

RESULTS

Physicochemical characteristics of test water

The physicochemical characteristics of the test water remained within acceptable ranges throughout the experimental period. The dissolved oxygen concentration ranged from 7.1 to 9.4 mg L^{-1}, while the water temperature varied between 21.4 and 27.6 °C. The pH of the test water ranged from 6.8 to 8.2. The electrical conductivity ranged from 240 to 307 µS cm^{-1}. Chloride concentration, total hardness, and total alkalinity were recorded in the ranges of 40-58 mg L^{-1}, 152-194 mg L^{-1}, and 264-298 mg L^{-1}, respectively. Overall, the physicochemical parameters of the test water remained relatively stable during the experimental period, indicating suitable water quality for maintaining the experimental animals.

λ-Cyhalothrin-induced oxidative stress and antioxidant response in blood

Exposure to λ-Cyhalothrin produced marked alterations in the oxidative stress and antioxidant status of the blood of *Channa punctatus* **(Fig. 1).** One-way ANOVA revealed significant differences among the experimental groups for LPO ($F = 135.2$, $p < 0.001$, $R^2 = 0.9232$), GSH ($F = 130.1$, $p < 0.001$, $R^2 = 0.9204$), and SOD ($F = 33.65$, $p < 0.001$, $R^2 = 0.7494$), demonstrating a significant overall treatment effect on all three biomarkers.

Blood LPO showed a pronounced concentration-related increase following λ-Cyhalothrin exposure **(Fig. 1A).** Compared with the negative control, LPO increased by 56.96%, 298.73%, and 506.33% in the LD, MD, and HD groups, respectively. However, Tukey's post-hoc analysis indicated that the increase at LD was not statistically significant (mean difference = 0.4500, $p > 0.05$), whereas significantly higher LPO levels were observed at MD and HD (mean differences = 2.360 and 4.000, respectively; both $p < 0.001$). The PC group exhibited a 931.65% increase in LPO relative to NC and showed a highly significant difference from NC ($p < 0.001$). Thus, lipid peroxidation was substantially enhanced by λ-cyhalothrin, particularly at the medium and high exposure concentrations.

In contrast to LPO, blood GSH content decreased following λ-Cyhalothrin exposure **(Fig. 1B).** GSH levels were reduced by 11.11% in both LD and MD and by 33.33% in HD compared with NC. Tukey's analysis showed that these reductions were statistically significant, with LD and MD differing significantly from NC ($p = 0.013$), while the reduction at HD was highly significant ($p < 0.001$). The PC group showed the greatest depletion, with GSH levels 66.67% lower than NC ($p < 0.001$). Compared with PC, GSH levels remained substantially higher in the λ-Cyhalothrin groups, being 166.67% higher in LD and MD and 100.00% higher in HD. These findings indicate a progressive depletion of the reduced glutathione pool, particularly at the highest λ-Cyhalothrin concentration.

A similar concentration-related decline was observed in blood SOD activity **(Fig. 1C).** SOD activity decreased by 6.64%, 17.58%, and 26.17% in the LD, MD, and HD groups, respectively, relative to NC. The reduction at LD was not statistically significant ($p = 0.787$), whereas significant and highly significant decreases were observed at MD ($p = 0.033$) and HD ($p < 0.001$), respectively. The PC group showed a marked 61.33% reduction in SOD activity relative to NC ($p < 0.001$). Thus, the decline in SOD activity became progressively more pronounced with increasing λ-Cyhalothrin concentration.

Overall, the blood biochemical profile demonstrated a coordinated increase in lipid peroxidation, accompanied by depletion of GSH and reduction of SOD activity following λ-Cyhalothrin exposure. The magnitude of these alterations generally increased with increasing concentration, with the most pronounced changes observed in the HD group. The simultaneous elevation of LPO and impairment of both enzymatic and non-enzymatic antioxidant defences indicates a substantial disturbance of the oxidative balance in the blood of *C. punctatus* following 7-day exposure to λ-cyhalothrin.

