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Original Article

Investigation on the cypermethrin-induced genotoxic, biochemical, and antioxidant gene expression modulations in the gill tissue of *Pethia conchonius*, a resident fish of river Teesta, India

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Abstract
Pesticides, an integral part of agricultural practices, contaminate aquatic bodies through surface runoff and leaching and threaten non-target organisms like fish. Synthetic pyrethroids, a widely used insecticide group have been shown to have detrimental effects on fish. *Pethia conchonius*, a non-target fish from river Teesta was used as a model organism to evaluate the detrimental effects of pyrethroid insecticide, cypermethrin on fish. The comet and micronucleus assays revealed cypermethrin-induced severe nuclear DNA damage showing three-fold decrease in % head DNA with a concomitant 70-fold increase in % tail DNA, and up to 21 times increase in MN frequency for SLC III at 96 hr exposure. The SOD, CAT, and GPx assays in gills and their mRNA expression analysis indicated down regulation of these anti-oxidant enzymes in a concentration and time-dependent response, with the lowest decrease being 4.2, 2.2, and 1.8-folds respectively for each of the enzymes at 96 hr of cypermethrin treatment in comparison to the control ($p > 0.05$). Subsequently, elevated levels of MDA in the gills of cypermethrin-treated *P. conchonius* further indicated increased oxidative stress and lipid peroxidation. Elevated oxidative stress and ROS levels can cause DNA damage and disrupt normal cell functioning, biochemical pathways, and cell cycle. The results indicated that cypermethrin is highly genotoxic and induces oxidative stress in the fish, supporting its hazardous effect on non-target organisms in aquatic ecosystems.

Keywords: biochemical analysis; comet assay; cypermethrin; gene expression; MN assay; *Pethia conchonius*

1 | INTRODUCTION

The widespread application of synthetic pesticides in agriculture has caused severe ecological and environmental repercussions, posing substantial health hazards to humans and other living organisms (El-Euony *et al.* 2020; Naiel *et al.* 2020). These pesticides infiltrate the air, soil, and water systems, leading to harmful effects on the non-target invertebrate and vertebrate species (Mahmood *et al.* 2016). Globally, around 2 million tonnes of pesticides are applied annually, with herbicides accounting for 47.5%, insecticides 29.5%, fungicides 17.5%, and other pesticides comprising 5.5% (De Bon *et al.* 2014). India consumes approximately 52,466.45 tonnes of pesticides annually, with West Bengal alone contributing 3,320.81

tonnes as per the report of the Government of India, 2022-2023. Organophosphates, neonicotinoids, and pyrethroids are the most commonly used pesticides (Nayak and Solanki 2021; GOI 2022, 2023).

Cypermethrin (CP), classified as a type-II synthetic pyrethroid, is extensively applied across agricultural fields, forestry practices, and household environments for controlling various pest species (Ullah *et al*. 2015; Farag *et al*. 2022). Chemically identified as a cyano-3-phenoxybenzyl ester of 2, 2-dimethyl-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate, CP is highly stable in the environment due to its extended half-life, leading to bioaccumulation and biomagnification across trophic levels (Rana *et al*. 2022). Although CP effectively eliminates target pests, its excessive and improper use results in agrochemical pollution, adversely impacting ecosystems and non-target organisms, particularly aquatic species (Saha and Kaviraj 2013).

The CP can infiltrate aquatic ecosystems via processes like surface runoff, irrigation flows, and leaching from nearby agricultural fields, contaminating aquatic bodies (Merga and Brink 2021). Reported concentrations of synthetic pyrethroids in surface water range from 0.1 to 1 µg L^{-1} (Merga and Brink 2021), while CP concentrations as high as 1 µg L^{-1} have been recorded in rainwater samples from Hisar, India (Kumari *et al*. 2007). In Southern Malawi, surface water from cotton-growing areas exhibited CP concentrations of 8.10 – 15.40 mg L^{-1} (Kanyika-Mbewe *et al*. 2020). Such contamination has been shown to induce severe toxic effects in aquatic organisms, including apoptosis, neurotoxicity, genotoxicity, and oxidative stress (Paravani *et al*. 2018, 2019; Parlak *et al*. 2018). In cyprinid fishes, CP exposure has resulted in immunotoxicity, biochemical disturbances, developmental malformations, and hepatotoxicity (Dawar *et al*. 2016; Soltanian and Fereidouni 2017; Jindal and Sharma 2020). Kumar *et al*. (2007) observed significant morphological, behavioral, and biochemical modifications along with oxidative stress, in freshwater fish *Channa punctatus* and *Clarias batrachus* exposed to CP.

To assess the sub-lethal impacts of pesticides, biomarkers such as the Comet Assay (single cell gel electrophoresis) and Micronuclei (MN) tests have been widely employed for the assessment of pesticide-induced DNA damage and nuclear abnormalities in aquatic species (Carrasco *et al*. 1990; Kapka-Skrzypczak 2011). Numerous studies have detected DNA fragmentation, strand breaks, and micronuclei formation in fishes exposed to synthetic pyrethroids (Selvi *et al*. 2013; Stara *et al*. 2015; Ullah *et al*. 2018). In addition, oxidative stress biomarkers such as reactive oxygen species (ROS), lipid peroxidation (LPO), and antioxidant enzyme activities (SOD, CAT, and GPX) have been used to determine cellular and molecular damage in CP-exposed fish (Vieira *et al*. 2016; Cong *et al*. 2020; Sharma and Jindal 2021).

Pethia conchonius (rosy barb), an indigenous freshwater fish of the Indian subcontinent, is widely distributed in rivers and ponds across India, Bangladesh, Nepal, Afghanistan, and Pakistan (Dahanukar 2015). Due to its high sensitivity to pollutants, *P. conchonius* is an excellent model organism for toxicological, biological, and biotechnological studies (Bhattacharya *et al*. 2005; Xiao *et al*. 2007; Patel *et al*. 2018). In West Bengal, rivers such as the Ganga, Hoogly, Deomani, and Karola have been reported to contain traces of CP (Singh *et al*. 2015; Mondal *et al*. 2018; Roy and Shamin 2020). The Teesta River, a major water body in North Bengal, traverses through districts like Darjeeling, Kalimpong, Jalpaiguri, and Coochbehar, famous for the monoculture of tea, rice, and other cash crops. Despite its ecological and economic significance, limited studies exist on the genotoxic and biochemical effects of CP on non-target aquatic organisms, particularly fish species inhabiting the Teesta River, in which contamination studies is sparse.

Considering the extensive use of CP and its potential risks to aquatic ecosystems, the present study focuses on the evaluation of the extent of the adverse impacts of CP on *P. conchonius* at nuclear, enzymatic, and molecular levels.

2 | METHODOLOGY

2.1 Experimental fish

The freshwater fish *P. conchonius* was selected for this study due to its wide availability, nutritional value, aesthetic appeal, and ease of maintenance in laboratory settings. Adult specimens were collected from two agriculturally influenced locations along the bank of river Teesta: site 1 (Gajoldoba Teesta Barrage, Jalpaiguri, West Bengal; 26.7515°N 88.5856°E) and site 2 (Domohoni, Jalpaiguri, West Bengal; 26.3430°N 88.4542°E). The live fish were transported to the laboratory under carefully maintained aerated conditions to minimize stress.

Upon arrival, the fish underwent a two-minute prophylactic treatment with 0.05% potassium permanganate ($KMnO_4$) to eliminate potential external infections. Fish with an average length and weight of 6.19 ± 0.78 cm and 3.90 ± 0.60 g (mean ± SD) was chosen, respectively for experiments.

Fish were housed in well-aerated glass aquaria (dimensions: 23×23×30 cm^3) at a controlled temperature of 25±2°C (mean ± SD) for 15 days for acclimatization, following OECD guidelines, (2019). During this period, the fish were fed commercial fish feed twice a day to ensure their health and stability. To maintain optimal water quality, waste materials, including faecal matter, were siphoned out regularly, thus preventing ammonia buildup in the aquaria.

