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ORIGINAL ARTICLE

Chlorpyrifos induced oxidative stress responses and alteration of acetylcholinesterase activity in the olfactory organ of freshwater minor carp, *Labeo bata*

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Abstract
The sub-lethal toxicity of Chlorpyrifos on selected oxidative stress biomarkers (malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and reduced glutathione (GSH)) and acetylcholinesterase (AChE) activity was examined in the olfactory organ of *Labeo bata* under sub-lethal exposures. Chlorpyrifos (CPF) was administered at predetermined exposure concentrations (21.93 and 36.54µg/L) and monitored on days 15 and 30 of the experimental periods. The experimental groups showed significantly higher values (P<0.05) of MDA, SOD, CAT, GST and GPx in CPF exposed fish compared to control. AChE and GSH were significantly lower in exposed fish. In general, biomarker depictions indicate that CPF exposures are capable of inducing stress-specific effects at the biochemical and physiological levels negatively impacting the overall health and longevity of such animals.

Keywords: Sub-lethal toxicity, Chlorpyrifos, Oxidative stress biomarkers, *Labeo bata*.

INTRODUCTION

The use of pesticides and herbicides has become an integral part of contemporary agricultural systems. Chlorpyrifos (CPF) is the most extensively used organophosphate insecticide (Sun & Chen 2008). CPF is frequently cited as a contaminant of surface and ground water in numerous countries (Miller et al., 2000; Banks et al. 2005; Du Preez et al. 2005; Murphy et al. 2006). Agriculture discharge and irrigation waters are the primary sources of these contaminants in aquatic ecosystems, where they degrade water quality. The widespread use of pesticides in agriculture has increased pesticide contamination in the environment. Changes in the chemical composition of freshwater's natural aquatic ecosystems can have a significant impact on fish. The toxic effects of a number of these substances or their metabolites are associated with oxidative stress (Winston & Di Giulio 1991). The role of reactive oxygen species (ROS) in pesticide toxicity is demonstrated by the fact that reactive oxygen species (ROS) generation from pesticides may result in oxidative stress (Banerjee et al. 1999; Das & Mukherjee, 2000; Elia et al. 2002; Sayeed et al. 2003; Saha et al. 2022b). Pesticide-induced oxidative stress has been the focus of toxicological research over the past decade (Akhgari et al. 2003; Cicchetti & Argentin 2003; Abdollahi et al. 2004).

CPF can result in oxidative stress, which can alter antioxidant or free oxygen radical-scavenging enzyme systems and generate free radicals (Almeida et al. 2005). Lipid peroxidation (LPO) has been postulated as one of the principal molecular mechanisms underlying pesticide-induced toxicity (Kehrer 1993). Antioxidant enzymes endeavour to alleviate the stress condition under these conditions. Some of the enzymes whose interactions with oxyradicals have been studied in fish include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and reduced glutathione (GSH). Avci et al. (2005) theorised that fish antioxidants could serve as useful biomarkers of exposure to aquatic pollutants. In addition to morphology, CPF influences the biochemical compositions of various tissues (Levin et al. 2011; Sledge et al. 2011; Stalin & Das 2012). When exposed to CPF, the activity of acetylcholinesterase (AChE) alters. It decreases AChE activity to an extent that has

a physiological effect (Jarvinen et al., 1983; Silva et al., 2013; Saha et al., 2018a; Saha et al., 2018b; Azadikhah et al., 2023). Pathiratne et al. (2008) and Yen et al. (2011) documented the well-known effect of CPF on fish brain AChE. The objective of the current study is to evaluate the AchE activity and oxidative stress responses in the olfactory system of the fish *L. bata* following sub-lethal exposure to the organophosphate pesticide chlorpyrifos.

MATERIAL AND METHODS

Experimental fish: *Labeo bata* (Hamilton 1822) (Cypriniformes, Cyprinidae) (length 15–18cm and weight 30–40g) were procured from a local fish farm at Santiniketan, West Bengal, India (Lat. 23°39′N, Long. 87°42′E). The fish were acclimatized to the laboratory conditions for 15 days during which the fish were fed with adequate amount of pellet feed.

Test chemical: Chlorpyrifos 20% EC (0, 0-diethyl-0-3, 5, 6-trichloro-2-pyridyl phosphorothioate) an organophosphate compound was procured in the trade name Tricel 20 % EC from Excel Crop Care Limited, Mumbai, India and based on the result of acute toxicity test two sublethal concentrations, 21.93µgL-1 (1/5th of 96h LC_{50}) and 36.54µgL-1(1/3rd of 96h LC_{50}) were used for this experiment (Samajdar & Mandal 2015).

Experimental design: Randomly selected acclimated fish were introduced to two sub-lethal chlorpyrifos concentrations (21.93µgL-1 and 36.54µgL-1) for periods of 15 days and 30 days, respectively, in the experimental aquariums (40L capacity) (Samajdar & Mandal 2015). For each set of the experiment, one control aquarium was kept with the same experimental conditions aside from chlorpyrifos contamination. Each experiment was carried out in triplicate (Saha et al. 2023). Fish were sacrificed at the conclusion of the experiment, and olfactory tissues from both control and treated fish were collected and processed for evaluation of acetylcholinesterase activity, oxidative stress, and antioxidant responses (Mukherjee et al. 2022).

Estimation of Acetylcholinesterase (AChE) activity: Olfactory tissues from treated fish and their respective control were procured separately and tissue of 10 fish of each group pooled together and homogenized (REMI homogenizer, model RQT-127A) taking 1:10 w/v tissue and 1 mM EDTA and 0.5% Triton X-100 containing 0.1 M phosphate buffer (pH 7.2). The homogenates were centrifuged at 12,000 g for 20 mins at 4°C by a centrifuge machine (Sigma 1-15K) and supernatant was taken. The colorimetric method was used right away to measure the AChE activity in the supernatant (Ellman et al. 1961).

Estimation of lipid peroxidation: The thiobarbituric acid reactive substances assay, which uses the Draper and Hadley method, was used to measure the level of malondialdehyde (MDA) in the tissue homogenates after centrifuging them at 8000 g for 15 minutes (1990). TCA (7.5g) was weighed and dissolved in concentrated HCl (1.14ml); TBA (0.188g) was then added to this solution, and the volume was then increased to 50 ml with distilled water. Then, 500 l of tissue supernatant and 1 ml of this buffer were added, and they were boiled for 10 minutes in a pot of boiling water. The solution was centrifuged at 1000 g for 10 minutes at 4 °C after cooling, and the precipitate was thrown away. At 532 nm, the absorbance of the supernatant was measured in a quartz cuvette in comparison to a blank. Using an extinction coefficient of 1.56 105 M-1 cm-1, the MDA equivalents of the sample were calculated and expressed in terms of nM MDA mg $protein^{-1}$.

Sample preparation for the estimation of antioxidative agents: The olfactory tissue of 10 fish from each group of treated and control fish was collected, pooled, and homogenised. The homogenates underwent a 20-minute, 13,000-g centrifugation at 4°C. The supernatants were removed after removing the cell debris and kept at -80°C until they were used for further analysis of both enzymatic and non-enzymatic antioxidative agents.