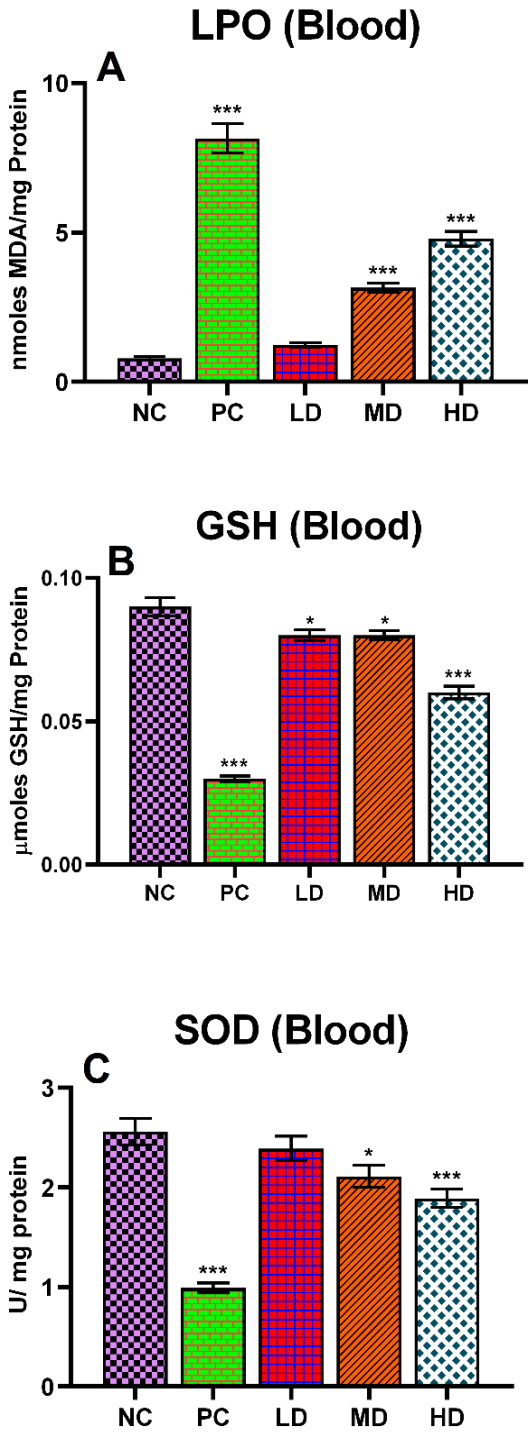

Fig. 1: Effect of 7-day λ-Cyhalothrin exposure on oxidative stress and antioxidant status in the blood of *Channa punctatus*. The figure shows changes in **(A)** lipid peroxidation (LPO), expressed as nmol MDA/mg protein; **(B)** reduced glutathione (GSH), expressed as µmol GSH/mg protein; and **(C)** superoxide dismutase (SOD) activity, expressed as U/mg protein, in fish exposed to low (LD; 0.495 µg/L), medium (MD; 0.990 µg/L), and high (HD; 1.980 µg/L) concentrations of λ-Cyhalothrin for 7 days. NC, negative control; PC, cyclophosphamide-positive control (4 mg/L). Data are presented as mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the negative control (NC).

λ-Cyhalothrin-induced oxidative stress and antioxidant response in liver

Seven-day exposure to λ-Cyhalothrin produced marked alterations in the oxidative stress and antioxidant status of the liver of *Channa punctatus* **(Fig. 2).** One-way ANOVA demonstrated significant differences among the experimental groups for LPO ($F = 141.0$, $p < 0.001$, $R^2 = 0.9261$), GSH ($F = 49.37$, $p < 0.001$, $R^2 = 0.8144$), and SOD ($F = 27.33$, $p < 0.001$, $R^2 = 0.7084$), indicating significant treatment-related alterations in all three oxidative-stress and antioxidant parameters.

Hepatic LPO showed a pronounced concentration-dependent increase following λ-Cyhalothrin exposure **(Fig. 2A).** Compared with the NC group, LPO increased by 70.48%, 260.00%, and 462.86% in the LD, MD, and HD groups, respectively. Tukey's post-hoc analysis revealed that the increase at LD was not statistically significant (mean difference = 0.7400, 95% CI: −0.4520 to 1.932, $p = 0.407$), whereas both MD and HD showed highly significant elevations compared with NC (mean differences = 2.730 and 4.860, respectively; both $p < 0.001$). The PC group exhibited the greatest increase in LPO, with an 831.43% elevation relative to NC ($p < 0.001$). Thus, although a numerical increase was evident at all λ-Cyhalothrin concentrations, significant hepatic lipid peroxidation was apparent from the medium concentration onward.