2.2 Chemicals used for study

The commercial formulation of Alpha-Cypermethrin 10%

EC (CAS number: 67375-30-8), branded as "Super Killer" manufactured by Dhanuka Agritech Limited, India, was procured from a local dealer. All other chemical reagents used were of analytical grade.

2.3 Physicochemical properties of water

The temperature, pH, total alkalinity, hardness, and dissolved oxygen (DO) of water used in aquaria were measured using standard methods (Bernet *et al*. 1999).

2.4 Experimental design

Based on the 96 hr LC_{50} value, three sub-lethal concentrations were determined to assess the toxic effects of CP on *P. conchonius*: SLC I ($1/50^{th}$ of LC_{50}) = 0.3408 µg L^{-1}, SLC II ($1/10^{th}$ of LC_{50}) = 1.704 µg L^{-1}, and SLC III ($1/5^{th}$ of LC_{50}) 3.408 µg L^{-1}. Twelve healthy fish (n = 12) were selected from the stock, starved for 24 hr, and carefully introduced into individual aquaria with 20 L water capacity.

The experimental setup included fish exposed to each sub-lethal concentration of CP for 96 hr, with dechlorinated pesticide-free water serving as control. To ensure accuracy and reliability, three replicates were maintained for each concentration. Three fish from each concentration group, including the control, were randomly sampled for analysis at regular intervals of 24, 48, 72, and 96 hr. The peripheral blood and gill tissues were collected and immediately processed for micronuclei, comet, enzymatic, and anti-oxidant gene expression assays. The experimental workflow is illustrated in Figure 1 to clarify and visualize the study design.

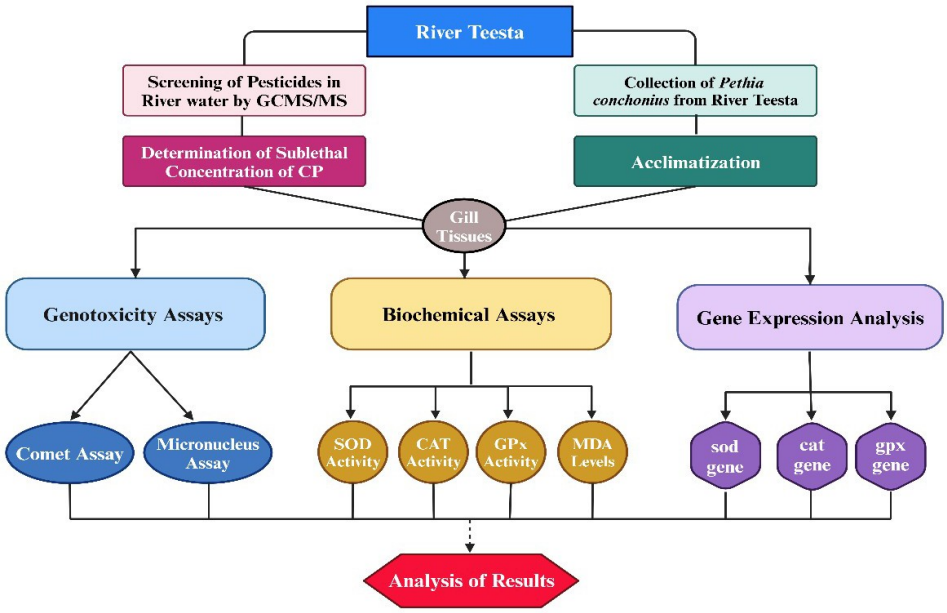

FIGURE 1 Diagrammatic representation of the experimental design.

2.5 Acute toxicity bioassay

The acute toxicity test was carried out to determine the median lethal concentration (LC_{50}) of CP for *P. conchonius* in the laboratory conditions as per OECD guidelines (Finney *et al*. 1971). Acclimatized fish specimens (n = 16 per concentration) were taken in four separate 23×23×30 cm^3 glass aquaria and starved for 24 hr before pesticide exposure. The preliminary range-finding bioassays were conducted to establish concentration gradients of CP for subsequent tests. A control (unexposed) was included in all experiments, and each experiment was replicated three times. Mortality was recorded at 24 hr intervals for 96 hr. For comparison, LC_{50} values and 95% confidence limits were calculated using Finney's Probit analysis method (1971).

2.6 Micronucleus (MN) assay

MN test was carried out on peripheral erythrocytes (whole blood) of the CP-treated and non-treated *P. conchonius*. Peripheral Erythrocytes (whole blood) were collected from 3 (n = 3) fish for each treatment group and the control at 24, 48, 72, and 96 hr intervals with the help of a 1 mL heparinized syringe and smeared on the clean, grease-free slides. The slides were air-dried, fixed in methanol for 20 min, and left to air-dry for 24 hr at room temperature. The slides were stained with 5% giemsa for 10 – 20 min and mounted with DPX (distyrene, plasticizer, and xylene) (Beninca 2006). Slides were observed under 100 × objective with 1000x magnification (Nikon Eclipse E200 microscope). The frequency of micronuclei and the other nuclear abnormalities (NA) like notched (NO) and blebbed (BL) nuclei were calculated as follows:

%MN&MN = (Number of cells containing micronuclei and nuclear abnormalities / Total number of cells scored) × 100

For each sub-lethal concentration and the control, 3000 cells per slide and a total of 9000 cells per specimen were counted. MNs were identified by their size, which was less than one-third of the main nucleus and had the same colour and intensity as the main nucleus. Other nu-

clear abnormalities like notched and blebbed nuclei were also scored. All the slides were coded and blindly scored.

2.7 Comet assay (alkaline single-cell gel electrophoresis)
The extent of DNA damage in the gill cells of *P. conchonius* exposed to three sub-lethal concentrations of CP was assessed by comet assay following Singh *et al*. (1998) with slight modifications.

2.7.1 Sample preparation: Gill tissues were dissected in ice-cold phosphate-buffered saline (PBS, pH 7.4) and homogenized using a micro-pestle to obtain a cell suspension. The cell suspension was transferred to eppendorf tubes for further processing. Pre-coated slides were prepared by smearing 50 µl of 1% agarose (in distilled water) on frosted slides, which were allowed to air-dry. Next, 200 µl of 1% agarose (in PBS) was layered on the pre-coated slides and covered with cover glasses. For embedding of the cells, 20 µl of the cell suspension was mixed with 80 µl of 0.5% low-melting agarose (in PBS). This mixture was smeared onto the precoated frosted slides and covered with cover glasses. Once solidified, an additional layer of 100 µl of 0.5% low-melting agarose (in PBS) was added, and the slides were again covered with cover glasses to ensure proper embedding.

2.7.2 Lysis and electrophoresis: After removing the cover glasses, the slides were immersed in 50 ml of cold lysis buffer (5M NaCl, 500 mM EDTA, 1M Tris-HCl, Triton X-100, and DMSO pH 10.0) for 1 – 2 hr. Following lysis, the slides were transferred to an electrophoresis unit to allow DNA unwinding in the electrophoresis buffer (NaOH and 500 mM EDTA; pH > 13) for 20 min. Electrophoresis was carried out at 18 V/300 mA for 10 min at 4°C.

2.7.3 Neutralization and staining: The slides were subsequently immersed in a neutralization buffer (1 M Tris-HCl; pH 7.5) for 5 min and dehydrated in absolute methanol for 5 min, followed by drying in an incubator at 50°C for 30 min. To visualize DNA damage, 50 µl of ethidium bromide (EtBr; 10 µg mL^{-1}) was evenly spread on the slides and covered by a glass.

2.7.4 Microscopic analysis: Extent of DNA damage was analyzed using a fluorescence microscope (Nikon Eclipse E200, Nikon) equipped with a barrier filter of 590 nm and an excitation filter of 510 – 560 nm connected to a CCD camera at 40X magnification. A total of 250 cells per concentration and duration were scored, with 50 cells per slide. The DNA damage was quantified in terms of %head DNA, %tail DNA, and tail length (L-tail) using an image analysis system with CaspLab software (version 1.2.3 b2).