Estimation of Superoxide dismutase activity (SOD): Giannopolitis & Ries (1977) measured total SOD activity by its ability to block photochemical reduction of nitroblue tetrazolium chloride (NBT). Shaking and illumination 1590 ml of 50 mM phosphate buffer (pH 7.8), 300 ml of 0.1 mM EDTA, 780 ml of 13 mM L-

methionine, 225 ml of 75 mM NBT, 5 ml of 2 mM riboflavin, and 100 ml tissue homogenate tubes started the process. A non-illuminated and illuminated sample without enzyme extract were made as controls, and the reaction mixture absorbance was measured at 560 nm. One unit of SOD activity (U) was needed to generate 50% NBT photo reduction rate inhabitation, which was represented as (U mg protein^{-1}).

Catalase assay (CAT): Aebi (1984) measured tissue supernatant catalase (CAT) activity using spectrophotometry. A cuvette contained 1960 mL of H_2O_2 Tris buffer (50 mM Tris-Cl, pH 8.0, 9 mM H2O2, 0.25 mM EDTA) and 40 L of supernatant. A UV-VIS spectrophotometer (Beckman Coulter, DU 730, Life Science) measured absorbance at 240 nm for 90 seconds at 15-second intervals. Reaction kinetics showed that the reaction rate peaked for 60 seconds at 2 mM H_2O_2. CAT activity was estimated using a standard curve from pure bovine liver CAT (2000–5000-unit mg protein-1).

Glutathione S-transferase activity (GST): Glutathione S transferase (GST) activity in olfactory tissue supernatant was measured spectrophotometrically using GSH (2.4mM) and 1-chloro-2, 4-dinitrobenzen (CDNB, 1 mM). Each test used 980µL of 0.1M Phosphate Buffer (pH-6.5), 10µL CDNB, and 10 µL GSH in a quartz cuvette. 100µL solution thrown away and 100 µL sample added immediately from this 1 mL combination. At 30°C for 5 minutes, the mixture's absorbance was measured at 340 nm every 60 seconds. Negative control was phosphate buffer saline (pH-6.5). Chen et al. (2013) employed successive dilutions of substrates (GSH and CDNB) for assay validation and to explore the effects of a specific GST inhibitor (coniferyl ferulate, CF). The assay's specificity was established when samples treated with 0.3 mM CF and varied GSH or CDNB concentrations showed the lowest GST activity. (Lowry et al. 1951)with BSA as standard protein was used to quantify sample protein content.

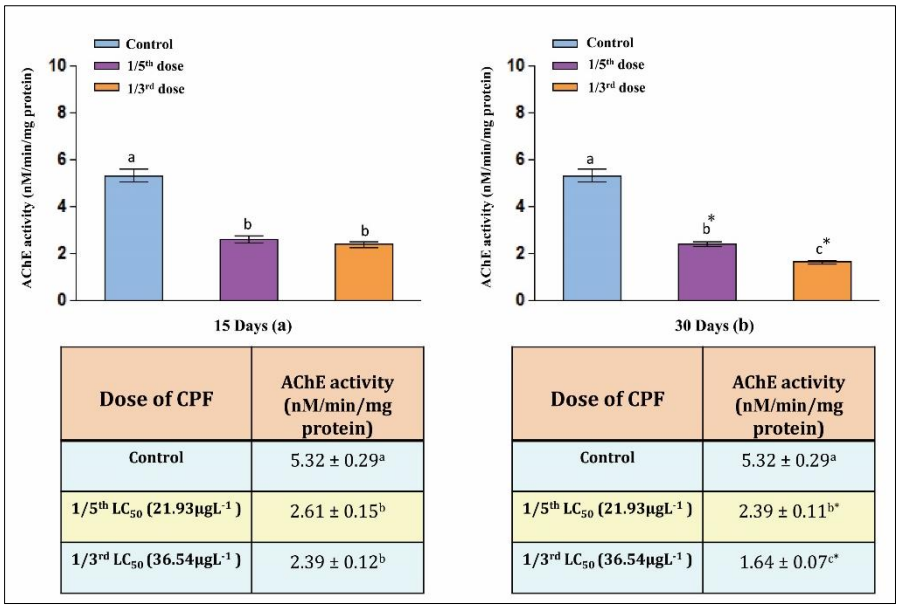
Glutathione peroxidase activity (GPx): Castro et al. (2008) assessed GPx activity spectrophotometrically. GPx substrate ortho-phenyl diamine (OPD) was serially diluted to validate the test. In summary, 100 µL sample was combined with 1 mL OPD in phosphate–citrate buffer (pH 5.0) and 0.9 mL 0.013% H_2O_2 (co-substrate) at room temperature for 30 minutes in dark. 0.4 mg mL-1 OPD in 0.05 M phosphate–citrate buffer, pH 5.0, maximised reaction rate. 492 nm absorbance was measured against blank.

Estimation of reduced Glutathion (GSH): Ellman (1959)'s approach assessed tissue extract GSH, a reliable non-enzymatic antioxidant. Tissue homogenate supernatant was centrifuged at 8000×g for 10 minutes at 4°C with an equal volume of 5% perchloric acid. The reaction mixture (2mL) [100µL supernatant, 1.88mL 0.1mol potassium–phosphate buffer (pH 8.0), and 0.02mL 4% DTNB] was incubated at room temperature for 3 minutes, then absorbance was measured at 412 nm at 60-second intervals for 5 minutes. Extrapolating from the GSH standard graph computed each sample's GSH level.

Protein estimation: The protein was estimated with Folin-Phenol (Folin ciocalteau) reagent following the reaction mixture containing 100µL tissue homogenate, 900µL distilled H_2O, and 5mL Lowry reagent mixed properly and incubated for 10 minutes. Then 500 µL Folin-Phenol [diluted in H_2O (1:2)] was added to the reaction mixture and incubated for 20 mins. The absorbance was measured at 660 nm using a UV-VIS spectrophotometer (Beckman Coulter, DU 730, Life Science) and compared with the standard curve to estimate the sample protein.

IBR Analysis: The authors combined all biomarker responses into a single "stress index" dubbed "Integrated Biomarker Response" (IBR) using a holistic approach (Beliaeff et al. 2022; Saha et al. 2022a). The integrated biomarker response (IBR) was computed by modifying an equation given in (Beliaeff et al. 2022; Samanta et al. 2018).

Statistical analysis: To find the significant difference at the 5% probability level, data were presented as mean standard error of mean and tested using one way analysis of variance (ANOVA) and Duncan's multiple range tests. Microsoft Excel 2019 for Windows was used for all of the analysis.



Dose of CPF	AChE activity (nM/min/mg protein)
Control	5.32 ± 0.29[a]
1/5th LC_{50} (21.93µgL^{-1})	2.61 ± 0.15[b]
1/3rd LC_{50} (36.54µgL^{-1})	2.39 ± 0.12[b]

Dose of CPF	AChE activity (nM/min/mg protein)
Control	5.32 ± 0.29[a]
1/5th LC_{50} (21.93µgL^{-1})	2.39 ± 0.11[b*]
1/3rd LC_{50} (36.54µgL^{-1})	1.64 ± 0.07[c*]

Fig.1. Acetylcholine esterase activity (AChE) in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