In contrast, hepatic GSH content progressively decreased with increasing λ-Cyhalothrin concentration **(Fig. 2B).** GSH levels declined by 3.88%, 17.80%, and 32.69% in the LD, MD, and HD groups, respectively, relative to NC. Tukey's analysis showed that the reduction at LD was not statistically significant (mean difference = 0.1200, 95% CI: −0.3106 to 0.5506, $p = 0.932$), whereas the MD group showed a significant decrease (mean difference = 0.5500, 95% CI: 0.1194–0.9806, $p = 0.006$) and the HD group exhibited a highly significant reduction (mean difference = 1.010, 95% CI: 0.5794–1.441, $p < 0.001$). The PC group showed a substantially greater 59.87% depletion of GSH relative to NC ($p < 0.001$). These findings demonstrate progressive depletion of the hepatic reduced-glutathione pool, with statistically significant alterations emerging at the medium and high λ-Cyhalothrin concentrations.

Hepatic SOD activity also exhibited a concentration-related decline **(Fig. 2C).** Relative to NC, SOD activity decreased by 5.02%, 13.32%, and 31.90% in the LD, MD, and HD groups, respectively. However, Tukey's analysis showed that the reductions at LD (mean difference = 0.6300, 95% CI: −1.743 to 3.003, $p = 0.942$) and MD (mean difference = 1.670, 95% CI: −0.7027 to 4.043, $p = 0.283$) were not statistically significant. In contrast, SOD activity was significantly reduced in the HD group by 31.90% compared with NC (mean difference = 4.000, 95% CI: 1.627–6.373, $p < 0.001$). The PC group showed a 60.45% reduction in SOD activity relative to NC ($p < 0.001$).

Overall, the hepatic response demonstrated a clear concentration-dependent increase in lipid peroxidation, accompanied by progressive depletion of GSH and reduction of SOD activity following λ-Cyhalothrin exposure. The most pronounced alterations were consistently observed in the HD group, in which LPO increased by 462.86%, while GSH and SOD decreased by 32.69% and 31.90%, respectively, relative to NC. Collectively, these findings indicate a substantial disruption of hepatic oxidative balance following 7-day λ-Cyhalothrin exposure, with the effects becoming statistically evident predominantly at the medium and high concentrations.