2.7.5 Protein estimation: Protein was estimated according to Lowry *et al*. (1951), using Bovine Serum Albumin (BSA) as standard. The standard curve of BSA was plotted using Reagent I and Reagent II and the absorbance (A) of the reaction mixtures with tissue homogenate was recorded at 660 nm spectrophotometrically. The amount of protein in the homogenate was used to determine various enzyme activity assay.

2.8 Level of malondialdehyde (MDA assay)
Malondialdehyde level was estimated spectrophotometrically in the gill-homogenate prepared by thiobarbituric acid (TBA) treatment following the method of Placer (1996) with slight modification. For spectrometric analysis, 0.1 ml of gill-homogenate, 0.1 ml SDS, 0.75 ml acetic acid buffer, 0.75 ml TBA, and 0.5 ml distilled water were mixed in a test tube and heated at 95°C for an hr. The mixture was cooled and centrifuged at 3000 rpm for 15 min, and the absorbance was recorded for the supernatant at 532 nm. For blank, tissue homogenate was replaced by distilled water. The results were expressed as nmol MDA g^{-1} of tissue.

2.9 Antioxidant enzyme assays
2.9.1 Superoxide dismutase (SOD) assay: SOD activity was measured in the gill homogenate of both the CP-treated and control *P. conchonius* following the method of Nishikimi *et al*. (1972). The tissue extract was prepared by homogenizing the gills in ice-cold PBS (pH 7.5) and centrifuging the homogenate at 10000 x g at 4°C for 10 min. The supernatant was collected for the study. 1720 µl of distilled water, 50 µl of Nitro Blue Tetrazolium (NBT) (50 µM), 100 µl Phenazine methosulfate, 50 µl of tissue homogenate, and 80 µl of NADH (78 µM) were mixed, and incubated for 5 min at room temperature, while the blank was prepared by replacing the homogenate with an equal volume of distilled water. Absorbance was measured at 560 nm using a spectrophotometer (Shimadzu, UV-1900i), and results were expressed in U mg^{-1} protein. Calculations were made using the formula:

Activity = (% inhibition / protein concentration) × dilution factor

2.9.2 Catalase (CAT) activity assay: CAT activity was measured using Aebi's method (1974). The tissue was homogenized in 0.1 M cold PBS (pH 8.0) and centrifuged at 700 x g for 5 – 10 min. Ethanol was added to the supernatant to a final concentration of 0.17 M (0.01 M ml^{-1}). The samples were incubated in an ice-water bath for 30 min. Then, 10% Triton X was added to a final concentration of 1%. A 100-fold dilution of the original homogenate was prepared by adding chilled PBS. 2 ml of sample and 1 ml of water was taken in a cuvette, and the absorbance was measured at 240 nm at 30-second intervals for 2 – 3 min. For blank, the sample was replaced with distilled water.

2.9.3 Glutathione peroxidase (GPx) activity assay: GPx activity was determined following the method of Flohe and Gunzler (1984) with minor modifications. The reaction mixture was prepared by mixing 500 µl 0.1 M phosphate buffer (pH 7.0), 100 µl enzyme sample (homogenate), 100 µl glutathione reductase (0.24 U), and 100 µl of 10 mM GSH. For blanks, the homogenate was replaced by the buffer. The reaction mixture was taken into a cuvette and preincubated for 10 min at 37°C followed by the addition of 100 µl of NADPH, and the hydrogen peroxide-independent consumption of NADPH was monitored for 3 min, spectrophotometrically. After that, 100 µl of pre-warmed hydrogen peroxide solution was added and the decrease in absorption was noted at 340 nm for about 5 min using a spectrophotometer (Shimadzu, UV-1900i). The activity was determined in terms of nmol min^{-1} mg^{-1} protein.

2.10 Antioxidant gene expression analysis

2.10.1 RNA extraction and cDNA synthesis: The gill tissues of the CP-treated and control *P. conchonius* were homogenized separately in ice-cold PBS (pH 7.5) with a micro-pestle in a 1.5 ml microfuge tube and centrifuged at 10,000x g at 4°C for 10 min. The supernatant was transferred to a clean autoclaved microfuge tube and stored at –20°C for gene expression analysis. RNA was extracted from the supernatant following the manufacturer's instructions (Invitrogen, Thermofisher Scientific) and Sambrook *et al*. (1989). The tissues were homogenized in TRIZOLTM reagent, and centrifuged to remove debris, polysaccharides, and DNA. The RNA-containing phase was purified by chloroform/isopropyl alcohol treatment followed by 70% ethanol wash. The RNA pellet was air-dried at room temperature for 5 – 10 min. The RNA quality was checked in 1.5% Agarose gel and spectrophotometrically at 260/280 nm (UV 1900i, Shimadzu, Japan). The RNA samples were treated with DNase I (Thermo Fisher Scientific) to remove any genomic DNA contamination. The cDNA was synthesized using the Go-Script Reverse Transcription Kit (Promega) from the purified RNA.

2.10.2 Expression analysis

The cat, sod, and gpx expressions were done by RT-PCR in Light Cycler 96 (Roche) using the SYBR Green system (Sigma-Aldrich). Expression of cat, sod, and gpx genes was quantified by the $2^{-\Delta\Delta ct}$ method according to Livak and Schmittgen (2001) with minor modifications using the housekeeping β-actin gene as an internal control. Each mRNA level was expressed as a ratio to β-actin mRNA. The PCR products were analyzed on 2% agarose gel stained with 0.5 µg ml^{-1} ethidium bromide (stock 10 mg ml^{-1}) with 100 bp DNA ladder as a size marker (Promega). Gels were visualized under a UV-transilluminator (TFX-20M, Life Technologies, India). The primers used for the expression analysis are given in Table 1.

Table 1 Primers used for mRNA gene expression analysis for superoxide dimustase (sod), catalase (cat), and glutathione peroxidase (gpx) genes in *Pethia conchonius*.

Genes	Primers	Product length	Accession No.	Reference
β actin	F 5'GGCTGTGCTGTCCCTGTA3′ R 5'GGGCATAACCCTCGTAGAT 3′	101 bp	OQ656379.1	Handa and Jindal (2020)
CAT	F 5'GTTTCCGTCCTTCATCCACTCT 3′ R 5'GACCAGTTTGAAAGTGTGCGAT 3′	190 bp	OQ675559.1	Designed by NCBI Primer BLAST
SOD	F 5'GTCGTCTGGCCTGTGGTGTT 3′ R 5'TCGTCAGTGGGCTAAGTGCTT3′	123 bp	OR853747.1	Designed by NCBI Primer BLAST
GPX	F 5'GGCACAACAGTCAGGGATTACACT3′ R 5'GGTGGGCGTTCTCACCATTCACT 3′	273 bp	ON733100.1	Zheng *et al.* (2012)

2.11 Data analysis

IBM SPSS Statistics Version 27 was used to analyze data with a significance level set at $p \leq 0.05$. The Shapiro-Wilk test was applied to assess normality, while Levene's test was used to check equal variances, with $p > 0.05$ indicating normal distribution and homoscedasticity. For normally distributed data with equal variances, ANOVA was used to compare means across the groups. Duncan's test served as a post-hoc analysis under these conditions, whereas Dunnett's T3 test was applied for unequal variances. Welch's ANOVA was utilized when equal variances were violated. For non-normally distributed data, the Kruskal-Wallis test was used to compare medians across groups. An independent *t*-test was also applied for normally distributed data with equal sample sizes, and the Mann-Whitney *U* test was employed for unequal sizes or non-normal data, assessing significant differences in distributions.

