Section: Research Paper

Biomarkers' responses of freshwater fish *Channa punctatus* (Bloch) exposed to Chlorpyrifos

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Abstract:
The current study aimed to investigate the effects of chlorpyrifos exposure on the activity of acetylcholinesterase (AChE), lipid peroxidation, reduced glutathione, oxidised glutathione, glutathione peroxidase and glutathione S transferase in the kidneys, liver, and gills tissues of Channa punctatus fish. The fish were treated with 3 sub-lethal concentrations of the chlorpyrifos viz. high dose-0.6 pppm, mild dose-0.3 ppm and low dose-0.16 ppm and a control group was kept to compare the results obtained. The results showed a significant decrease in AChE activity in all three tissues of fish, which was dose-dependent and time-dependent. The study also found a significant increase in the concentration of malondialdehyde (MDA), a marker of lipid peroxidation, in all three tissues of fish exposed to chlorpyrifos, which was dose-dependent and time-dependent. The results indicated a significant dose- and time-dependent increase in GSH levels in the kidney, liver, and gills of the fish exposed to chlorpyrifos, while GSSG levels were also elevated, suggesting oxidative stress in the tissues. GPx activity was significantly altered in all three tissues, with higher doses and longer exposure times having greater effects. GST activity was significantly increased in all treatment groups, with the highest activity observed in the high dose group at all-time points. These results suggest that chlorpyrifos exposure can significantly inhibit AChE activity and induce oxidative stress in different tissues of C. punctata fish, which may lead to adverse effects on their health and survival and highlights the importance of monitoring and regulating pesticide use in aquatic environments.
Keywords: Channapunctatus, Chlorpyrifos, AChE, Lipid peroxidation, Glutathione.

1. INTRODUCTION

Due to the expansion of agriculture and the indiscriminate use of insecticides brought on by the rise in global food demand, fish populations have decreased and chemicals have been contaminated (Lizano et al., 2017). The most prevalent pesticides in agriculture are organophosphates, which are hazardous to non-target animals, especially fish (Budiyono et al., 2021). The usage of pesticides causes environmental problems, and the aquatic pollution they generate has a negative impact on fish mortality (Lizano et al., 2017). In 1930s, Germany was the first country to introduce organophosphate pesticides (Amin et al., 2022).

In India, a number of insect pests are successfully managed using the organophosphate pesticide chlorpyrifos (CPF) (Duraisamy et al., 2018). It is a broad-spectrum pesticide that works well on agricultural crops and is effective against foliar insects and subterranean termites. It is commonly employed to safeguard crops and is licensed for the control of several pests (Rathod and Garg, 2017). Chlorpyrifos is widely used, which causes it to enter surface water and groundwater. This might potentially pollute water bodies and negatively impact aquatic life, particularly fish, by harming their neurological and respiratory systems and altering their metabolic networks (Gulati et al., 2015).

In commercial agro-farming methods around the world, the organophosphate derivative chlorpyrifos is frequently employed as an insect repellent (Hasanuzzaman et al., 2018) and it is counted as India's second most popular means of synthetic pest control (Stalin et al., 2019). Due to its inexpensive cost, availability in a variety of formulations, and effectiveness in little doses, it is widely used in Bangladesh (Ali et al., 2020; Ihsan et al., 2018).

Reactive oxygen species (ROS) produced by CPF in fish are known to cause oxidative stress, which in turn causes lipid peroxidation (LPO), one of the molecular processes underlying its toxicityAcute hydrolysis of the neurotransmitter acetylcholine to choline and acetate at cholinergic synapses is known to occur in fish, where it is known that CPF inhibits acetylcholinesterase (AChE) activity. As a result, fish suffer from impaired nerve impulses and general ill health (Deb and Das 2021). Fish gills, liver, and brain exhibit enhanced lipid peroxidation and a considerable reduction of AChE activity as a result of exposure to chlorpyrifos.

Key antioxidants like reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GPx), and glutathione S-transferase (GST) have been found to change with chlorpyrifos exposure in fish. A tripeptide called GSH is essential for cellular protection against oxidative stress. GSH participates in the detoxification of xenobiotics, such as pesticides, and serves as a ROS scavenger. The detoxification of ROS and xenobiotics produces GSSG, the oxidized form of GSH. A reduction in the GSH/GSSG ratio indicates increasing oxidative stress, and it is employed as a measure of oxidative stress (Jones, 2006). GPx is an antioxidant enzyme that facilitates the conversion of lipid peroxides and hydrogen peroxide into alcohols and water, respectively. Cells are crucially shielded from oxidative damage by GPx. A class of enzymes known as GST catalyzes the conjugation of GSH to xenobiotics, increasing their water solubility and facilitating excretion from the body. GST is essential for the detoxification of xenobiotics, such as pesticides (Marins et al., 2020).