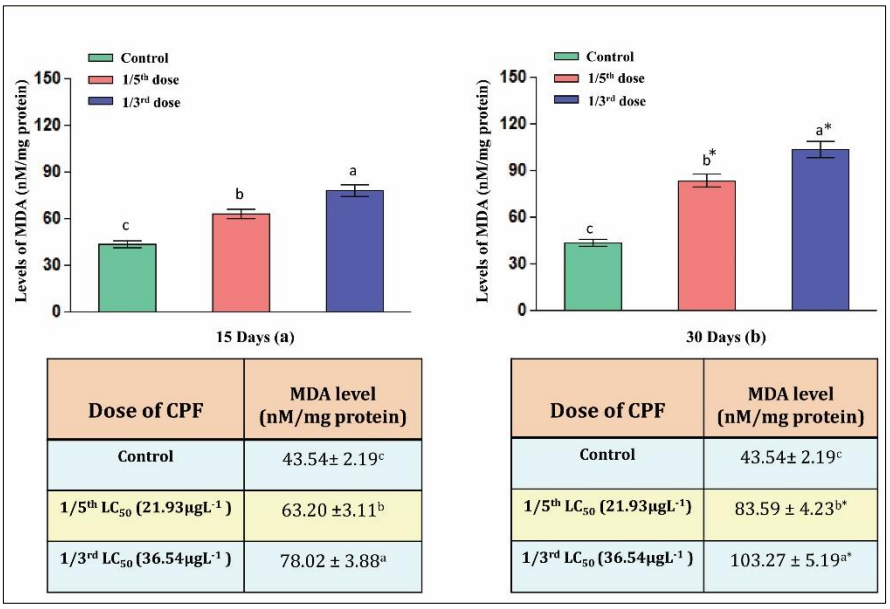
RESULTS AND DISCUSSION

Acetylcholinesterase activity (AChE): Exposure to chlorpyrifos inhibited the activity of acetylcholinesterase in the olfactory organ of the studied fish. Following 15 days of sublethal exposure to CPF, olfactory tissue in both treated groups exhibited a significant inhibition of acetylcholinesterase activity compared to the control group. However, the AChE inhibition in the treated groups was not statistically significant (Fig. 1a). The olfactory tissues of fish exposed to CPF for 30 days demonstrated a significant decrease in AChE with increasing CPF exposure concentration (Fig. 1b).

OP compounds inhibit AChE to accumulate acetylcholine in synapses, which overstimulates postsynaptic cholinergic receptors in the neuromuscular junction, causing muscle fasciculation and paralysis (Mileson et al. 1998). CPF decreases olfactory tissue AChE activity in both treated groups relative to controls. AChE activity decreased dose-dependently in guppy fish Poecilia reticulata (Van der Wel & Welling 1989; Sharbidre et al. 2011a) found that *Poecilia reticulate* AChE activity was 90% inhibited after 6 days of sublethal CPF treatment. Topal et al. (2016) extensively researched the effects of CPF on rainbow trout brains and found similar AChE inhibition. Xing et al. (2012a) reported that chlorpyrifos inhibited AChE in common carp brains. *Oryzias latipes* brain AChE activity decreased significantly with CPF (Khalil et al., 2013). In larval zebrafish brains, chlorpyrifos inhibited AChE.

Methyl parathion suppressed *B. cephalus* brain AChE in (Almeida et al. 1997). Methyl parathion exposure lowered AChE activity in rat tissues in Celik et al. (2009). Xu et al. (2011) found AChE inhibition in neuromuscular junctions and cholinergic synapses of all aquatic creatures exposed to organophosphate pesticides. CPF attacking AChE's active serine hydroxyl group disrupts its structure (Sharbidre et al. 2011b). Since the hydroxyl group cannot be hydrolyzed, the enzyme is inactivated. Thompson et al. (2004) and Da Cuna et al. (2011) showed that CPF causes irreversible AChE inhibition, which gathers acetylcholine and causes neurotoxic changes, including nerve impulse transmission and energy metabolism in the nervous system. CPF exposure inhibited AChE, depriving acetylcholine and overstimulating cholinergic neurons. Both exposure concentrations strongly reduce AChE activity. Reduced AChE activity accumulates acetylcholine in synapses, impairing critical tasks like swimming, eating, and general behaviour (Glusczak et al. 2006).

Lipid peroxidation (MDA): Sub-lethal chlorpyrifos exposure (1/3rd and 1/5th of the 96-hour LC_{50} value)



Dose of CPF	MDA level (nM/mg protein)
Control	43.54± 2.19^{c}
1/5th LC_{50} (21.93µgL^{-1})	63.20 ±3.11^{b}
1/3rd LC_{50} (36.54µgL^{-1})	78.02 ± 3.88^{a}

Dose of CPF	MDA level (nM/mg protein)
Control	43.54± 2.19^{c}
1/5th LC_{50} (21.93µgL^{-1})	83.59 ± 4.23^{b*}
1/3rd LC_{50} (36.54µgL^{-1})	103.27 ± 5.19^{a*}

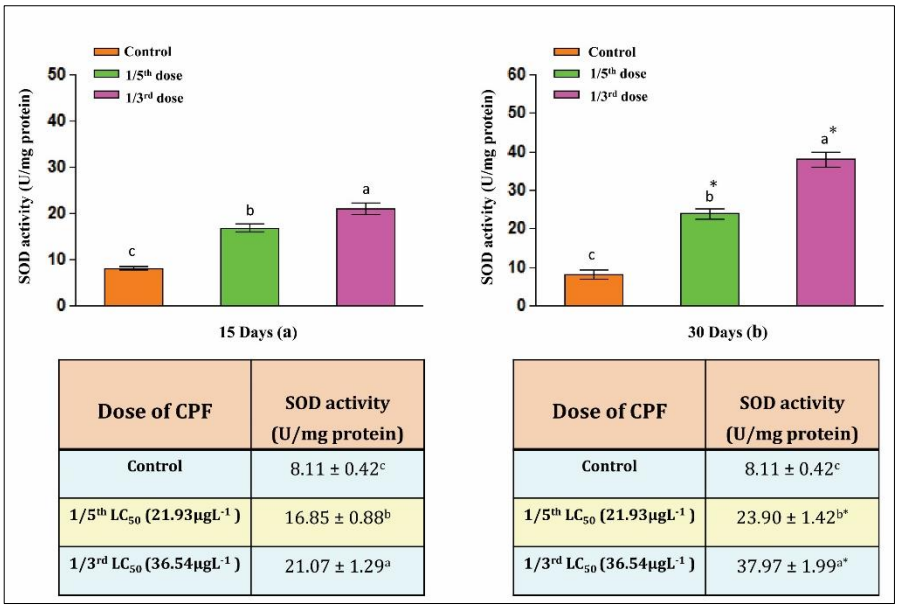
Fig.2. Levels of Malondialdhyde (MDA) in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

for 15 and 30 days resulted in lipid peroxidation in the olfactory organ, as indicated by an increase in malondialdehyde (MDA) levels. Malondialdehyde (MDA) levels increased substantially after 15 days of exposure to CPF in both treated groups compared to the control (Fig. 2a). After 30 days of exposure, the olfactory tissue MDA level was significantly higher than the control (Fig. 2b). Lipid peroxidation in the tissue was also duration dependent, and fish exposed for 30 days showed a significantly greater increase in MDA than the treatment group exposed for 15 days.

Pesticides promote lipid peroxidation in many organs and other biological consequences in experimental animals (Sharma et al. 2005). Pesticides disrupt cellular membranes by lipid peroxidation, which also indicates cellular component oxidative damage. Pesticide exposure increases liver lipid peroxidation (Datta et al. 1994; Sharma et al. 2005). Chlorifos generates reactive oxygen species (ROS) that increase oxidation and lipid peroxidation by oxidising polyunsaturated fatty acids. This study shows that chlorpyrifos (CPF) treatment generates reactive oxygen species and promotes lipid peroxidation in *Labeo bata* olfactory tissue by significantly increasing malodialdehyde (MDA). Lipid peroxidation and MDA levels increased in CPF-exposed common carp brain and kidney tissues (Xing et al. 2012b) and atrazine-exposed zebrafish Danio rerio liver tissues (Jin et al. 2010) CPF enhances hepatic LPO in normal rats (Tuzmen et al. 2008). Chlorpyrifos, a lipophilic drug, may increase LPO by directly interacting with the plasma membrane (Hazarika et al. 2003).