3 | RESULTS

3.1 Acute toxicity analysis

No mortality was recorded in the control group. The LC_{50} value of CP was estimated by recording the percentage of fish mortality at the different test concentrations (10, 15, 20, 25, and 30 µg L^{-1}) for 96 hr. The 96 hr LC_{50} value of CP for *P. conchonius* was calculated to be 17.04 µg L^{-1} by Probit analysis.

3.2 Micronuclei assay

Micronuclei (MN) and other nuclear abnormalities, notch (NO), and bleb (BL) in the peripheral erythrocytes of the CP-exposed *P. conchonius* were higher than the control with no significant differences and formed a single homogeneous subset.

The MN, NO, and BL frequencies were 0.422 ± 0.024, 0.156 ± 0.015, and 0.311 ± 0.028 at 24 hr, which showed an increasing trend after 48 and 72 hr of exposure. Highest frequencies of MN, NO, and BL were recorded as 2.489 ± 0.031, 0.556 ± 0.029, and 1.544 ± 0.036 at 96 hr in the treatment group SLC I. A similar dose and time-dependent increase in MN, NO and BL was observed in the treatment groups SLC II and SLC III. The SLC III showed extensive nuclear damage in the MN, NO, and BL with frequency of 0.844 ± 0.024, 0.222 ± 0.015, and 0.589 ± 0.034; 1.356 ± 0.020, 0.333 ± 0.025, and 0.611 ± 0.020; 2.289 ± 0.048, 0.511 ± 0.029, and 1.533 ± 0.038; and 3.289 ± 0.077, 1.444 ± 0.024, and 2.133 ± 0.025, respectively over the treatment period than control. The MN, NO, and BL in the CP-exposed groups were significantly higher ($p < 0.05$) compared to the control ($p < 0.05$) with 21, 11.8, and 27 times increase in the SLC III group at 96 hr indicating a pronounced dose- and time-dependent damage (Table 2, Figure 2).

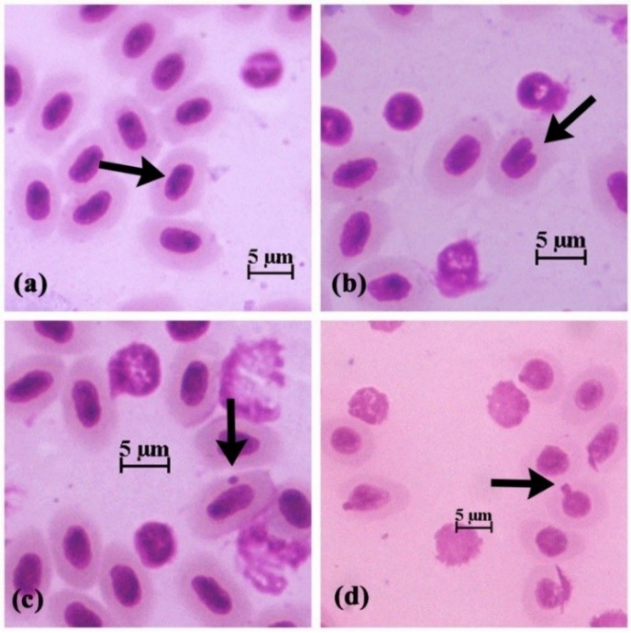

FIGURE 2 Microphotographs of normal nucleated cells in control (a), Notched nucleus (b), Micronucleated cell (c), and Blebbed nucleus (d), in peripheral erythrocytes of exposed cypermethrin groups (bar = 5µm).

TABLE 2 The frequency of different nuclear abnormalities in erythrocytes (Mean ± SE) in different duration and sub-lethal concentrations (SLC) I, II and III of cypermethrin (CP).

Exposure duration	Concentrations of CP			
(hr)	Control	SLC I	SLC II	SLC III
Micronuclei%				
24	0.133±0.010^{a1}	0.422±0.024^{b1}	0.678±0.039^{c1}	0.844±0.024^{d1}
48	0.156±0.011^{a1}	0.811±0.031^{b2}	1.133±0.019^{c2}	1.356±0.020^{d2}
72	0.144±0.006^{a1}	1.433±0.025^{b3}	1.611±0.024^{c3}	2.289±0.048^{d3}
96	0.156±0.006^{a1}	2.489±0.031^{b4}	2.622±0.071^{b4}	3.289±0.077^{c4}
Notched%				
24	0.100±0.010^{a1}	0.156±0.015^{a1}	0.244±0.015^{b1}	0.222±0.015^{b1}
48	0.111±0.011^{a1}	0.278±0.024^{b2}	0.267±0.017^{b1}	0.333±0.025^{b2}
72	0.133±0.010^{a1}	0.333±0.010^{b2}	0.489±0.031^{c2}	0.511±0.029^{c3}
96	0.122±0.006^{a1}	0.556±0.029^{b3}	0.515±0.024^{b2}	1.444±0.024^{c4}
Blebbed%				
24	0.056±0.006^{a1}	0.311±0.028^{b1}	0.522±0.064^{b1}	0.589±0.034^{c1}
48	0.067±0.010^{a1}	0.544±0.036^{b2}	0.622±0.039^{b1}	0.611±0.020^{b1}
72	0.067±0.010^{a1}	0.700±0.042^{b3}	1.122±0.044^{c2}	1.533±0.038^{d2}
96	0.078±0.006^{a1}	1.544±0.036^{b4}	1.622±0.034^{b3}	2.133±0.025^{c3}

Different lowercase letters indicate different homogeneous subsets ($p < 0.05$) along the concentration of CP within the same duration of exposure. Numbers indicate different homogeneous subsets ($p < 0.05$) along the exposure duration within same concentration.

3.3 Comet assay

Gill tissues collected from CP-exposed *P. conchonius* exhibited a marked increase in DNA damage across all the exposed groups compared to the control groups. The extent of DNA damage was quantified in terms of % head DNA, % tail DNA, and tail length (Ltail). Control groups demonstrated no significant differences and formed a single homogeneous subset.

The % head DNA was recorded to be the highest at 24 hours after exposure (95.521 ± 0.825, 92.992 ± 0.745, and 84.088 ± 2.118) with subsequent decrease over the experimental period with % head DNA of 47.403 ± 2.391,

32.823 ± 2.214, and 31.068 ± 1.453 at 96 hr, respectively for SLC I, II, and III showing a progressive reduction with increasing concentration and exposure duration. These reductions represented a threefold decrease ($p < 0.05$) compared to the controls, highlighting the deleterious impact of CP (Figure 2, Table 1). On the other hand, a gradual increase in the %Tail DNA was noted in the CP-treated groups SLC I, II and III, respectively at 24, 48, 72 and 96 hr of exposure than control with a minimum of 5.30-fold increase in the SLC I at 24 hr and a maximum of 70-fold increase in SLC III at 96 hr with values 52.989 ± 2.579, 66.981 ± 2.402, and 70.893 ± 1.843 ($p < 0.05$). The % head and tail DNA showed a dose and time-dependent change in response to CP exposure.

Similarly, the tail length observed in the gill cells showed a concentration and time-dependent increase for SLC I, II, and III. The results showed significantly higher Ltail with a minimum 5.6-fold to a maximum 12.5-fold increase in the SLC I and III, respectively, at 24 – 96 hr of exposure than the control. The damage parameters indicated mild to extensive DNA damage, revealing the genotoxic potential of CP (Table 3, Figure 3).

3.4 MDA analysis

In contrast to antioxidant enzymes, MDA levels in the CP-treated groups (SLC I, II, and III) exhibited a significant induction in a dose and time-dependent response compared to the control groups. Significantly increased MDA levels were noted in SLC I, II, and III treated groups at 24, 48, 72, and 96 hr respectively. The lowest increase was recorded as 0.251 ± 0.019, 0.322 ± 0.022, 0.442 ± 0.010, and 0.832 ± 0.041, while the highest MDA values were 0.555 ± 0.035, 1.016 ± 0.061, and 1.326 ± 0.064 in the SLC I, II, and III, respectively, at 24 and 96 hr of exposure in comparison to the control (Figure 4). Thus, a 27-fold, 34-fold, and 44-fold increase at 96 hr of exposure indicates elevated oxidative stress in CP-exposed fish.