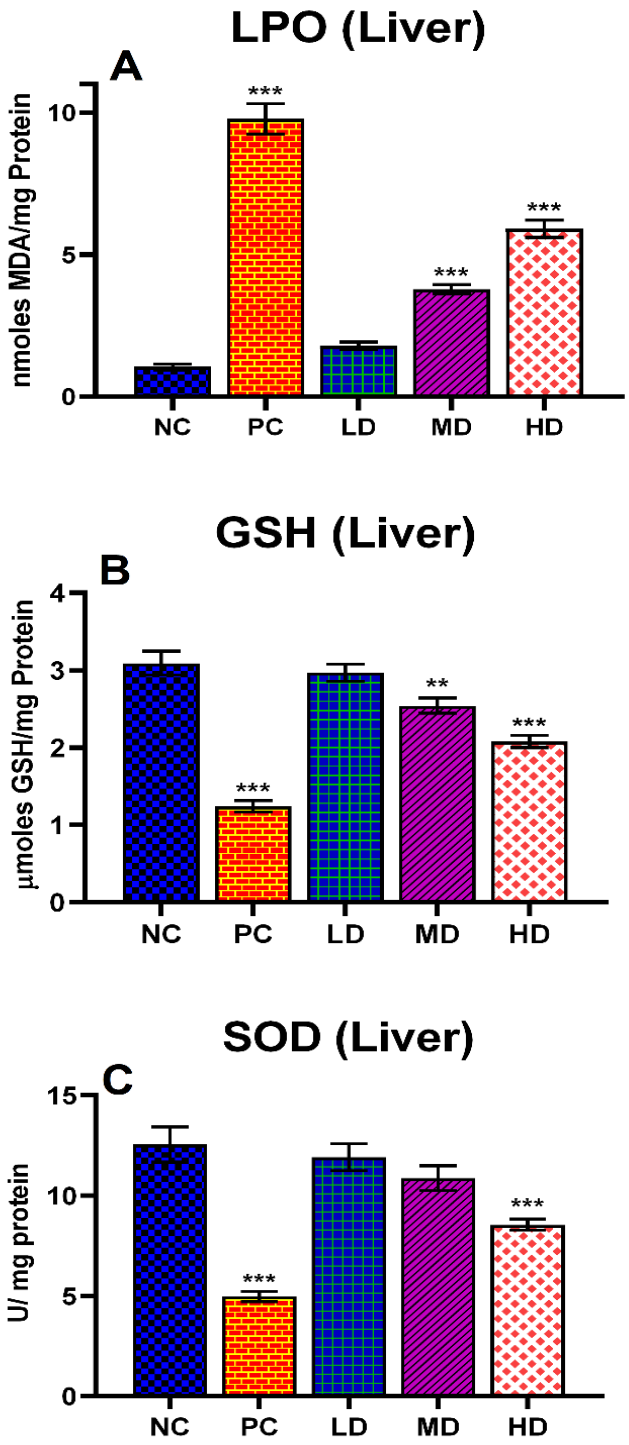

Fig. 2: Effect of 7-day λ-Cyhalothrin exposure on oxidative stress and antioxidant status in the liver of *Channa punctatus*. The figure shows changes in **(A)** lipid peroxidation (LPO), expressed as nmol MDA/mg protein; **(B)** reduced glutathione (GSH), expressed as µmol GSH/mg protein; and **(C)** superoxide dismutase (SOD) activity, expressed as U/mg protein, in fish exposed to low (LD; 0.495 µg/L), medium (MD; 0.990 µg/L), and high (HD; 1.980 µg/L) concentrations of λ-Cyhalothrin for 7 days. NC, negative control; PC, cyclophosphamide-positive control (4 mg/L). Data are presented as mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the negative control (NC).

λ-Cyhalothrin-induced micronucleus formation in erythrocytes of *Channa punctatus*

One-way ANOVA demonstrated a highly significant difference in MN frequency among the experimental groups ($F = 235.9$, $p < 0.001$, $R^2 = 0.9545$), indicating a strong overall treatment effect on micronucleus formation. The treatment factor accounted for approximately 95.45% of the variability in MN frequency.

Tukey's multiple-comparison analysis showed that the PC group exhibited a highly significant increase in MN frequency compared with NC (mean difference = −1.825, 95% CI: −2.016 to −1.634, $p < 0.001$), confirming the responsiveness of the assay to the positive control. Among the λ-cyhalothrin-exposed groups, the LD group did not differ significantly from NC (mean difference = −0.1750, 95% CI: −0.3664 to 0.01637, $p = 0.088$), despite its 269.23% numerical increase in MN frequency. In contrast, both MD and HD exhibited highly significant increases in MN frequency compared with NC (MD: mean difference = −0.3950, 95% CI: −0.5864 to −0.2036; HD: mean difference = −0.8650, 95% CI: −1.056 to −0.6736; both $p < 0.001$).

Importantly, Tukey's test also demonstrated significant differences among all three λ-Cyhalothrin concentrations. MN frequency was significantly higher in MD than LD (mean difference = −0.2200, 95% CI: −0.4114 to −0.02863, $p = 0.017$), while HD showed significantly higher MN frequency than both LD (mean difference = −0.6900, $p < 0.001$) and MD (mean difference = −0.4700, $p < 0.001$). Thus, the genotoxic response increased significantly with increasing λ-Cyhalothrin concentration.

Numerically, MN frequency increased from 0.065% in NC to 0.24%, 0.46%, and 0.93% in LD, MD, and HD, corresponding to increases of 269.23%, 607.69%, and 1,330.77%, respectively. The PC group showed the greatest response, with MN frequency increasing by 2,807.69% relative to NC. Furthermore, MN frequencies in LD, MD, and HD remained 87.30%, 75.66%, and 50.79% lower than PC, respectively.

Overall, the results demonstrate a strong and statistically significant concentration-dependent induction of micronucleus formation by λ-cyhalothrin, with the significant genotoxic response becoming evident at the medium concentration and increasing progressively through the high concentration. The representative micronucleated erythrocyte shown in **Fig. 3**, together with the quantitative data presented in **Table 2**, provides morphological and numerical evidence of λ-cyhalothrin-associated chromosomal/genetic damage in *C. punctatus* erythrocytes.

Table 2: Incidence of micronuclei (MN) in erythrocytes of *Channa punctatus* exposed to different concentrations of λ-Cyhalothrin and cyclophosphamide (4 mg/L) for 7 days.