Superoxide dismutase activity (SOD): Superoxide dismutase (SOD) activity was increased in the nasal tissue after it was exposed to chlorpyrifos. After 15 days of exposure to CPF, the SOD activity in the olfactory organ was significantly higher in both treatment groups than in the control group. And the SOD activity was much higher in the fish exposed to the higher concentration than in the control group or the group exposed to the lower concentration (Fig. 3a). After 30 days of treatment, a significant, concentration-dependent rise in SOD was seen (Fig. 3b). The quantity and length of exposure had a big effect on how much SOD was working.

Parvez & Raisuddin (2006) suggest that pro-oxidant and antioxidant defence mechanisms result in oxidative stress imbalance after numerous metabolic pathways. Organophosphate pesticides cause oxidative stress, which generates free radicals and alters antioxidant enzyme levels, which scavenge them (Sharma et al. 2005; Rajeswary et al. 2007). Rai & Sharma (2007) extensively study the antioxidant



Dose of CPF	SOD activity (U/mg protein)
Control	8.11 ± 0.42^{c}
1/5th LC_{50} (21.93µgL^{-1})	16.85 ± 0.88^{b}
1/3rd LC_{50} (36.54µgL^{-1})	21.07 ± 1.29^{a}

Dose of CPF	SOD activity (U/mg protein)
Control	8.11 ± 0.42^{c}
1/5th LC_{50} (21.93µgL^{-1})	23.90 ± 1.42^{b*}
1/3rd LC_{50} (36.54µgL^{-1})	37.97 ± 1.99^{a*}

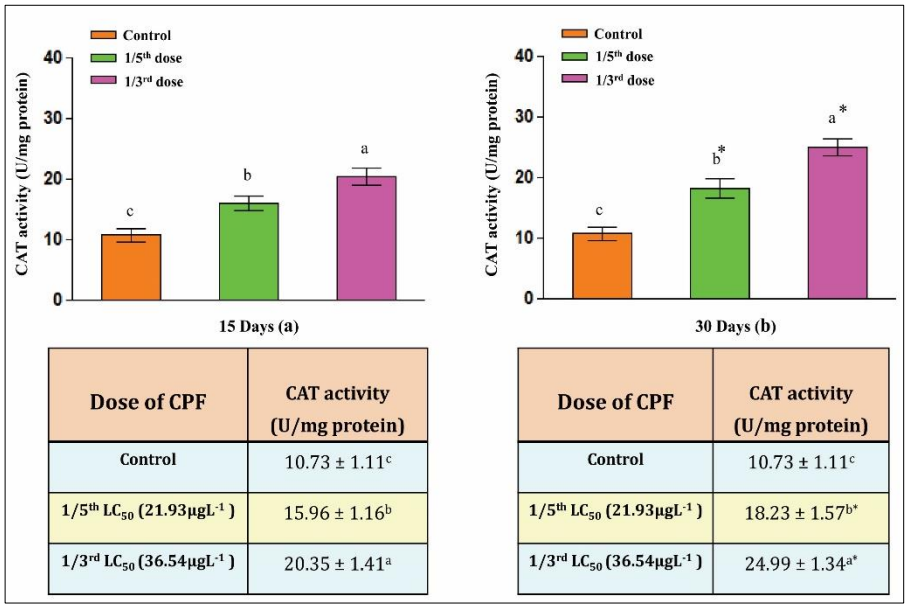
Fig.3. Superoxide dismutase (SOD) activity in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

defense mechanism for environmental monitoring biochemical indicators. Both treated groups had higher olfactory tissue SOD activity than the control group in this study. After CPF exposure, *Poecilia reticulata* guppy tissues showed similar results. Keramati et al. (2010) found identical MP and CPF-induced SOD activity in *O. mykiss* tissues. Hexachlorobenzene exposure also decreased *C. carpio* hepatic SOD activity (Regoli et al. 2003). SOD enzyme activity is fish's first line of defence against oxidative stress; hence it can be employed as a biomarker for pesticide-induced ROS formation (Yamano & Morita 1995). SOD converts O2- to H2O2. Thus, the higher SOD levels in CPF-exposed fish's olfactory tissues may neutralise superoxide radical O2- generated by CPF-induced oxidative stress.

Catalase activity (CAT): Catalase activity (CAT) in the olfactory tissue of both treated groups increased in CPF-exposed fish compared to the control group after 15 days (Fig. 4a). The concentration and duration of exposure to CPF both significantly increased CAT activity after 30 days (Fig. 4b). SOD and CAT are the enzymes that condense ROS produced during bioactivation of xenobiotics in aquatic creature tissues, according to (Doyotte et al. 1997). Dichlorvos treatment to *Ictalurus nebulosus* increases CAT activity dose-dependently (Bebe and Panemangalore, 2003) and in CPF-exposed *Poecilia reticulata* liver tissue (Sharbidre et al. 2011b). found that common carp exposed to furadan induced CAT as an (Varga and Matkovics, 1997)adaptive response. John et al. (2001) suggest that higher super oxide anions(O2–) and H_2O_2 levels may cause SOD and CAT activity to rise. SOD converts O2- to H2O and H2O2, while CAT detoxifies them. SOD–CAT is the initial defence against oxygen toxicity (Pandey et al. 2003) and a biomarker for ROS generation (Van der Oost et al. 2003). A considerable increase in CAT activity in the olfactory tissue of CPF-exposed *Labeo bata* confirms that chlorpyrifos exposure induces ROS in the tissue dependent on concentration and time.

Glutathione *S*-transferase (GST) activity: In the olfactory organ, chlorpyrifos induced Glutathione-S-transferase (GST) activity. GST showed a significant (P<0.05) increase with increasing exposure concentration after 15 days of exposure (Fig. 5). Similarly, the 30-days exposed group demonstrated a statistically significant (P<0.05) increase in GST activity in the olfactory organ of treated fish compared to the control. GST activity was considerably higher in fish exposed to high concentrations compared to those exposed to low concentrations (Fig. 5).