Due to their sensitivity to xenobiotic stress, fish can serve as an appropriate bioindicator of contaminated settings since studies on the effects on fsh can offer quantitative information on the ecological integrity (Stalin et al., 2019). Due to its ease of handling and year-round availability, the Indian freshwater teleost *Channa punctatus* (Bloch) (Anabantiformes) is regarded as an appropriate model for xenobiotics-related risk assessment. Given that the species is widely distributed in shallow waterways, close to agriculture fields, it is most likely to come into touch with agrochemicals (Bhattacharjee et al., 2020). Due to their separate roles as active absorption sites and sites of xenobiotic metabolism, fish kidney, gills, and liver are thought to be suitable for bio-monitoring of toxic stress at the cellular level.

2. MATERIALS AND METHODS

2.1 Fish maintenance

Fish *Channa punctatus* was purchased from the local market of Meerut. They were kept in continuously aerated dechlorinated tap water in a static system. First, the fish were acclimatized for two weeks. During acclimatization, the fish were fed once a day with commercial fish pellets. After the acclimation period, the fish were allocated to aquariums with a capacity of 25L and divided into four groups.

2.2 Chemical

A commercial-grade pesticide, CPF, manufactured by Indian Farmers Fertilizer Co-operative Limited, Allahabad, were bought from an agrochemical retailer of Meerut.

2.3 Experimental setup

The fish were acclimatized for two weeks prior to the treatment. The LC50 value was calculated. The fish were distributed into a control group (no chlorpyrifos treatment) and three treatment groups.

Based on the LC50 value, the fish were treated with three sub lethal concentrations of the pesticide, i.e., a high dose of 0.6 ppm, a mild dose of 0.3 ppm, and a low dose of 0.16 ppm for 7, 14, 21, and 28 days respectively. After regular exposure to each dose, fish were sacrificed, and tissues from the fish were collected and analyzed. Tissues extracted were gills, kidneys, and liver. The results of the treatment group were compared with the results of control group of fish.

2.4 Biomarkers

To study the activity of acetylcholinesterase, the substrate Acetylcholine was used, and reactions were monitored at 420 nm using a spectrophotometer. 0.1 M potassium phosphate buffer (pH 8.0), 0.34 mM DTNB, 0.5 mM substrate and 100 L tissue homogenate made up the reaction mixture (3 mL). The control tests replaced the substrates with 20 µl of demineralized water. All tests were performed in duplicate. The quantity of enzyme catalyzing the hydroxylation of 1 µmol of substrate per minute (U = µmol min^{-1}) was defined as one unit of enzyme activity.

The measurements of MDA levels assessed LPO. Trichloroacetic acid, thiobarbituric acid, and hydrochloric acid make up the TCA-TBA-HCl reagent.

Samples were mixed with TCA-TBA-HCl. In a boiling water bath, the solution was heated for 15 minutes. The flocculent precipitate was removed after cooling by centrifugation. The sample's absorbance was measured at 535 nm against a blank that included all the reagents except the sample.

$$Malondialdehyde\left(\frac{\mu mol}{l}\right) = \frac{(Absorbance\ of\ sample)}{\varepsilon \times L} \times D$$

GSH was determined following Ellman, 1959 method. Oxidised glutathione was determined following Ohmori et al., 1981. GPx activity was calculated following the method of Paglia and Valentine, 1967 and Glutathione S transferase activity was determined following Habig *et al.*,1974.

2.5 Statistical Analyses

Statistical analyses were performed using a one-way analysis of variance (ANOVA) to compare means. Data are presented as mean ± S.D., and the differences were considered to be significant at a probability level of $p<0.05$ between treatments and controls.