GROUPS *(Dosage/Concentration)*	No of fish observed	No of cells observed	MN frequencies (%) ± SEM
Negative control (NC) ***(0 µg/L λ-cyhalothrin)***	10	10056	0.065 ± 0.009
Positive control (PC) ***Cyclophosphamide (4 mg/L)***	10	10067	1.89 ± 0.076***
LD **λ-Cyhalothrin** ***(0.495 µg/L)***	10	10112	0.24 ± 0.028
MD **λ-Cyhalothrin** ***(0.990 µg/L)***	10	10214	0.46 ± 0.038***
HD **λ-Cyhalothrin** ***(1.980 µg/L)***	10	10190	0.93 ± 0.057***

Values represent mean ± SEM. A total of approximately 10,000 erythrocytes were evaluated per treatment group. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. $p < 0.001$ versus the negative control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the negative control (NC).

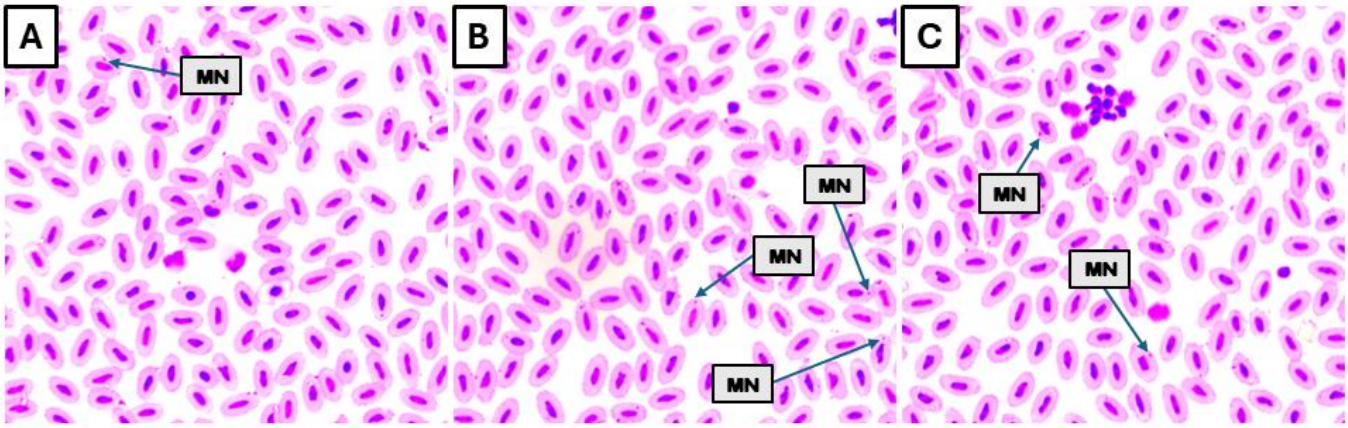

Fig. 3: Representative photomicrographs of erythrocytes of *Channa punctatus* showing micronuclei (MN) in different cells. **(A–C)** Micronucleated erythrocytes displaying distinct, small, round extranuclear bodies with staining characteristics comparable to the main nucleus.

λ-Cyhalothrin-induced DNA damage in erythrocytes of *Channa punctatus* assessed by the alkaline SCGE/comet assay

The alkaline single-cell gel electrophoresis (SCGE)/comet assay revealed a marked increase in DNA damage in the erythrocytes of *Channa punctatus* following 7-day exposure to λ-cyhalothrin. One-way ANOVA demonstrated highly significant differences among the experimental groups for both tail length ($F = 346.8$, $p < 0.001$, $R^2 = 0.9686$) and Olive tail moment (OTM) ($F = 755.0$, $p < 0.001$, $R^2 = 0.9853$), indicating strong treatment-associated effects on DNA migration. The SCGE findings, including tail length and OTM, are summarised in **Table 3**, with representative comet photomicrographs illustrating the observed DNA migration patterns presented in **Fig. 4.**

Tail length increased progressively with increasing λ-Cyhalothrin concentration, from 4.51 ± 0.89 a.u. in the NC group to 13.18 ± 2.49, 19.07 ± 3.17, and 34.37 ± 4.06 a.u. in the LD, MD, and HD groups, respectively. These values corresponded to increases of 192.24%, 322.84%, and 662.08%, respectively, relative to NC. Tukey's post-hoc analysis showed that the increase was statistically significant at all three exposure concentrations, with LD ($p = 0.028$), MD ($p < 0.001$), and HD ($p < 0.001$) all differing significantly from NC. The PC group exhibited the greatest response, with tail length reaching 97.08 ± 12.84 a.u., representing a 2,052.66% increase relative to NC ($p < 0.001$).