3.5 Antioxidant enzyme analysis

Sub-lethal toxicity of CP was also evaluated by assessing the antioxidant enzyme (CAT, SOD, and GPx) activity and the level of MDA in the gills of CP-treated and control *P. conchonius*. CAT, SOD, and GPx activities and the levels of MDA did not show any significant differences at different time intervals in the control and formed a single subset "a1", while the CP-treated groups showed significant changes. CP-treated groups SLC I, II, and III exhibited a noticeable reduction in the CAT, SOD and GPx activities in the gill tissue.

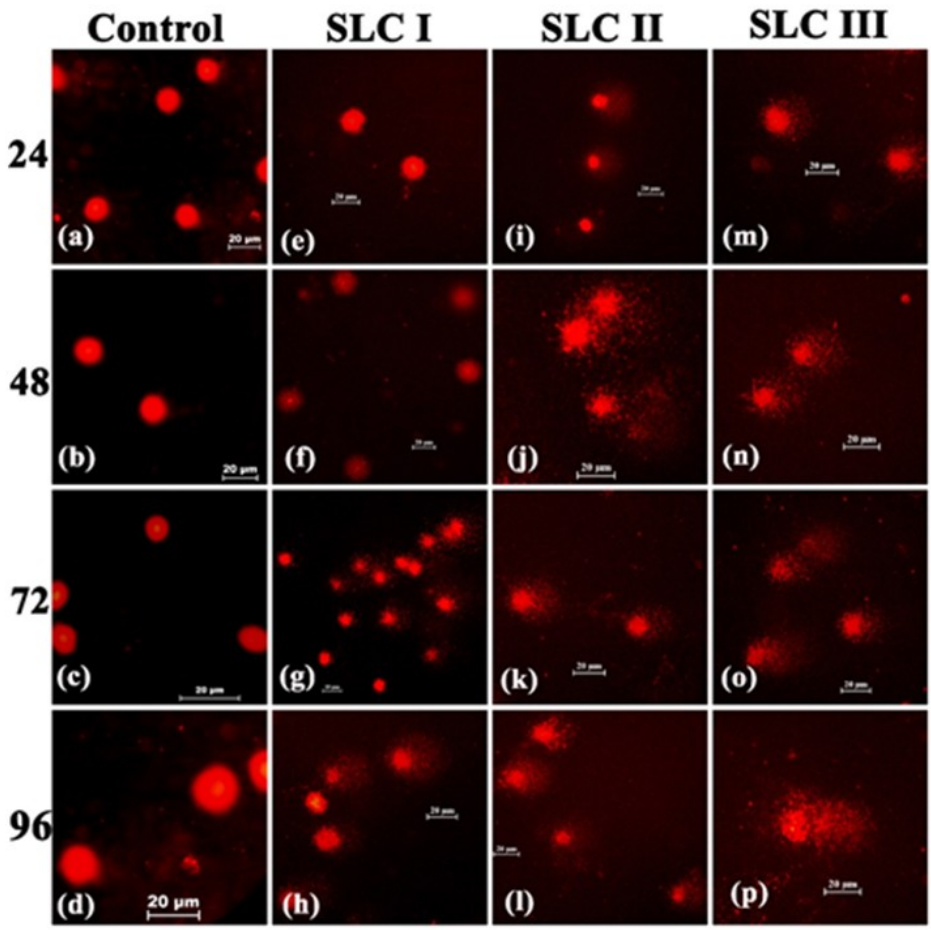

FIGURE 3 Microphotographs showing the results of SCGE in the gill cells of *Pethia conchonius*: Normal cells of the control group (a-d) and damaged gill cells in SLC I (e-h), SLC II (i-l) and SLC III (m-p) for 24 – 96 hr exposure of cypermethrin (bar = 20µm).

TABLE 3 Percentage (%) of head DNA, tail DNA, and tail length (L tail) (Mean ± SE) of control and exposed specimens of *Pethia conchonius* at sub-lethal concentrations (SLC) I, II, and III of cypermethrin.

Exposure duration (hr)	Concentrations			
	Control	SLC I	SLC II	SLC III
% head DNA				
24 hr	99.160±0.299^{a1}	95.521±0.825^{b1}	92.992±0.745^{c1}	84.088±2.118^{d1}
48 hr	99.599±0.105^{a1}	76.414±2.669^{b2}	58.668±2.928^{c2}	56.436±3.918^{c2}
72 hr	99.151±0.278^{a1}	61.223±2.313^{b3}	49.899±2.737^{c3}	46.070±2.480^{c3}
96 hr	99.506±0.155^{a1}	47.403±2.391^{b4}	32.823±2.214^{c4}	31.068±1.453^{c4}
% tail DNA				
24 hr	0.840±0.299^{a1}	4.479±0.825^{b1}	7.408±0.937^{c1}	15.912±2.118^{d1}
48 hr	0.869±0.203^{a1}	26.331±3.388^{b2}	41.332±2.928^{c2}	43.564±3.911^{c2}
72 hr	0.849±0.278^{a1}	44.337±3.356^{b3}	50.101±2.737^{bc3}	53.930±2.626^{c3}
96 hr	0.893±0.257^{a1}	52.989±2.579^{b4}	66.981±2.402^{c4}	68.932±1.843^{c4}
Tail length (L tail)				
24 hr	3.061±0.035^{a1}	17.120±1.062^{b1}	31.240±0.694^{c1}	50.180±1.090^{d1}
48 hr	3.200±0.064^{a1}	83.039±1.649^{b2}	99.596±2.378^{c2}	132.471±2.427^{d2}
72 hr	3.120±0.046^{a1}	146.220±4.936^{b3}	189.313±3.116^{c3}	215.128±5.216^{d3}
96 hr	3.189±0.076^{a1}	246.824±3.228^{b4}	267.745±3.308^{c4}	375.430±9.770^{d4}

Different superscript lowercase letters indicate values with different homogeneous subsets along the concentration of CP within the same duration of exposure, which significantly differ at ($p < 0.05$) obtained from the Kruskal-Walli's test followed by Dunn's posthoc analysis. Different superscript numbers indicate values with different homogeneous subsets along the exposure duration within the same concentration which significantly differ at $p < 0.05$ obtained from the Kruskal-Walli's test followed by Dunn's posthoc analysis.

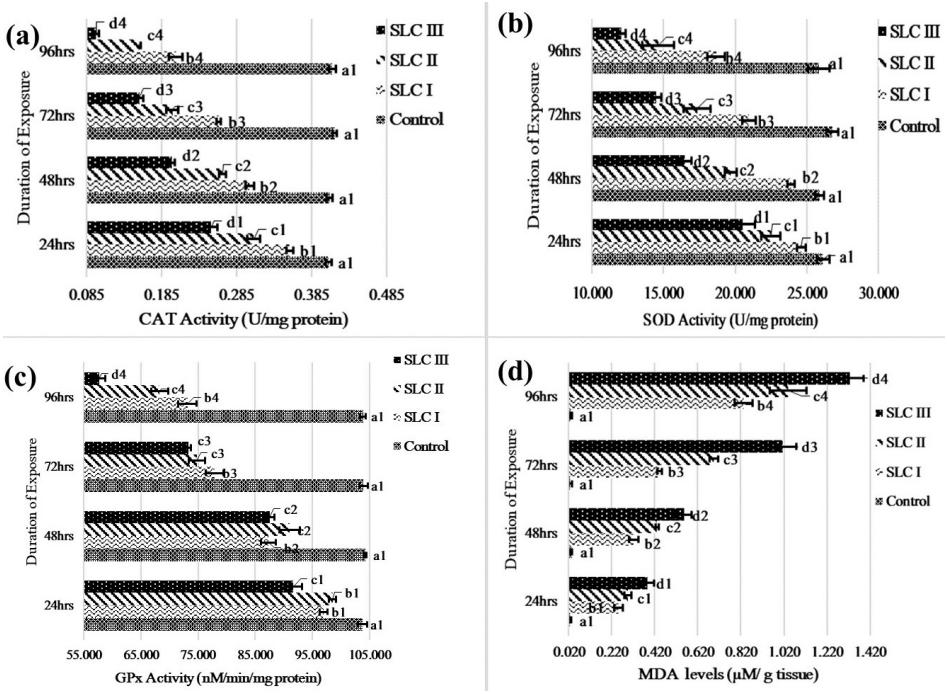

FIGURE 4 Graphical presentations of the activities of antioxidant enzymes CAT, SOD, and GPx and levels of MDA in the gills of *Pethia conchonius* exposed to three sub-lethal concentrations (SLC) of cypermethrin. Different superscript letters indicate values with different homogeneous subsets that significantly differ at ($p < 0.05$)