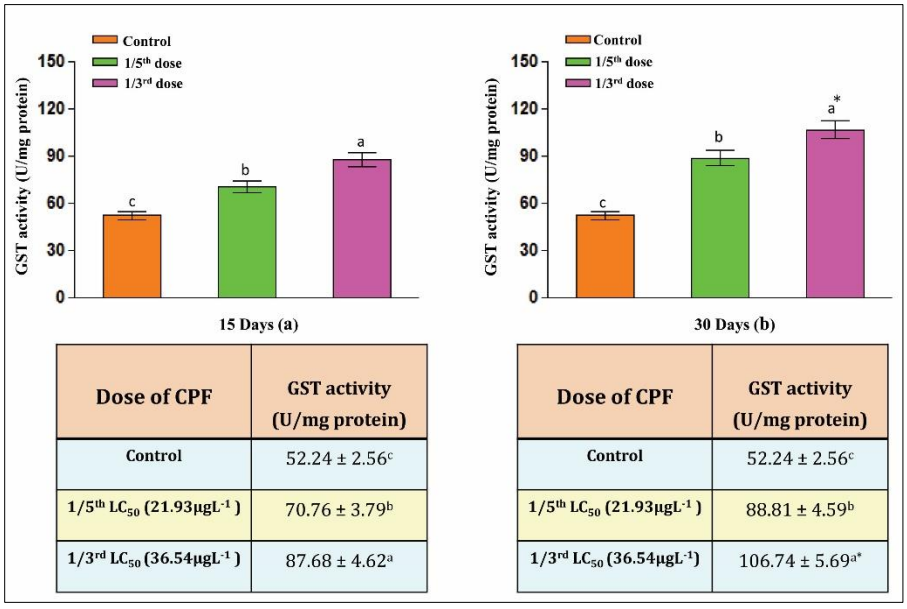
GST detoxifies and excretes xenobiotics and their



Dose of CPF	CAT activity (U/mg protein)
Control	10.73 ± 1.11^{c}
1/5th LC_{50} (21.93µgL^{-1})	15.96 ± 1.16^{b}
1/3rd LC_{50} (36.54µgL^{-1})	20.35 ± 1.41^{a}

Dose of CPF	CAT activity (U/mg protein)
Control	10.73 ± 1.11^{c}
1/5th LC_{50} (21.93µgL^{-1})	18.23 ± 1.57^{b*}
1/3rd LC_{50} (36.54µgL^{-1})	24.99 ± 1.34^{a*}

Fig.4. Catalase (CAT) activity in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.



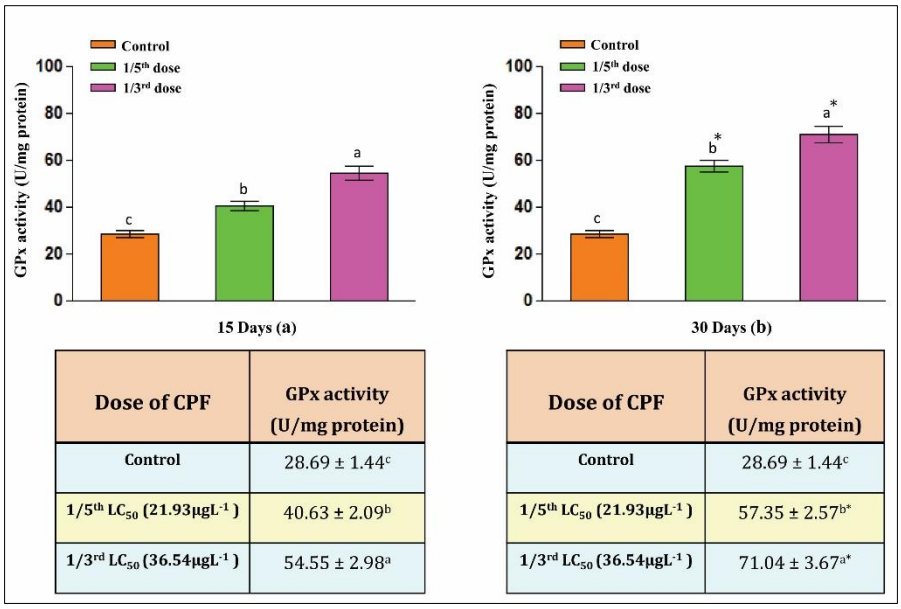
Dose of CPF	GST activity (U/mg protein)
Control	52.24 ± 2.56^{c}
1/5th LC_{50} (21.93µgL^{-1})	70.76 ± 3.79^{b}
1/3rd LC_{50} (36.54µgL^{-1})	87.68 ± 4.62^{a}

Dose of CPF	GST activity (U/mg protein)
Control	52.24 ± 2.56^{c}
1/5th LC_{50} (21.93µgL^{-1})	88.81 ± 4.59^{b}
1/3rd LC_{50} (36.54µgL^{-1})	106.74 ± 5.69^{a*}

Fig.5. Glutathione S-transferase (GST) activity in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

metabolites, including organophosphate insecticides (Jokanović 2001). It is also a biomarker for the environmental impact of organic xenobiotics that cause oxidative stress (Livingstone, 1998). GST protects tissue from oxidative stress (Fournier et al., 1992; Banerjee et al., 1999). *Labeo bata* exposed to CPF had higher GST levels in their olfactory tissue than the control group. GST activity increased compared to control, suggesting chlorpyrifos detoxification by this enzyme. Improved GST activity in tissues may imply the establishment of a defensive mechanism to neutralise the toxic effects of CPF and a more organised pesticide toxicity defence. Methyl parathion exposure altered GST in *Brycon cephalus* tissues (Monteiro et al. 2006). After CPF exposure, GST activity increased in tissues, accelerating detoxification.

Glutathione peroxidase (GPx) activity: After being exposed to chlorpyrifos, glutathione peroxidase (GPx) activity in the smell organ was sped up. Labeo bata



Dose of CPF	GPx activity (U/mg protein)
Control	28.69 ± 1.44^{c}
1/5th LC_{50} (21.93µgL^{-1})	40.63 ± 2.09^{b}
1/3rd LC_{50} (36.54µgL^{-1})	54.55 ± 2.98^{a}

Dose of CPF	GPx activity (U/mg protein)
Control	28.69 ± 1.44^{c}
1/5th LC_{50} (21.93µgL^{-1})	57.35 ± 2.57^{b*}
1/3rd LC_{50} (36.54µgL^{-1})	71.04 ± 3.67^{a*}

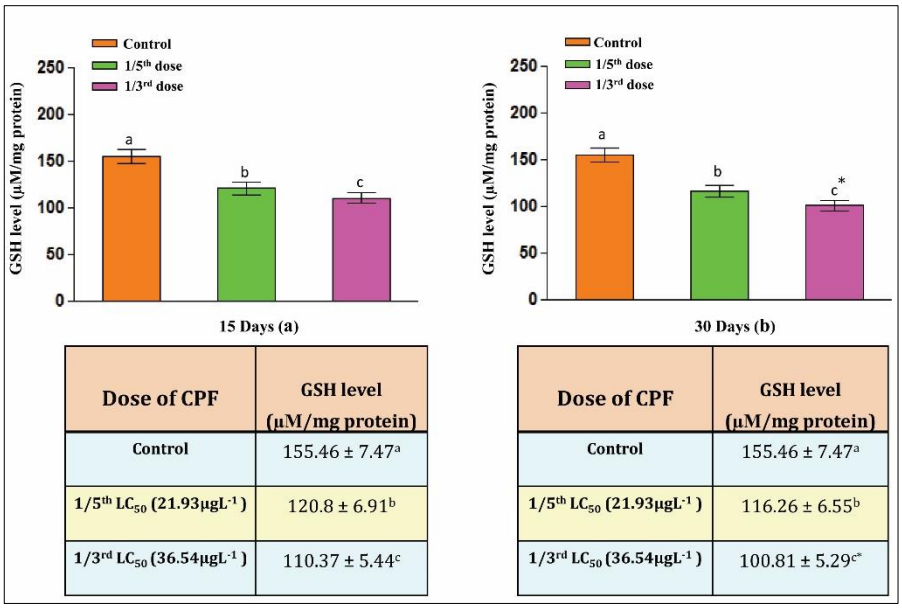
Fig.6. Glutathione Peroxidase (GPx) activity in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

olfactory tissue exposed to CPF for 15 days showed a significant increase in GPx activity in both treated groups compared to the control group (Fig. 6a). After 30 days of being exposed to CPF, the treated groups showed a significant rise in GPx activity (Fig. 6b). When the 30 days treated groups were compared to the control groups and the 15-day exposed groups, the 30 day treated groups showed a significant rise.