3. RESULTS

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	7.376±0.077	2.903±0.299[a]	3.135±0.023[a]	3.232±0.033[a]
14	7.069±0.088	2.471±0.247[ab]	2.927±0.049[a]	3.132±0.033[a]
21	7.031±0.046	2.422±0.509[ab]	2.611±0.035[ab]	2.931±0.052[a]
28	7.384±0.054	1.515±0.146[b]	1.603±0.052[b]	1.914±0.046[b]

Table 1. AChE activity (U/min/mg of protein) in kidneys of *C. punctata* upon exposure to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	12.052±0.085	3.522±0.157[a]	3.871±0.295[a]	4.716±0.169[a]
14	12.112±0.035	3.372±0.192[a]	3.670±0.319[a]	3.944±0.545[ab]
21	11.581±0.100	1.729±0.095[b]	1.741±0.187[b]	2.274±0.264[b]
28	11.219±0.103	1.466±0.191[b]	1.541±0.338[b]	1.844±0.196[b]

Table 2. AChE activity (U/min/mg of protein) in liver of *C. punctata*upon exposure to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at p< 0.05 are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	6.271±0.050	5.024±0.253^{a}	5.797±0.122^{a}	5.979±0.078^{a}
14	6.101±0.053	4.865±0.113^{a}	5.582±0.068^{a}	5.915±0.051^{a}
21	6.241±0.063	4.034±0.128^{ab}	4.422±0.151^{b}	4.798±0.118^{ab}
28	6.222±0.158	3.954±0.104^{b}	4.150±0.134^{b}	4.372±0.052^{ab}

Table 3. AChE activity (U/min/mg of protein) in gills of *C. punctata* upon exposure to chlorpyrifos. Data is represented as mean ±S.D. and n=7. The significant differences within the same group at p< 0.05 are denoted by different letters.

The results show that chlorpyrifos exposure caused a significant decrease in AChE activity in all three tissues of *C. punctata* fish in a dose-dependent and time-dependent manner. In the kidney tissue, the AChE activity decreased significantly after 7 days of exposure to high, mild, and low doses of chlorpyrifos compared to the control group, and this effect persisted until the end of the experiment as shown in table 1. In the liver tissue, the AChE activity also decreased significantly after 7 days of exposure to high, mild, and low doses of chlorpyrifos compared to the control group. The decrease in AChE activity continued to decrease until the end of the experiment as shown in table 2. In the gills tissue, the AChE activity decreased significantly after 7 days of exposure to high, mild, and low doses of chlorpyrifos compared to the control group. However, the effect was more pronounced in the high-dose group, and it continued to decrease until the end of the experiment as shown in table 3.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	1.474±0.011	1.628±0.068^{ab}	1.186±0.059^{b}	1.173±0.040^{b}
14	1.314±0.073	2.269±0.038^{a}	1.660±0.033^{ab}	2.327±0.029^{ab}
21	1.609±0.073	2.205±0.073^{a}	1.776±0.091^{a}	2.737±0.048^{a}
28	1.558±0.019	2.551±0.059^{a}	2.038±0.088^{a}	2.776±0.038^{a}

Table 4. MDA concentration (µM/L) in the kidneys of *C. punctata* upon exposure to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at p< 0.05 are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	1.423±0.051	1.750±0.019^{b}	2.397±0.040^{a}	1.423±0.077^{a}
14	1.423±0.019	2.365±0.058^{ab}	2.455±0.029^{a}	2.186±0.073^{ab}
21	1.615±0.088	2.641±0.011^{ab}	2.577±0.077^{a}	2.442±0.038^{a}
28	1.641±0.044	3.410±0.056^{a}	2.564±0.062^{a}	2.494±0.155^{a}

Table 5. MDA concentration (µM/L) in the liver of *C. punctata* upon exposure to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at p< 0.05 are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	1.365±0.019	1.417±0.078^{b}	1.218±0.095^{b}	1.212±0.058^{b}
14	1.571±0.029	1.821±0.059^{ab}	1.647±0.048^{ab}	1.417±0.073^{ab}

21	1.622±0.040	2.404±0.069[a]	2.410±0.091[a]	2.013±0.059[a]
28	1.686±0.029	1.891±0.116[ab]	1.853±0.073[ab]	1.577±0.051[ab]

Table 6. MDA concentration (µM/L) in the gills of *C. punctata* upon exposure to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