A similar concentration-related pattern was observed for OTM. OTM increased from 3.07 ± 0.45 a.u. in NC to 5.07 ± 0.67, 8.19 ± 0.17, and 13.64 ± 2.01 a.u. in LD, MD, and HD, respectively. Relative to NC, these represented increases of 65.15%, 166.78%, and 344.30%, respectively. However, Tukey's analysis indicated that the increase at LD was not statistically significant ($p = 0.125$), whereas significant increases were observed at MD and HD ($p < 0.001$ for both). The PC group showed the highest OTM (42.08 ± 3.49 a.u.), corresponding to a 1,270.36% increase relative to NC ($p < 0.001$).

The representative comet images in **Fig. 4** provide visual evidence of the observed DNA migration, with progressively greater comet-tail formation apparent in the λ-cyhalothrin-exposed groups. The parallel increases in tail length and OTM demonstrate a consistent concentration-related enhancement of DNA migration following λ-Cyhalothrin exposure. Notably, tail length detected a significant alteration even at the LD concentration, whereas OTM showed statistical significance from the MD concentration onward, indicating somewhat greater sensitivity of tail length to the lowest exposure level under the present experimental conditions. Overall, the SCGE findings demonstrate that 7-day λ-Cyhalothrin exposure induced concentration-dependent DNA damage in erythrocytes of *C. punctatus*, with the most pronounced response occurring at the HD concentration.

Table 3: Effect of 7-day λ-Cyhalothrin exposure on DNA damage in erythrocytes of *Channa punctatus* assessed by the alkaline SCGE/comet assay.

Experimental group	Treatment/ concentration	Tail length (a.u.)	Olive tail moment (OTM) (a.u.)
NC	λ-cyhalothrin (0 µg/L)	4.51 ± 0.89	3.07 ± 0.45
PC	Cyclophosphamide (4 mg/L)	97.08 ± 12.84***	42.08 ± 3.49***
LD	λ-Cyhalothrin (0.495 µg/L)	13.18 ± 2.49*	5.07 ± 0.67
MD	λ-Cyhalothrin (0.990 µg/L)	19.07 ± 3.17***	8.19 ± 0.17***
HD	λ-Cyhalothrin (1.980 µg/L)	34.37 ± 4.06***	13.64 ± 2.01***

Values are expressed as mean ± SEM. NC, negative control; PC, positive control; LD, low dose (0.495 µg/L); MD, medium dose (0.990 µg/L); HD, high dose (1.980 µg/L); SCGE, single-cell gel electrophoresis; OTM, Olive tail moment; a.u., arbitrary units. Tail length and OTM represent the respective parameters of DNA migration obtained from the comet assay. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the negative control (NC).