Together, superoxide dismutase, GPx, and CAT make up the main enzyme defence against the damage caused by free radicals. GPx is an important enzyme in the glutathione redox cycle. It links reduced glutathione to xenobiotic substrates via a sulfhydryl group in order to detoxify them (Samiec et al. 1998). GPx activity could be caused by an increase in the production of H_2O_2 from O_2. By making H_2O_2, (Hamed et al. 1999) were able to make GPx work when xenobiotics were present and get rid of peroxides. When atrazine and chlorpyrifos were given to the fish *Gambusia affinis*, the amount of GPx went up by a lot (Xing et al. 2012a). So, in this study, the fact that the CPF-exposed fish had a significant increase in GPx activity in their olfactory tissues shows that GPx induction is a defense mechanism that gets rid of CPF and free radicals from the olfactory system.

Level of Reduced Glutathione (GSH): In the case of GSH, the situation was somewhat different; the 15-day exposed group displayed a significant decrease with increasing pesticide concentration (Fig. 7a). After 30 days of exposure to CPF, a significant decrease was observed with increasing CPF concentration (Fig. 7b). The group exposed to a higher concentration showed a significant decrease as exposure duration increased.

Hasspieler et al. (1994) and Sies (1999) found that GSH plays a central role in the detoxification of toxicants and the prevention of cellular oxidative stress. Because GSH is the substrate of the GST enzyme, an increase in its activity was associated with a decrease in GSH levels in tissues. In the present investigation, GSH levels in the olfactory tissues of CPF-exposed *Labeo bata* decreased significantly. Similar outcomes were observed in methyl parathion-exposed *Brycon cephalus* .After 24h of exposure to dichlorvos, an organophosphorus insecticide known to induce oxidative damage, Varga & Matkovics (1997) found decreased levels of GSH in the liver and muscle of carp. Increased utilization of GSH, which can be converted into oxidized glutathione, and inefficient GSH regeneration may contribute to the decline of GSH. The GPx enzymes catalyze the reduction of H_2O_2 and organic peroxides to water and their respective stable alcohols using GSH as a



Dose of CPF	GSH level (µM/mg protein)
Control	155.46 ± 7.47[a]
1/5th LC_{50} (21.93µgL^{-1})	120.8 ± 6.91[b]
1/3rd LC_{50} (36.54µgL^{-1})	110.37 ± 5.44[c]

Dose of CPF	GSH level (µM/mg protein)
Control	155.46 ± 7.47[a]
1/5th LC_{50} (21.93µgL^{-1})	116.26 ± 6.55[b]
1/3rd LC_{50} (36.54µgL^{-1})	100.81 ± 5.29[c*]

Fig.7. Reduced glutathione (GSH) activity in the olfactory organ of control and CPF exposed *L.bata*. (a) after 15 days exposure (b) after 30 days exposure.

reducing agent. GSH is oxidized in this process, which is required for GPx activity.

Integrated biomarker response: The IBR index was used to compare the total stress on *L. bata* over various exposure durations (15 and 30days). In comparison to the T1 and T2 exposure groups, the control exposure group had the lowest values of the IBR index for AchE, MDA, SOD, CAT, GST, GPx and GSH biomarker parameters for *L. bata* (Table 1). For these parameters, the IBR indices are higher in fish exposed to CPF compared to those in the control group, indicating toxicity-related adverse responses. As demonstrated by this index, the order of toxic effects resulting from chlorpyrifos exposure is T2 > T1 > control.

With increasing chlorpyrifos concentrations, the score values for measures including AchE and oxidative stress (MDA, SOD, CAT, GST, GPx, GSH) biomarkers increased in *L. bata*. With toxicant exposure durations (15days and 30 days), a comparable rising trend was observed (Fig. 8a-b). In Figure 8a and b, the transformed data for all of the investigated biomarkers are displayed as star plots. The star plots reveal a hazardous response gradient because they depict a gradual decrease in form reduction or increase in shape size for each experimental group. The T2 CPF exposure group was the most influenced, followed by T1, with the largest sized star plot and highlighted region, while the control group, with the smallest shaped and highlighted area, was the least impacted.

By combining multiple biomarker signals, the IBR method provides a straightforward method for assessing the overall health of a population (Dhara et al. 2021b; Saha et al. 2022c; Sharma et al. 2023). In spite of the extensive variety of biomarker sets utilized in the index computations, additional research utilizing the IBR index revealed comparable toxicological effects. IBR's visual component emphasizes its strength as a species- and toxin-specific technique that enables rapid qualitative assessment across variables and categories. This technique has been used to demonstrate the global effect of a variety of xenobiotic compounds on a number of species, including fish (Dhara et al. 2021a; Saha et al. 2024).

The exposure concentrations used in this investigation represent intermediate environmental concentration possibilities in areas impacted by human activity. The amplitude of effects observed in this study for this species may be attributed to CPF's diverse bioaccumulation (Varo et al. 2002; Jiang et al. 2022), biotransformation (Lakhshmi et al. 2008; Supreeth et al. 2017), and biomagnification (Bhende et al. 2022)

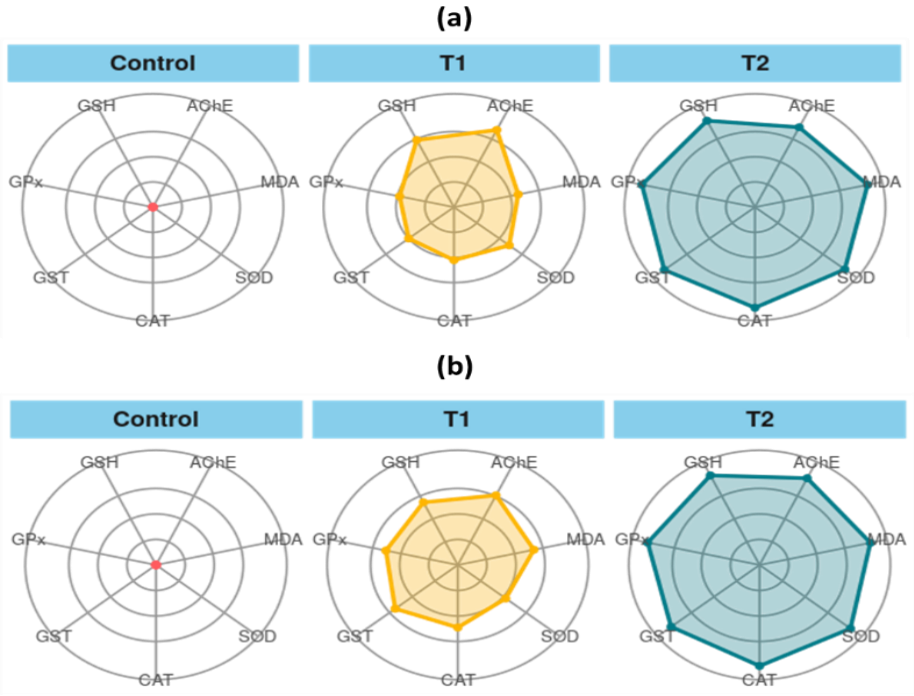


Fig.8. IBR plots for assessing AchE and oxidative stress (MDA, SOD, CAT, GST, GPx, GSH) biomarker responses in *L. bata* exposed to different CPF concentration (T1: 21.93 and T2: 36.54 mg/l) over (a) 15 days and (b) 30days exposure period.