The CAT activities were 0.356 ± 0.005, 0.303 ± 0.005, 0.261 ± 0.003, and 0.204 ± 0.009; 0.308 ± 0.009, 0.266 ± 0.005, 0.200 ± 0.008, and 0.155 ± 0.002; and 0.251 ± 0.008, 0.198 ± 0.004, 0.155 ± 0.005, and 0.098 ± 0.004 in the CP-treated SLC I, II, and III, respectively at 24, 48, 72 and 96 hr of exposure with a significant reduction of 1.14 – 4.2 folds ($p < 0.05$) (Figure 4).

Similarly, reduced SOD activity of 24.610 ± 0.312, 23.901 ± 0.251, 20.936 ± 0.468, and 18.680 ± 0.616; 22.507 ± 0.665, 19.683 ± 0.409, 17.337 ± 0.919, and 14.637 ± 1.103, and 20.510 ± 0.840, 16.493 ± 0.465, 14.470 ± 0.375, and 12.063 ± 0.301 was observed in the

exposed groups SLC I, II and III respectively at 24, 48, 72, and 96 hr of exposure. The SOD activities were significantly lower than the control (26.133 ± 0.467, 25.873 ± 0.306, 26.770 ± 0.413, and 25.827 ± 0.741) at the same time intervals ($p > 0.05$). The results exhibited an overall 1.30, 1.70, and 2.10-fold decrease in the treatment groups in a dose and time-dependent response (Figure 4).

GPx was noted to be 97.013 ± 0.641, 87.337 ± 1.271, 77.907 ± 1.516, and 73.147 ± 1.586; 98.553 ± 0.575, 91.073 ± 1.770, 74.777 ± 1.391, and 68.247 ± 1.498, and 91.683 ± 1.516, 87.557 ± 0.833, 73.290 ± 0.485, and 57.657 ± 1.100 in the SLC I, II and III at 24, 48, 72, and 96 hr of CP exposure, respectively with significantly lower activity in the exposed groups than control (Figure 4). The reduction in GPx activity was 1.42, 1.50, and 1.80-fold in the treated groups. Results indicated an overall inhibitory effect of CP on the three antioxidant enzymes in the gills.

3.6 Antioxidant gene expression analysis (cat, sod, and gpx expression)

The results of the antioxidant enzyme activity in the *P. conchonius* groups exposed to the three sub-lethal concentrations of CP were validated by the cat, sod, and gpx gene expression analysis.

RT-PCR analysis of cat, sod, and gpx mRNA transcripts in the gill tissues of CP-treated *P. conchonius* revealed a significant reduction in the transcript levels. The cat mRNA was 0.94 ± 0.02, 0.84 ± 0.02, 0.82 ± 0.01, and 0.80 ± 0.01; 0.92 ± 0.00, 0.83 ± 0.00, 0.88 ± 0.02, and 0.78 ± 0.06; and 0.90 ± 0.01, 0.83 ± 0.00, 0.87 ± 0.01, and 0.64 ± 0.09 in SLC I, II and III, respectively at 24, 48, 72, and 96 hr of exposure compared to control showing 1.25, 1.28 and 1.5-fold reduction (Figure 5). A decrease was observed in all treated groups as early as 24 hours; however, a significant decrease was observed after 72 hr of exposure. The results suggested downregulation of the cat gene in all three treated groups ($p < 0.05$), which is comparable to the catalase enzyme activity (Figure 4).

On the other hand, the sod mRNA transcripts of 0.88 ± 0.01, 0.84 ± 0.04, 0.60 ± 0.01, and 0.43 ± 0.01; 0.86 ± 0.03, 0.76 ± 0.06, 0.56 ± 0.06, and 0.43 ± 0.01; and, 0.81 ± 0.01, 0.66 ± 0.01, 0.47 ± 0.03, and 0.19 ± 0.02 were observed in the SLC I, II and III with a 13 – 57%, 15 – 57%, and 20 – 81% reduction, respectively at 24, 48, 72 and 96 hr (Figure 5) compared to the control.

Similarly, reduced gpx mRNA transcripts of 0.79 ± 0.01, 0.85 ± 0.04, 0.61 ± 0.03 and 0.44 ± 0.01 were observed in SLC I, while SLC II and SLC III showed the gpx transcripts of 0.77 ± 0.03, 0.77 ± 0.07, 0.58 ± 0.04, and 0.43 ± 0.01 and 0.72 ± 0.01, 0.67 ± 0.02, 0.47 ± 0.04, and 0.30 ± 0.13, respectively at 24, 48, 72 and 96 hr of CP treatment with a significant reduction of 22 – 70% ($p < 0.05$). The results strongly indicated a dose and time-dependent response in the cat, sod, and gpx expression leading to downregulation of the genes in the exposed groups (Figure 5).

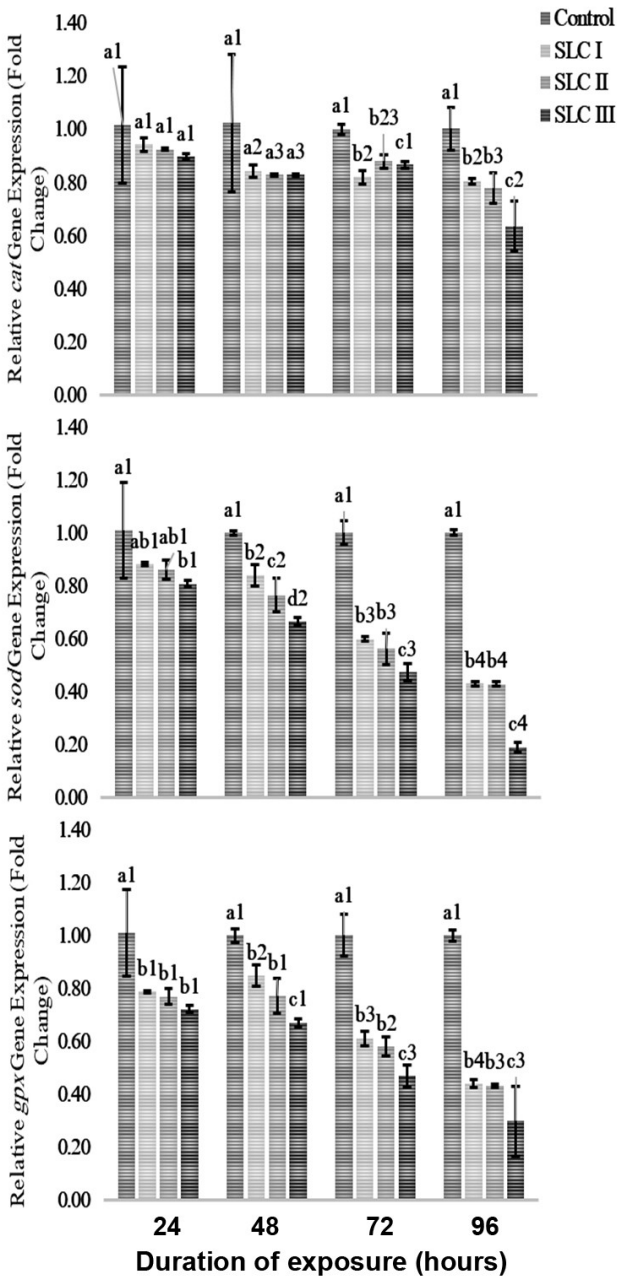

FIGURE 5 Fold-changes recorded in the cat, sod, and gpx gene expression in the gill tissues of CP-exposed *Pethia conchonious*. Different superscript letters indicate values with different homogeneous subsets that significantly differ at $p < 0.05$.