In the kidney tissue, the high dose of chlorpyrifos resulted in significantly higher MDA concentration compared to the mild and low doses and the control group at all-time points. The MDA concentration increased gradually over time in all groups as shown in table 4.In the liver tissue, the high dose of chlorpyrifos resulted in significantly higher MDA concentration compared to the mild and low doses and the control group at all-time points. The MDA concentration increased over time in all groups, with the highest concentration observed in the high dose group at the 28th day as shown in table 5.In the gills tissue, the high dose of chlorpyrifos resulted in significantly higher MDA concentration compared to the mild and low doses and the control group at all-time points except for the 21st day, where the mild and high doses showed similar MDA concentration. The MDA concentration increased over time in all groups, with the highest concentration observed in the mild dose group at the 21st day as shown in table 6.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	4.025±0.070	11.182±0.039[b]	8.444±0.291[b]	5.608±0.829[b]
14	5.211±0.048	10.826±0.073[b]	8.185±0.127[b]	7.168±0.209[ab]
21	5.557±0.029	11.020±0.036[b]	8.003±0.047[b]	7.309±0.133[ab]
28	6.790±0.071	19.041±0.422[a]	12.901±0.121[a]	11.829±0.062[a]

Table 7. Reduced glutathione (GSH) content in nM/ml/mg of protein in kidneys of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	3.897±0.057	7.286±0.020[b]	6.749±0.132[ab]	4.734±0.089[b]
14	4.038±0.031	6.409±0.063[b]	5.739±0.250[b]	4.831±0.095[b]
21	4.000±0.066	7.939±0.108[b]	7.544±0.273[ab]	5.579±0.136[ab]
28	3.999±0.091	17.921±0.221[a]	9.258±0.101[a]	7.092±0.239[a]

Table 8. Reduced glutathione (GSH) content in nM/ml/mg of protein in liver of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	1.889±0.018	1.571±0.068[b]	1.044±0.131[b]	0.805±0.101[b]
14	1.904±0.010	2.203±0.073[ab]	1.104±0.138[b]	0.997±0.176[b]
21	2.291±0.017	2.773±0.077[ab]	1.952±0.224[ab]	1.405±0.214[ab]
28	2.772±0.017	3.272±0.000[a]	2.737±0.161[a]	2.710±0.353[a]

Table 9.Reduced glutathione (GSH) content in nM/ml/mg of protein in gills of *C. punctata* exposed to chlorpyrifos.Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

The GSH content in the kidney of the fish exposed to chlorpyrifos for 7, 14, 21, and 28 days showed a significant increase in the high dose group as compared to the control group. However, the mild and low dose groups showed a significant increase at 28 days as shown in table 7. The GSH content in the liver of the fish exposed to chlorpyrifos for 7, 14, 21, and 28 days showed a significant increase in the high and mild dose groups as compared to the control group. The low dose group also showed a significant increase at 21 and 28 days as shown in table 8. The GSH content in the gills of the fish exposed to chlorpyrifos for 7, 14, 21, and 28 days showed a increase in the high and mild dose groups as compared to the control group. The low dose group also showed a significant decrease at 7 days as shown in table 9 and the GSH content in gills were not affected much. Overall, the GSH content in the tissues of the fish was significantly affected by chlorpyrifos exposure in a dose- and time-dependent manner. The results suggest that chlorpyrifos exposure affects the GSH content in the kidney, liver, and gills of *C. punctata* in a dose- and time-dependent manner.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	1.489±0.349	$6.114±0.316^{d}$	$5.414±0.146^{b}$	$3.734±0.081^{b}$
14	1.504±0.161	$14.011±0.464^{c}$	$10.262±0.150^{ab}$	$7.714±0.144^{ab}$
21	2.205±0.212	$15.221±0.380^{b}$	$13.686±0.115^{ab}$	$9.812±0.200^{ab}$
28	1.127±0.071	$16.367±0.388^{a}$	$14.390±0.289^{a}$	$10.558±0.197^{a}$

Table 10. Oxidised glutathione (GSSG) content in nM/ml/mg of protein in kidneys of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	2.204±0.021	$7.858±0.067^{c}$	$4.975±0.029^{b}$	$3.336±0.093^{b}$
14	3.983±0.015	$12.871±0.134^{b}$	$8.319±0.108^{ab}$	$6.818±0.105^{ab}$
21	4.066±0.053	$19.003±0.134^{b}$	$9.387±0.140^{ab}$	$7.704±0.010^{ab}$
28	5.386±0.109	$20.511±0.403^{a}$	$12.319±0.117^{a}$	$11.271±0.270^{a}$