Table 1. IBR mean values for several biomarkers across CPF exposure concentrations.

Exposure duration	Exposure concentration	AChE	MDA	SOD	CAT	GST	GPx	GSH
15day	Control	0	0	0	0	0	0	0
	T1	1.70	1.13	1.22	1.05	0.99	0.96	1.47
	T2	1.76	1.99	1.98	1.99	2.01	1.99	1.90
30day	Control	0	0	0	0	0	0	0
	T1	1.51	1.34	1.05	1.23	1.38	1.27	1.36
	T2	1.88	1.95	2.00	1.98	2.02	1.97	1.95

capabilities. The oxidative stress effects observed in this study confirm the potential hazards of CPF pollution to invertebrates, fish, and mammals (Zhou et al. 2007; Duarte-Restrepo et al. 2020; Sharma et al. 2017; Botté et al. 2012).

CONCLUSION

This study found that sub-lethal CPF exposure to fish olfaction can damage olfactory-mediated functioning. Chlorpyrifos exposure inhibits acetylcholinesterase (AChE), causing neurotoxicity and olfactory signal transduction. Chlorpyrifos exposure causes ROS, oxidative stress, lipid peroxidation, and peripheral olfactory organ tissue damage. SOD, CAT, and GPx are increased in treated groups to scavenge and neutralise ROS and reduce oxidative stress. However, persistent exposure at high CPF concentrations overrides the detoxifying process, causing tissue damage in the fish's olfactory organ. The significant damage reflected by evaluated parameters in CPF exposure groups compared to control portends risks to the health of local fish populations, including *Labeo bata* in aquatic systems adjacent to agrarian landscapes.

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REFERENCES

Abdollahi, M.; Ranjbar, A.; Shadnia, S.; Nikfar, S. & Rezaie, A. 2004. Pesticides and oxidative stress: a review. Medical Science Monitor 10(6): 141-147.

Aebi, H. 1984. Catalase in vitro, Methods in enzymology No. 105. Elsevier. pp. 121-126.

Akhgari, M.; Abdollahi, M.; Kebryaeezadeh, A.; Hosseini,R. & Sabzevari, O. 2003. Biochemical evidence for free radicalinduced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Human and Experimental Toxicology 22(4): 205-211.

Almeida, L.; Aguiar, L.H. & Moraes, G., 2005. Effect of methyl parathion on the muscle and brain acetylcholinesterase activity of matrinxã (*Brycon cephalus*). Ciência Rural 35: 1412-1416.

Almeida, M.G.; Fanini, F.; Davino, S.C.; Aznar, A.E.; Koch, O.R. & Barros, S.D.M. 1997. Pro-and anti-oxidant parameters in rat liver after short term exposure to hexachlorobenzene. Human and Experimental Toxicology 16(5): 257-261.

Avci, A.; Kaçmaz, M. & Durak, İ. 2005. Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicology and Environmental Safety 60(1): 101-105.

Azadikhah, D.; Yalsuyi, A.M.; Saha, S.; Saha, N.C. & Faggio, C. 2023. Biochemical and Pathophysiological Responses in Capoeta capoeta under Lethal and Sub-Lethal Exposures of Silver Nanoparticles. Water 15(3): 585.

Banerjee, B.D.; Seth, V.; Bhattacharya, A.; Pasha, S.T. & Chakraborty, A.K. 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology letters 107(1-3): 33-47.

Banks, K.E.; Hunter, D.H. & Wachal, D.J. 2005. Chlorpyrifos in surface waters before and after a federally mandated ban. Environment international 31(3): 351-356.

Bebe, F. N. & Panemangalore, M. 2003. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. Journal of Environmental Science and Health, Part B 38(3): 349-363.

Castro, R.; Piazzon, M.C.; Noya, M.; Leiro, J.M. & Lamas, J. 2008. Isolation and molecular cloning of a fish myeloperoxidase. Molecular Immunology 45(2): 428-437.

Celik, I. & Isik, I. 2009. Neurotoxic effects of subacute exposure of dichlorvos and methyl parathion at sublethal dosages in rats. Pesticide Biochemistry and Physiology 94(1):1-4.

Chen, C.; Wu, C.; Lu, X.; Yan, Z.; Gao, J.; Zhao, H.,= & Li, S. 2013. Coniferyl ferulate, a strong inhibitor of glutathione S-transferase isolated from radix Angelicae sinensis, reverses multidrug resistance and downregulates P-glycoprotein. Evidence-Based Complementary and Alternative Medicine 2013.

Cicchetti, R. & Argentin, G. 2003. The role of oxidative stress in the in vitro induction of micronuclei by pesticides in mouse lung fibroblasts. Mutagenesis 18(2): 127-132.

Da Cuña, R.H.; Vazquez, G.R.; Piol, M.N.; Guerrero, N.V.; Maggese, M.C. & Nostro, F.L.L. 2011. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in *Cichlasoma dimerus* (Teleostei, Perciformes). Ecotoxicology and Environmental Safety 74(4): 1065-1073.

Das, B. & Mukherjee, D. 2000. Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham.). Toxicology Letters 114(1-3): 11-18.

Datta, C.; Gupta, J. & Sengupta, D. 1994. Interaction of organophosphorus insecticides phosphamidon & malathion on lipid profile & acetylcholinesterase activity in human erythrocyte membrane. The Indian journal of Medical Research 100: 87-89.

Dhara, K.; Saha, S.; Chukwuka, A.V.; Pal, P.; Saha, N.C. & Faggio, C. 2021a. Fluoride sensitivity in freshwater snail, *Bellamya bengalensis* (Lamarck, 1882): An integrative biomarker response assessment of behavioral indices, oxygen consumption, haemocyte and tissue protein levels under environmentally relevant exposure concentrations. Environmental Toxicology and Pharmacology 89: 103789

Dhara, K.; Saha, S.; Pal, P.; Chukwuka, A.V., Panigrahi, A.K.; Saha, N.C. & Faggio, C. 2021b. Biochemical, physiological (haematological, oxygen-consumption rate) and behavioural effects of mercury exposures on the freshwater snail, *Bellamya bengalensis*. Comparative Biochemistry Physiology Part C: Toxicology and Pharmacology 251: 109195

Doyotte, A.; Cossu, C.; Jacquin, M.-C.; Babut, M. & Vasseur, P. 1997. Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*.

Aquatic Toxicology 39(2): 93-110.

Du Preez, L.H.; Van Rensburg, P.J.; Jooste, A.; Carr, J.; Giesy, J.P.; Gross, T.S.; Kendall, R.; Smith, E.; Van Der Kraak, G. & Solomon, K. R. 2005. Seasonal exposures to triazine and other pesticides in surface waters in the western Highveld corn-production region in South Africa. Environmental Pollution 135(1): 131-141.

Elia, A.C.; Waller, W.T. & Norton, S.J. 2002. Biochemical responses of bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. Bulletin of environmental contamination and toxicology Letters 68: 809-816.

Ellman, G.L.,; Courtney, K.D.; Andres Jr, V. & Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7(2): 88-95.

Ellman, G.L. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82(1): 70-77.

Fournier, D.; Bride, J.M.; Poirie, M.; Berge, J.B. & Plapp, F.W. 1992. Insect glutathione S-transferases. Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. Journal of Biological Chemistry 267(3): 1840-1845.

Giannopolitis, C.N. & Ries., S.K. 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology 59(2): 309-314.