4 |DISCUSSION

Pesticides or insecticides are indispensable components in agriculture that minimize crop loss due to pest infestations, especially in tropical nations like India with favorable climatic conditions for arthropod pests to thrive (Rajak *et al*. 2023). Although pesticides are applied to control targeted arthropod pests, they also contaminate the envi-

ronment (air, water, and soil) adversely affecting several non-target organisms, including humans (Gibbons *et al*. 2015; Mahmood *et al*. 2016; Amenyogbe *et al*. 2021). Pesticides with prolonged half-life are persistent and can permeate food chains and bioaccumulate in the consumers of higher trophic levels, jeopardizing the entire ecosystem if utilized improperly (Rana *et al*. 2022). Synthetic pyrethroids, such as CP, are globally used in agriculture. The CP is a 4th generation halogenated type II pyrethroid shown to be neurotoxic to the target organisms and extends the opening of sodium channels in the central nervous system, leading to hypo-polarization and hyper-excitation of the neurons that cause muscle deficits (Kumar *et al*. 2007; Majumdar and Kaviraj 2017). Pyrethroid-induced toxicity in non-target organisms like rodents and freshwater fishes exposed to CP at sub-lethal doses has been reported by several workers (Wolansky *et al*. 2008).

Rivers act as the principal water storage system in terrestrial ecosystems, making them vulnerable to the introduction of xenobiotics, including pesticides from agricultural areas. Pesticides or insecticides typically reach rivers through precipitation, irrigation, soil leaching, drainage systems, or direct run-off (Merga and Brink 2021).

The Teesta River, a significant watercourse in the northern part of West Bengal (North Bengal) of India, originates in the Himalayas of Sikkim and traverses several regions of the state. The river drains areas defined by monoculture tea cultivation, rice fields, and cash crops like nuts and vegetables. However, increasing population density and industrialization in the area, inappropriate disposal of untreated sewage, and the unregulated use of pesticides and fertilizers in agricultural practices are heavily polluting the river Teesta (Acharjee *et al*. 2013). The current study evaluates the effects of CP on *P. conchonius* from the Teesta River using multiparameter approaches by exposing the organism to SLC of CP.

4.1 Genotoxicity assays

4.1.1 Comet assay: In the current study, reduced % head DNA and concomitant increase of % tail DNA and the LTail in the gills of CP-exposed *P. conchonius* in a concentration and time-dependent response indicated elevated DNA damage. Maximum damage occurred after 96 hr of CP exposure for all SLC. In a similar study Taju *et al*. (2014) reported a 1.3%, 2.5%, 1.8%, 1.6%, and 2.1% increase of tail DNA in five distinct fish cell lines, respectively exposed to CP compared to control. Jin *et al*. (2011) have demonstrated a substantial increase in tail DNA, tail length, and Olive tail moment in gills of zebrafish at a low dose of 0.3 µg L^{-1} of CP. Additionally, Poletta *et al*. (2013) showed that fish exposed to CP in their natural habitat had a much higher DNA damage index (DI) in their gill cells. The DI values were 239.62 ± 6.21 at a concentration of 0.150 µg L^{-1} and 270.63 ± 2.09 at a concentration of 0.300 µg L^{-1}, compared to a control with DI = 150.25 ± 4.38, indicating significantly higher DNA damage in response to CP exposure.

4.1.2 MN assay: At all concentrations, a dose and time-dependent increase of MN, NO, and BL in the CP-treated *P. conchonius* indicated that CP is genotoxic inducing abnormal cell division. Ansari *et al*. (2011) have shown significantly higher MN in CP-exposed *Channa punctatus* ($p < 0.05 – 0.001$), along with other cytogenetic abnormalities such as chromosomal and chromatid breaks. Sharma and Jindal (2020) have recorded the genotoxic effects of sub-lethal concentrations of commercial-grade CP in *Catla catla*, revealing a dose-dependent rise in the frequency of micronuclei, percentage of DNA damage, and erythrocyte abnormalities, such as bridging, deformed nuclei, notched and lobed nuclei, after 45 days of exposure. Significantly higher MN frequency was reported in the CP-exposed *Prochilodus lineatus* than control (Davico *et al*. 2020). Micronuclei are formed as a result of DNA lesions or unstable cell division and are considered a sign of genotoxic events and chromosomal instability (Amjad *et al*. 2018). The results of MN assay on a variety of fish species, such as *Cheirodon interruptus*, *Garra rufa*, *Channa punctatus*, and *Danio rerio* showing elevated micronuclei frequency are comparable with our findings, demonstrating the genotoxic effects of pyrethroid insecticides like lambda-cyhalothrin, atrazine, or CP on fish due to changing oxidative environment in the cell, alkylating effect of the pesticide or cell cycle defects (Campana *et al*. 1999; Cavas and Gozukara 2003; Jin *et al*. 2011; Amjad *et al*. 2018).

In conclusion, the results underscore a clear concentration and time-dependent escalation in DNA damage in the gill cells of *P. conchonius* upon exposure to CP. The progressive reduction in Head DNA, coupled with significant increases in tail DNA and tail length, highlights the genotoxic potential of CP and its deleterious impact on aquatic organisms.

4.1.3 Biochemical analysis in gill tissues of *Pethia conchonius*: CP exposure disrupts oxidative balance in fish by impairing antioxidant enzyme activities and inducing oxidative stress, particularly in the gills, which are in direct contact with water (Paravani *et al*. 2019). Antioxidant enzymes such as SOD, CAT, and GPx play a crucial role in mitigating oxidative stress (Yang *et al*. 2020; Benli and Celik 2021). SOD, the primary defense against oxidative stress in animals, catalyses the dismutation of superoxide anions into hydrogen peroxide, which is further detoxified by CAT or GPx (McCord and Fridovich 1969; Winston and Di Gulio 1991, Mishra and Devi 2014). A significant reduction of SOD activity (6 – 31% and 22 – 53%) in the gills of CP-treated *P. conchonius* is consistent with previous studies on zebrafish, demonstrating CP-induced inhibition of SOD activity (Paravani *et al*. 2019). Moreover, the role of

CAT is also important in neutralizing reactive oxygen species (ROS) by breaking down hydrogen peroxide into oxygen and water (Beers and Sizer 1952; Kirkman *et al*. 1987; Abbott *et al*. 2009). A significant dose and time-dependent reduction in CAT activity in CP-treated *P. conchonius* may result in elevated ROS activity and subsequent oxidative stress. Davico *et al*. (2020) have also shown decreased CAT activity in *Prochilodus lineatus* exposed to 0.15 µg L^{-1} and 0.30 µg L^{-1} CP. Studies on zebrafish exposed to CP, endosulfan, and imidacloprid have also demonstrated CAT inhibition, leading to oxidative damage (Paravani *et al*. 2018; Muazzam *et al*. 2019; Paravani *et al*. 2019). The reduction in CAT activity is associated with the binding of toxicants to the enzyme's -SH groups and the accumulation of ROS (Ruas *et al*. 2008), making it a sensitive biomarker of oxidative stress in fish. CP has also been reported to reduce SOD and CAT activity in various fish cell lines, including IEG, CB, ICG, LRG, and CSG, by 2.2-fold, 4.1-fold, 2.7-fold, 3.1-fold, and 3.5-fold, respectively, at a concentration of 20 ng ml^{-1} (Taju *et al*. 2014). Such alterations in enzyme activity may be an adaptive cellular response to pesticide-induced stress (Livingstone 2001).