Table 11. Oxidised glutathione (GSSG) content in nM/ml/mg of protein in liver of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	0.510±0.018	$9.421±0.157^{b}$	$6.886±0.356^{c}$	$5.310±0.059^{b}$
14	1.110±0.078	$11.665±0.062^{ab}$	$9.339±0.284^{b}$	$8.015±0.496^{ab}$
21	1.213±0.020	$15.386±0.062^{a}$	$12.058±0.447^{ab}$	$9.983±0.186^{ab}$
28	1.489±0.127	$19.694±0.215^{a}$	$15.400±0.189^{a}$	$13.088±0.189^{a}$

Table 12. Oxidised glutathione (GSSG) content in nM/ml/mg of protein in gills of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

The results show the levels of oxidised glutathione (GSSG) in kidney, liver and gills tissues of *C. punctata* after exposure to different doses of chlorpyrifos for varying time periods. In general, the results indicate that exposure to chlorpyrifos caused a dose- and time-dependent increase in GSSG

levels in all tissues. This suggests that chlorpyrifos exposure causes oxidative stress in the tissues of *C. punctata*. Looking at the kidney results, it can be seen that the GSSG levels were significantly higher in the high dose group compared to the other groups at all time points, indicating a strong effect of the highest dose of chlorpyrifos on the kidney tissue. The mild and low dose groups also showed significantly higher GSSG levels compared to the control group at all time points, indicating that even lower doses of chlorpyrifos are capable of causing oxidative stress in the kidneys (table 10). The liver results show a similar pattern, with the high dose group having significantly higher GSSG levels compared to the other groups at all time points, indicating a strong effect of the highest dose of chlorpyrifos on the liver tissue. The mild and low dose groups also showed significantly higher GSSG levels compared to the control group at all time points, indicating that even lower doses of chlorpyrifos are capable of causing oxidative stress in the liver (table 11). The gills results also show a similar pattern, with the high dose group having significantly higher GSSG levels compared to the other groups at all time points. However, the mild and low dose groups only showed significantly higher GSSG levels compared to the control group at later time points (21 and 28 days), indicating a delayed effect of chlorpyrifos on the gill tissue (table 12).

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	0.527±0.36	$20.944±0.955^{b}$	$11.130±0.838^{c}$	$5.350±0.000^{c}$
14	2.851±0.125	$22.452±0.904^{ab}$	$11.161±0.000^{c}$	$6.105±0.661^{c}$
21	3.202±0.075	$24.222±0.893^{ab}$	$12.449±0.674^{b}$	$8.061±0.537^{b}$
28	5.485±0.186	$35.903±0.000^{a}$	$21.993±0.828^{a}$	$13.228±0.881^{a}$

Table 13. Glutathione peroxidase (GPx) activity in mM/mg of protein in kidney of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	8.945±0.151	$117.049±2.753^{b}$	$120.135±0.942^{d}$	$51.157±2.986^{c}$
14	9.218±0.081	$141.612±0.778^{b}$	$164.491±2.217^{c}$	$136.166±1.348^{b}$
21	9.098±0.172	$87.796±0.252^{c}$	$213.845±2.205^{b}$	$149.229±0.000^{a}$
28	9.051±0.239	$260.317±2.478^{a}$	$290.179±4.296^{a}$	$277.778±2.834^{a}$

Table 14. Glutathione peroxidase (GPx) activity in mM/mg of protein in liver of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	5.154±0.214	$30.972±1.676^{b}$	$23.595±1.858^{b}$	$6.614±1.432^{c}$
14	6.016±0.324	$35.505±1.809^{ab}$	$29.478±1.964^{ab}$	$12.283±1.636^{b}$
21	15.748±0.911	$39.683±0.000^{ab}$	$34.873±2.083^{ab}$	$16.042±1.462^{ab}$
28	16.521±0.394	$49.923±0.000^{a}$	$46.296±2.291^{a}$	$24.197±1.676^{a}$

Table 15. Glutathione peroxidase (GPx) activity in mM/mg of protein in gills of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