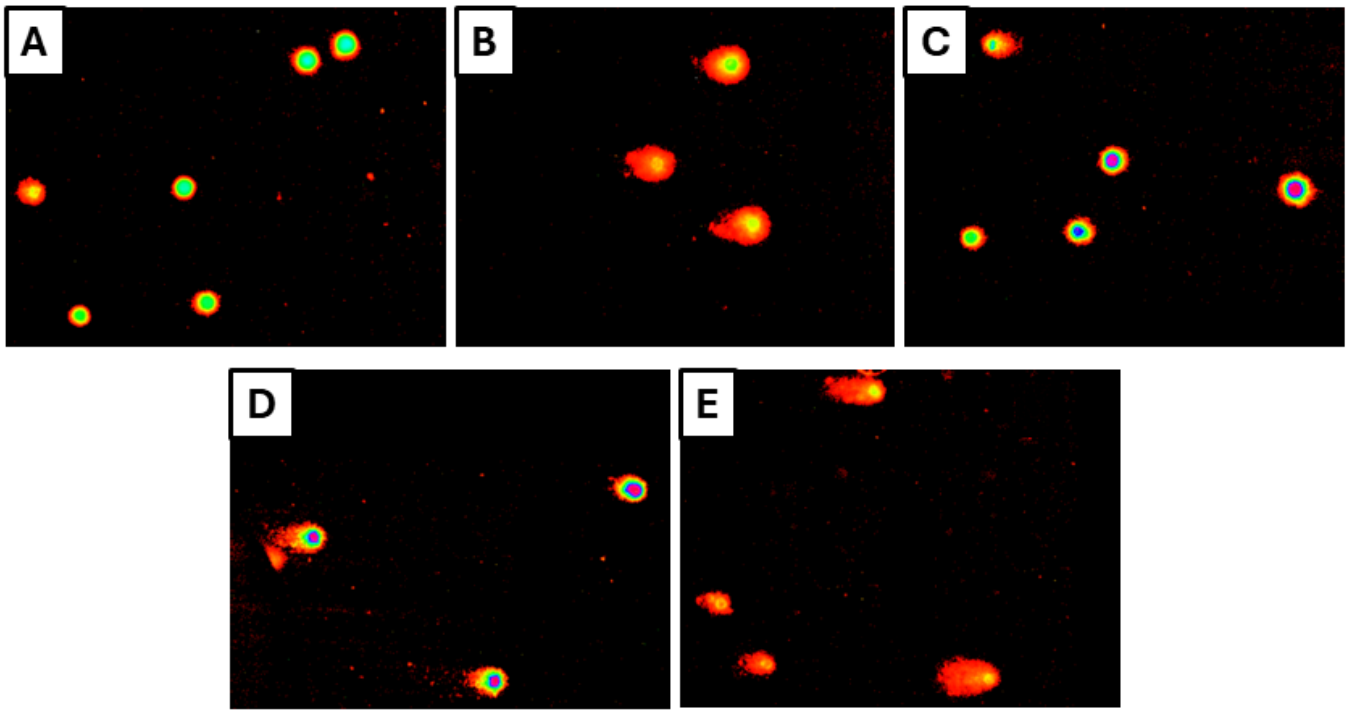

Fig. 4: Representative fluorescence photomicrographs of erythrocytes from *Channa punctatus* showing DNA damage assessed by the alkaline single-cell gel electrophoresis (SCGE)/comet assay following 7-day exposure to λ-cyhalothrin. **(A)** Negative control (NC; 0 µg/L λ-cyhalothrin), **(B)** positive control (PC; cyclophosphamide, 4 mg/L), **(C)** low-dose group (LD; 0.495 µg/L λ-cyhalothrin), **(D)** medium-dose group (MD; 0.990 µg/L λ-cyhalothrin), and **(E)** high-dose group (HD; 1.980 µg/L λ-cyhalothrin). Increasing migration of DNA from the nucleoid, manifested as an elongated comet tail, indicates increasing DNA damage.

Discussion

The findings indicate that short-term exposure to λ-Cyhalothrin produces concentration-dependent oxidative stress and genotoxicity in *Channa punctatus*. This pattern is biologically plausible and is broadly consistent with the literature showing that pyrethroids, and λ-Cyhalothrin in particular, disrupt antioxidant homeostasis, enhance lipid peroxidation, and damage nuclear DNA in aquatic organisms (Campana *et al*., 1999; Kumar *et al*., 2012; Alvim & Martinez, 2019).

The progressive rise in LPO in blood and liver indicates that λ-Cyhalothrin promoted membrane oxidative damage, most likely through excess reactive oxygen species generation that exceeded cellular antioxidant capacity (El-Demerdash, 2007; Clasen *et al*., 2018; Fouzai *et al*., 2019). A comparable increase in lipid peroxidation after pyrethroid exposure has been reported in *Channa punctatus* exposed to deltamethrin, where oxidative stress was evident across tissues, and in λ-cyhalothrin-exposed fish in which LPO rose in a dose-dependent manner (Sayeed *et al*., 2003; Kumar *et al*., 2012; Venturini *et al*., 2018).

The concomitant depletion of GSH and SOD in the present study supports the view that antioxidant defences were consumed or inhibited during detoxification of λ-cyhalothrin-induced oxidant burden (El-Demerdash, 2007; Venturini *et al*., 2018; Fouzai *et al*., 2019). This response agrees with several pesticide studies in fish showing reduced SOD and glutathione-dependent protection under oxidative challenge, including pendimethalin-exposed tissues of *Channa punctata* and λ-cyhalothrin-exposed erythrocytes or digestive tissues in other models (El-Demerdash, 2007; Tabassum *et al*., 2016).

Some studies have reported increased GSH after pyrethroid exposure rather than depletion, indicating that glutathione responses can be biphasic and depend on tissue, dose, and exposure duration (Sayeed *et al*., 2003; Ansari *et al*., 2009; Venturini *et al*., 2018). Such divergence does not weaken the present findings; instead, it suggests that early or moderate exposure may transiently induce compensatory glutathione synthesis, whereas stronger or sustained exposure, as reflected here particularly in the liver, can shift the system toward antioxidant exhaustion and overt peroxidative injury (Parvez & Raisuddin, 2005; Nwani *et al*., 2013; Venturini *et al*., 2018).