GPx, responsible for reducing hydrogen peroxide and lipid hydroperoxides using glutathione (GSH), prevents oxidative damage caused by lipid peroxidation (Van der Oost *et al*. 2003; Lushchak *et al*. 2016). The observed 7-12% and 30-50% reduction in GPx activity in CP-treated *P. conchonius* suggests downregulation of the gpx gene and increased oxidative damage in gill tissues, potentially leading to chronic respiratory syndromes (Lubos *et al*. 2011). A concentration and time-dependent decrease in GPx activity has also been reported in CP-exposed *Cyprinus carpio* (Yonar *et al*. 2011). Reduced CAT and GPx activity led to hydrogen peroxide accumulation and elevated lipid peroxidation, further exacerbating oxidative stress (Haliwell and Gutteridge 1986). GPx inhibition has been observed in several fish species, including *Prochilodus lineatus* (Vieira *et al*. 2019), zebrafish (Kizilkaya *et al*. 2023), and *Labeo rohita* (Marigoudar *et al*. 2013). However, CP has also been reported to upregulate GPx, Cu / Zn SOD, Mn-SOD, and CAT as well as DNA damage and increased apoptosis in the liver of zebrafish when exposed to concentrations up to 3 µg L^{-1} for 4 – 8 days (Jin *et al*. 2011). Therefore, it can be assumed effects of CP depend on several factors, such as tissue-specificity, and physiological conditions.

Malondialdehyde (MDA), a key intermediate product of lipid peroxidation (LPO), is widely recognized as a biomarker for oxidative stress caused by pesticides (Anila *et al*. 2021; Golomb *et al*. 2021). Significantly higher MDA levels in the gills of CP-exposed *P. conchonius* indicate elevated lipid peroxidation and oxidative stress leading to excessive ROS. Increased ROS may cause oxidative damage, such as nuclear and DNA damage. Increased MDA levels in response to CP exposure have been documented in vivo studies of several fish species, including *Tor putitora* (Ullah *et al*. 2014) and *Danio rerio* (Paravani *et al*. 2019) as well as in vitro studies on five fish cell lines (Taju *et al*. 2014). Elevated LPO levels have also been reported in the liver of juvenile *Clarias gariepinus* exposed to either CP or under combined exposure to CP and Pb; the latter resulting in more damage (Adeyemi *et al*. 2014). Additionally, mixtures of pyrethroids, such as λ-cyhalothrin and CP, have been shown to induce oxidative stress in *Clarias batrachus*, as indicated by increased MDA levels in brain and liver tissues (Kumar *et al*. 2014). Therefore, CP has the potential to induce oxidative stress and damage which may contribute to chronic respiratory problems and tissue damage in *P. conchonius*, emphasizing the potential ecological risks of pesticide contamination in aquatic environments.

4.1.4 Antioxidant gene expression analysis

The expression analysis of sod, cat, and gpx genes in the gills of the CP-exposed *P. conchonius* readily supported the results of the antioxidant enzyme assays. A reduction of 8 – 36% in cat mRNA transcripts in the gills of CP-treated fish than control indicated down-regulation of the cat gene due to cypermethrin exposure. Similar to our study, Wang *et al*. (2020) showed down-regulation of cat and sod genes in embryonic zebrafish exposed to a combination of beta-cypermethrin and thiacloprid. In contrast, several studies have demonstrated the induction of cat mRNA transcription in different developmental stages of *Danio rerio* exposed to varying doses of CP (Chow 2009; Paravani *et al*. 2018; Davico *et al*. 2020;) indicating a tissue-specific upregulation of the gene as an adaptive response to the environmental contaminants (Livingstone 2001). Similarly, a 13 – 57%, 15 – 57%, and 20 – 81% reduction in sod mRNA expression in the gills of CP-treated *P. conchonius* compared to the control across all sublethal concentrations of cypermethrin indicated down-regulation of sod. Yuan *et al*. (2023) have reported a significant reduction in mRNA levels of antioxidant genes like sod in the gill tissues of *Carassius auratus* exposed to CP. In contrast, Hiransuchalert *et al*. (2019) have shown elevated mRNA transcripts of CuZn-sod and Mn-sod in Asian Seabass (*Lates calcarifer*) following 7 days of CP exposure. Alterations in the activities of antioxidant enzymes Cu/Zn-SOD and Mn-SOD are considered indicators of oxidative stress (Jin *et al*. 2010). ROS accumulation in cells can cause an upregulation of sod gene expression as an activated antioxidant defense in fish. However, the inhibitory effect of pesticides has also been recorded and has been theorized to be due to binding of the pesticide or its metabolites to the SOD-active site inhibiting the enzymes normal antioxidant functions (Prasanth *et al*. 2009).

Gpx is an important phase II biotransformation en-

zyme known to detoxify environmental toxicants like polyaromatic hydrocarbons, pesticides, and other reactive intermediates formed in the phase I biotransformation pathway (Trute *et al*. 2007; Xu *et al*. 2013). In the present study, 22 – 56%, 24 – 57%, and 29 – 70% reduction in gpx mRNA transcripts in all the treatment groups indicated down-regulation of gpx in the gills of *P. conchonius*, i.e., CP acts as a strong inhibitor of transcription. A similar study conducted by Xing *et al*. (2012) on zebrafish showed a tissue-specific response to CP exposure. Recently, Yuan *et al*. (2023) have reported a significant reduction of gpx mRNA in the gill tissues of CP-treated *Carassius auratus*. Another antioxidant gene, GST, was shown to be upregulated in zebrafish embryos, by 2.5 to 2.8-fold when exposed to CP (Yuan *et al*. 2023). These studies confirm that pyrethroids, influence gene expression in a tissue-specific, species-specific, and/or development-specific manner and can either upregulate the expression to alleviate the oxidative stress or produce an inhibitory effect on the gene. Changes in the expression of antioxidant genes upon xenobiotic exposure, such as sod and cat and gpx might be regulated as a response to ROS stress in fishes (Liu *et al*. 2015). These reports across various species demonstrate that pesticides can modulate gene expression in a tissue-specific manner.

5 | CONCLUSIONS

River Teesta is not only contaminated by anthropogenic xenobiotics like agricultural pesticides/insecticides, but the non-target organisms that thrive in these freshwater systems also face a threat due to increased contamination. Cypermethrin, among other insecticides, induces damage at cellular and DNA levels in *P. conchonius*, as evidenced by larger DNA comets and elevated frequencies of MN and NA. SOD, CAT, and GPx inhibition in the gills show that the fish are subjected to oxidative disbalance upon exposure to the cypermethrin, as also confirmed by the gene expression profiling of sod, cat, and gpx. The rise of MDA levels further suggests elevated oxidative stress, leading to cellular damage. The study can also be extended to other species to monitor pesticide toxicity. *P. conchonius* is a key species within the local food chain and an essential bioindicator of aquatic health; the results of this study will offer valuable insights into the effects of CP contamination on aquatic organisms and help elucidate its broader ecological consequences in pesticide-polluted environments. The results emphasize the need for the effective use of pesticides/insecticides, their management, and regular environmental monitoring to mitigate or reduce the detrimental effect of the pesticide(s) on non-target organisms including the human population that depend on these organisms and the river water for sustenance. This study also opens future directions for genotoxic studies in these regions of the country.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

D. Dutta, A. Ray, J. Nag, and E. Bhattacharya carried out the original study, followed by statistical analysis by D. Dutta. The main author of the manuscript is D. Dutta. As the supervisor of the Genetics and Molecular Biology Laboratory, Department of Zoology at the University of North Bengal, M. Bahadur edited and reviewed the work. UGC provided partial support for this work. Upon request, the corresponding author will provide the data obtained during this investigation. The writers have no financial or non-financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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