The data represents the Glutathione peroxidase (GPx) activity in kidney, liver, and gill tissues of Channa punctatus fish exposed to chlorpyrifos at different doses and for different durations. The results indicate that GPx activity is significantly altered in response to exposure to chlorpyrifos in all three tissues. In the kidney tissue, GPx activity was significantly higher in all exposure groups compared to the control group, with the highest activity observed in the high dose group at all time points (table 13). In the liver tissue, GPx activity showed a dose and time-dependent response, with the highest activity observed in the high dose group at all time points (table 14). In the gill tissue, GPx activity was significantly higher in all exposure groups compared to the control group, with the highest activity observed in the high dose group at all time points (table 15). Overall, these results suggest that higher doses and longer exposure times may have greater effects on GPx activity in fish tissues.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	0.185±0.036	$0.739±0.002^{d}$	$0.647±0.003^{b}$	$0.513±0.005^{a}$
14	0.224±0.058	$1.027±0.020^{c}$	$0.648±0.005^{b}$	$0.430±0.003^{a}$
21	0.299±0.050	$1.384±0.008^{b}$	$0.684±0.002^{b}$	$0.467±0.005^{b}$
28	0.360±0.096	$1.770±0.010^{a}$	$0.940±0.006^{a}$	$0.499±0.004^{b}$

Table 16. Glutathione S transferase (GST) activity in U/mg of protein in kidneys of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	0.612±0.006	$0.942±0.015^{c}$	$0.786±0.009^{d}$	$0.690±0.002^{b}$
14	0.605±0.081	$1.434±0.000^{b}$	$1.050±0.023^{c}$	$0.811±0.004^{ab}$
21	0.603±0.005	$2.015±0.021^{ab}$	$1.439±0.011^{b}$	$1.125±0.012^{a}$
28	0.637±0.001	$2.361±0.025^{a}$	$2.143±0.003^{a}$	$1.063±0.009^{a}$

Table 17. Glutathione S transferase (GST) activity in U/mg of protein in liver of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

Days	Control	High Dose (0.6 ppm)	Mild Dose (0.3 ppm)	Low Dose (0.16 ppm)
7	0.454±0.007	$1.773±0.004^{b}$	$1.434±0.008^{d}$	$1.067±0.009^{c}$
14	0.453±0.014	$2.054±0.041^{b}$	$1.778±0.014^{c}$	$1.329±0.007^{b}$
21	0.591±0.016	$2.960±0.018^{ab}$	$2.116±0.005^{b}$	$1.391±0.015^{b}$
28	0.594±0.024	$3.596±0.021^{a}$	$3.065±0.016^{a}$	$1.836±0.018^{a}$

Table 18. Glutathione S transferase (GST) activity in U/mg of protein in gills of *C. punctata* exposed to chlorpyrifos. Data is represented as mean ± S.D. and n=7. The significant differences within the same group at $p< 0.05$ are denoted by different letters.

The data presented shows the GST activity in the kidney, liver, and gills of fish *Channa punctatus* exposed to different concentrations of chlorpyrifos for different time periods. In the kidney tissue, the GST activity was significantly increased in all treatment groups compared to the control group at all-time points. The highest GST activity was observed in the high dose group at all-time points. The

mild dose and low dose also showed increased GST activity compared to the control, but the difference was not as pronounced as in the high dose group (table 16). In the liver tissue, the GST activity was also significantly increased in all treatment groups compared to the control group at all-time points. The highest GST activity was observed in the high dose group at all-time points, followed by the mild dose and low dose (table 17). In the gill tissue, the GST activity was significantly increased in all treatment groups compared to the control group at all-time points. The highest GST activity was observed in the high dose group at all-time points, followed by the mild dose and low dose (table 18). Overall, the results suggest that exposure to chlorpyrifos leads to increased GST activity in the kidney, liver, and gills of *Channa punctatus.*