The more pronounced hepatic response observed here is also plausible because liver is a major site of xenobiotic biotransformation and oxidative metabolism in fish. Previous studies in freshwater fish have repeatedly shown marked pesticide-associated oxidative injury in liver, including increased LPO, altered antioxidant enzymes, protein oxidation, and persistent morphological damage after λ-Cyhalothrin exposure (Clasen *et al*., 2018; Venturini *et al*., 2018).

The increase in micronucleated erythrocytes demonstrates that λ-Cyhalothrin exposure produced chromosomal damage or mitotic disturbance in circulating blood cells. This finding is strongly supported by earlier work showing that λ-Cyhalothrin induced micronuclei in fish erythrocytes and validated the MN assay as a useful biomarker for aquatic genotoxic pollutants (Campana *et al*., 1999; Alvim & Martinez, 2019).

The comet assay results further show that λ-Cyhalothrin caused primary DNA strand damage, with tail length increasing even at the lowest tested concentration and OTM rising from the medium dose onward. This agrees with reports that pyrethroids and other pesticides produce concentration- and time-dependent DNA migration in fish blood and tissue cells, and that comet analysis is highly sensitive for detecting early genotoxic injury before fixed chromosomal lesions become fully expressed (Ali *et al*., 2008; Ismail *et al*., 2014; Alvim & Martinez, 2019).

The combined rise in LPO, MN frequency, and comet parameters supports a mechanistic link between oxidative stress and genotoxicity. Earlier studies in *Channa punctata* erythrocytes exposed to deltamethrin concluded that oxidative stress contributes, at least in part, to pyrethroid-induced genotoxic damage, while broader fish toxicology literature similarly associates pesticide-generated reactive oxygen species with DNA injury (Ansari *et al*., 2009; Nwani *et al*., 2010; Nwani *et al*., 2013).

An important strength of the present work is the integrated use of biochemical and cytogenetic endpoints, which provides a more coherent toxicological picture than any single marker alone. Earlier studies in *Channa punctatus* and other freshwater fishes likewise support the combined application of antioxidant biomarkers with MN and comet assays for detecting sublethal pesticide stress and for environmental biomonitoring (Ali *et al*., 2008; Nwani *et al*., 2009; Nwani *et al*., 2013).

The observation that measurable damage occurred at sublethal fractions of the 96-h LC_{50} is environmentally relevant because pyrethroids can affect non-target fish well below acutely lethal concentrations. Prior studies have shown that λ-Cyhalothrin can be highly toxic even at low µg/L ranges, can drive oxidative and genetic injury in fish, and can persist sufficiently to produce bioaccumulation or incomplete recovery after exposure (Venturini *et al*., 2018; Clasen *et al*., 2018; Alvim & Martinez, 2019).

However, the present study examined a 7-day laboratory exposure under semi-static conditions and therefore does not capture longer-

term adaptation, recovery, or field-level mixture effects. This is relevant because previous studies have shown non-linear time courses for DNA damage, persistence of some biomarker alterations after recovery, and, in some cases, synergistic toxicity when λ-Cyhalothrin occurs with other pesticides (Ali *et al*., 2009; Venturini *et al*., 2018; Alvim & Martinez, 2019). Overall, the results support that λ-Cyhalothrin exerts significant short-term toxic effects in *Channa punctatus.*

Conclusion

Seven-day sublethal exposure to λ-Cyhalothrin induced concentration-dependent oxidative stress and genotoxicity in *Channa punctatus*, characterised by increased lipid peroxidation, impaired antioxidant defences, and elevated micronucleus formation and DNA damage. The integrated biomarker responses demonstrate the sensitivity of *C. punctatus* to λ-cyhalothrin. Thus, the present study adds evidence that blood and liver oxidative biomarkers, together with MN and comet assays, are effective tools for assessing sublethal λ-Cyhalothrin toxicity in freshwater fish.

Author Contributions

Muzamil Liakat Mir: Conceptualisation, methodology, investigation, data curation, formal analysis, and writing—original draft. **Aamir Majeed:** Methodology, investigation, formal analysis, interpretation, and writing—review and editing. **G.G.H.A. Shadab:** Conceptualisation, supervision, project administration, interpretation, and writing—review and editing. **Khalida Hassan:** Review and editing.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data produced and/or examined during this research can be obtained from the corresponding authors upon a reasonable request.

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