4. Discussion

The present study investigated the effects of chlorpyrifos exposure on the activity of acetylcholinesterase (AChE), lipid peroxidation and oxidative stress in the kidney, liver, and gills tissues of *C. punctata* fish. The results showed a significant decrease in AChE activity in all three tissues of fish, which was dose-dependent and time-dependent. This result is in line with other research that showed comparable effects of pesticide exposure on AChE activity in fish tissues (Xing et al., 2010; Topal et al., 2017; Singh et al., 2018). The breakdown of the neurotransmitter acetylcholine is prevented by the binding of organophosphates (OPs), which are substrate analogs of acetylcholine (ACh). A continuous signal resulting from the buildup of acetylcholine results in a variety of altered behavioral patterns and nerve functions (Bhattacharya, 1993; Kirby et al., 2000). Acetylcholine's cleavage into choline and acetate by the crucial enzyme AChE makes it a potential target for poisonous substances (Schmidel et al., 2014). AChE inhibition might be brought on by decreased AChE expression (Xing et al., 2013) and alters the neurological system in a harmful manner (Da Cuna et al., 2011). AChE activity is largely inhibited by organophosphates (Cavanagh and Barnes, 2008). The observed decrease in AChE activity in fish tissues raises the possibility that exposure to chlorpyrifos may harm fish's cholinergic system, which would have a negative impact on their survival and general health. When AChE activity is inhibited, cholinergic nerves are overstimulated, which can cause behavioral abnormalities like tremors, seizures, and irregular or slow swimming (Beauvais et al., 2001; Fernández-Vega et al., 2002). Organophosphate and carbamate exposure is typically linked to the inhibition of AChE activity; however, research suggest that other pesticides and metals may also block this enzyme (Barillet et al., 2007; Pretto et al. al., 2011). According to Barhoumi et al. (2014) and Vieira et al. (2016), several field investigations in the most affected area found a similar outcome. It was discovered that fenitrothion exposure caused AChE inhibition in the brain and muscular tissues of *Dicentrarchus labrax* (Almeida et al., 2010). Following 15 days of exposure to chlorpyrifos (CPF) and monocrotophos, AChE activity gradually decreased in the brain and muscle tissues, indicating that the exposure time may affect the enzyme's activity (Narra et al., 2017).

Another crucial biological process that was examined in the current study is lipid peroxidation. Cell membranes are harmed by the process of lipid peroxidation, which also produces reactive oxygen species (ROS), which can result in oxidative stress and a number of clinical disorders (Halliwell and Gutteridge, 2015). Malondialdehyde (MDA), a measure of lipid peroxidation, significantly increased in concentration in all three tissues of fish exposed to chlorpyrifos, and this rise was dose- and time-dependent. The present study's findings demonstrated that exposure to chlorpyrifos increased lipid peroxidation with the intensity and length of exposure influencing the degree of harm. These results are in line with other research on fish, including *Ctenopharyngodon idellus*, that found similar effects of chlorpyrifos on lipid peroxidation (Kaur and Jindal, 2017) and *Oncorhynchus mykiss* exposed to propiconazole (Li et al., 2011), *Danio rerio* exposed to atrazine (Blahova et al., 2013), *Carassius*

auratus exposed to sencor (Husak et al., 2015). In a study by Topal et al. (2017), fish exposed to imidacloprid doses had MDA levels in their brain tissues that were noticeably greater than those of the control group. The rise in MDA concentration that was detected shows that fish tissues may experience oxidative stress as a result of exposure to chlorpyrifos, which may result in a number of pathological diseases. Oxidative stress is a condition when there is an imbalance between the creation of ROS and the antioxidant defense system's capacity to neutralize those ROS (Sies, 1993). Han et al. (2016) observed that *Danio rerio* LPO increased after four weeks of exposure to the azoxystrobin. Chlorpyrifos exposure over an extended period of time may cause fish to experience chronic oxidative stress, which may cause DNA damage and lipid peroxidation.

It has been reported that chlorpyrifos exposure can cause oxidative stress in fish, leading to alterations in antioxidant defenses such as GSH, GSSG, GPx, and GST. GSH, a significant non-enzymatic antioxidant, is crucial for the detoxification of ROS in the liver. It works closely with GST to both promote the removal of specific chemicals and other reactive molecules from the cells and directly detoxify specific ROS (Sk and Bhattacharya, 2006). Narra et al. (2017) found a gradual increase in GSH levels in hepatic tissue and hypothesized that this would be a cell's adaptive response to combat the rise in oxidative stress. Similar outcomes were seen in *Brycon cephalus* fish that had been subjected to the OP insecticide Folisuper (Monteiro et al., 2009). GSH inhibits apoptosis and does so through a variety of methods, including detoxification, redox-sensitive cell signaling pathways, modification of cellular redox status, and interaction with pro- and anti-apoptotic signals (Masella et al., 2005). According to the current study, the tissues' increased GSH levels may constitute an adaptive response to offset the toxicity brought on by chlorpyrifos. The results of this study show that exposure to chlorpyrifos can cause substantial alterations in the antioxidant defense system of *C. punctatus*, which can result in oxidative stress and tissue damage. According to the findings, exposure to chlorpyrifos causes oxidative stress by increasing the levels of GSSG in all organs in a dose- and time-dependent manner. This result is in line with earlier studies that demonstrated that chlorpyrifos is a powerful inducer of oxidative stress in a variety of organisms (Mansour and Mossa, 2010). The liver data follow a similar pattern, with the high dose group consistently exhibiting higher GSSG levels than the other groups. This result is in line with other studies that indicated the liver was a target organ for chlorpyrifos damage (Mansour and Mossa, 2010). By utilising cellular GSH as a substrate to detoxify H2O2 and lipid peroxides into less reactive species at the membrane level, GPx enzymes inhibit the increasing production of free radicals and offer vital cellular defense against oxidative stress and lipid peroxidation (Sankar et al., 2012). GPx is a crucial antioxidant enzyme that can remove ROS and guard cells against oxidative damage. GPx activity rises in response to oxidative stress. This circumstance might represent a fish's adaption to oxidative circumstances as a result of chemical exposure (Lenartova et al., 1997). This study's increased GPx activity is consistent with prior findings in rainbow trout fish (Topal et al., 2017). According to the enhanced GPx activity in fish tissues exposed to chlorpyrifos, fish may activate their antioxidant defense mechanisms in response to the oxidative stress this organophosphate causes. Additionally, it has been stated that an increase in GPx activity demonstrates that the antioxidant system can be activated as a result of excessive peroxide production (Modesto and Martinez, 2010). Xenobiotics or their metabolites are conjugated with glutathione by the phase II enzyme GST, which reduces their toxicity and increases their excretion efficiency. Use of this biomarker in biomonitoring investigations is widespread (Scarcia et al., 2014: Loro et al., 2015; do Amaral et al., 2020; Marins et al., 2020). In a study, it was discovered that the gills, the liver, and the muscles had increased GST activity (Severo et al., 2023). Fish exposed to atrazine also showed enhanced GST activity in their gills. Here, the researchers concluded that the spikes in antioxidant enzyme activity were an effort to reduce the impairment of lipids and proteins brought on by exposure to herbicides (Silva et al., 2023). The increased activity of

the GST enzyme in liver tissue demonstrates the liver's important function in the detoxification of xenobiotics (Basha and Rani, 2003). Increased GST activity is a certain sign that the enzyme was activated during phase II biotransformation of xenobiotics, either by the detoxification of hydrogen peroxides or by the conjugation of excretion process. Additionally, *O. mossambicus* exposed to monocrotophos (Rao, 2006) and *O. niloticus* treated to diazinon (Uner et al., 2007) showed elevated GST. Embryos of *Danio rerio* exposed to water samples from agricultural areas showed higher GST activity and lipid peroxidation levels when exposed to samples that included more pesticides, according to research by Stringini-Severo et al. (2020).

5. Conclusion

The study concludes by highlighting the detrimental effects of chlorpyrifos on *C. punctata* fish, demonstrating that contact with this pesticide can drastically decrease the activity of acetylcholinesterase and enhance lipid peroxidation in several fish tissues. According to these results, chlorpyrifos poses a serious risk to the wellbeing and survival of aquatic life as well as the aquatic environment as a whole. This study adds to the mounting data that chlorpyrifos exposure leads to oxidative stress in *C. punctata* tissues, affecting the kidney, liver, and gill tissues. According to the results, even modest dosages of chlorpyrifos can result in oxidative stress in these tissues, which could be harmful to the survival and health of *C. punctata* and other species exposed to this pesticide. Overall, the study's findings indicate that chlorpyrifos exposure might cause oxidative stress and tissue damage in fish by upregulating the GSH concentration and antioxidant defense systems like GPx activity. The current work shows that *C. punctatus* kidney, liver, and gill exposure to chlorpyrifos results in dose-dependent activation of GST activity. These results demonstrate the potential of AChE, lipid peroxidation, GSH, GSSG, GPx, and GST activity as a biomarker of environmental contaminant exposure in aquatic animals. The findings of this study demonstrate the need for improved control and management of the use of chlorpyrifos and other organophosphorus insecticides in order to reduce their negative effects on aquatic ecosystems. The study also highlights the need of keeping an eye on the wellbeing and survival of aquatic organisms in regions where chlorpyrifos and other pesticides are applied. In order to reduce the negative impact that pesticides and other dangerous chemicals have on the environment and its people, it is crucial to monitor and regulate their use.

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Conflict of Interest

The authors declare that they have no competing interests.

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