Glusczak, L.; dos Santos Miron, D.; Crestani, M.; da Fonseca, M.B.; de Araújo Pedron, F.; Duarte, M.F. & Vieira, V.L.P. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotoxicology and Environmental Safety 65(2): 237-241.

Hamed, R.R.; Elawa, S.E.; Farid, N.M. & Ataya, F.S. 1999. Evaluation of detoxification enzyme levels in Egyptian catfish, *Clarias lazera*, exposed to dimethoate. Bulletin of Environmental Contamination and Toxicology 63(6): 789-796.

Hasspieler, B.M.; Behar, J.V. & Digiulio, R.T. 1994. Glutathione-dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameriurus nebulosus*). Ecotoxicology and Environmental Safety. 28(1): 82-90.

Hazarika, A.; Sarkar, S.N.; Hajare, S.; Kataria, M. & Malik, J.K. 2003. Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology 185(1-2): 1-8.

Jarvinen, A.W.; Nordling, B.R. & Henry, M.E. 1983. Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetylcholinesterase inhibition. Ecotoxicology and Environmental Safety 7(4): 423-434.

Jin, Y.; Zhang, X.; Shu, L.; Chen, L.; Sun, L.; Qian, H. & Fu, Z. 2010. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere 78(7): 846-852.

John, S.; Kale, M.; Rathore, N. & Bhatnagar, D. 2001. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. The Journal of Nutritional Biochemistry 12(9): 500-504.

Jokanović, M. 2001. Biotransformation of organophosphorus compounds. Toxicology 166(3): 139-160.

Kehrer, J.P. 1993. Free radicals as mediators of tissue injury and disease. Critical reviews in Toxicology 23(1): 21-48.

Keramati, V.; Jamili, S.H. & Ramin, M. 2010. Effect of diazinon on catalase antioxidant enzyme activity in liver tissue of *Rutilus rutilus*.

Khalil, F.; Kang, I.J.; Undap, S.; Tasmin, R.; Qiu, X.; Shimasaki, Y. & Oshima, Y., 2013. Alterations in social behavior of Japanese medaka (*Oryzias latipes*) in response to sublethal chlorpyrifos exposure. Chemosphere 92(1): 125-130.

Levin, E.D.; Sledge, D.; Roach, S. Petro, A. Donerly, S. & Linney, E. 2011. Persistent behavioral impairment caused by embryonic methylphenidate exposure in zebrafish. Neurotoxicology and Teratology 33(6): 668-673.

Livingstone, D. 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 120(1): 43-49.

Lowry, O., N. Rosebrough, A. L. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1): 265-275.

Mileson, B.E.; Chambers, J.E.; Chen, W.; Dettbarn, W.; Ehrich, M.; Eldefrawi, A.T.; Gaylor, D.W.; Hamernik, K.; Hodgson, E. & Karczmar, A.G. 1998. Common mechanism of toxicity: a case study of organophosphorus pesticides. Toxicological Sciences 41(1): 8-20.

Miller, S.M.; Sweet, C.W.; DePinto, J.V. & Hornbuckle,

K.C. 2000. Atrazine and nutrients in precipitation: results from the Lake Michigan mass balance study. Environmental Science & Technology 34(1): 55-61.

Monteiro, D.A.; De Almeida, J.A.; Rantin, F.T. & Kalinin, A.L. 2006. Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comparative Biochemistry and Physiology Part C: Toxicology 143(2): 141-149.

Mukherjee, D.; Saha, S.; Chukwuka, A.V.; Ghosh, B.; Dhara, K.; Saha, N.C. & Faggio, C. 2022. Antioxidant enzyme activity and pathophysiological responses in the freshwater walking catfish, *Clarias batrachus* Linn under sub-chronic and chronic exposures to the neonicotinoid, Thiamethoxam®. Science of The Total Environment 836: 155716

Murphy, M.B.; Hecker, M.; Coady, K.K.; Tompsett, A.R.; Jones, P.D.; Du Preez, L.H. & Giesy, J.P. 2006. Atrazine concentrations, gonadal gross morphology and histology in ranid frogs collected in Michigan agricultural areas. Journao of Aquatic Toxicology 76(3-4): 230-245.

Pandey, S.; Parvez, S.; Sayeed, I.; Haque, R.; Bin-Hafeez, B. & Raisuddin, S. 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Science of the Total Environment Contamination 309(1-3): 105-115.

Parvez, S. & Raisuddin, S. 2006. Effects of paraquat on the freshwater fish *Channa punctata* (Bloch): non-enzymatic antioxidants as biomarkers of exposure. Archives of Environmental Contamination and Toxicology 50:392-397.

Pathiratne, A.L.W.H.U.; Chandrasekera, L.W.H.U. & De Seram, P.K.C. 2008. Effects of biological and technical factors on brain and muscle cholinesterases in Nile tilapia, O*reochromis niloticus*: implications for biomonitoring neurotoxic contaminations. Archives of environmental Contamination and Toxicology Letters 54: 309-317.

Rai, D.K. & Sharma, B. 2007. Carbofuran-induced oxidative stress in mammalian brain. Molecular Biotechnology 37: 66-71.

Rajeswary, S.; Kumaran, B.; Ilangovan, R.; Yuvaraj, S.; Sridhar, M.; Venkataraman, P. & Aruldhas, M.M. 2007. Modulation of antioxidant defense system by the environmental fungicide carbendazim in Leydig cells of rats. Reproductive Toxicology 24(3-4): 371-380.

Regoli, F.; Winston, G.W.; Gorbi, S.; Frenzilli, G.; Nigro, M.; Corsi, I. & Focardi, S. 2003. Integrating enzymatic responses to organic chemical exposure with total oxyradical absorbing capacity and DNA damage in the European eel *Anguilla anguilla*. Environmental Toxicology and Chemistry: An International Journal. 22(9): 2120-2129.

Saha, S., Saha, N.C.; Chatterjee, A.; Banerjee, P.; Garai, P.; Sharma, P.; Patnaik, L.; Nayak, S.; Dhara, K.; Chukwuka, A. & Faggio, C. 2023. Integrated multi-biomarker responses in Mozambique tilapia, *Oreochromis mossambicus* under acute and chronic Diazinon® exposures. Chemistry and Ecology 39(3): 235-255

Saha, S.; Chukwuka, A.V.; Mukherjee, D.; Dhara, K..; Pal, P. & Saha, N.C. 2022a. Physiological (haematological, growth and endocrine) and biochemical biomarker responses in air-breathing catfish, *Clarias batrachus* under long-term Captan® pesticide exposures. Environmental Toxicology and Pharmacology 90: 10381

Saha, S.; Chukwuka, A.V.; Mukherjee, D.; Saha, N.C. & Adeogun, A.O. 2022b. Hydrological connectivity, surface water quality and distribution of fish species within sub-locations of an urban oxbow lake, East India. Watershed Ecology and the Environment 4: 44-58.

Saha, S.; Dhara, K.; Pal, P.; Saha, N.C.; Faggio, C. & Chukwuka, A.V. 2022c. Longer-Term Adverse Effects of Selenate Exposures on Hematological and Serum Biochemical Variables in Air-Breathing Fish *Channa punctata* (Bloch, 1973) and Non-air Breathing Fish *Ctenopharyngodon Idella* (Cuvier, 1844): an Integrated Biomarker Response Approach. Biological Trace Element Research 201(7): 3497-3512.

Saha, S.; Mukherjee, D. & Saha, N.C. 2018a. Studies on acute toxicity and behavioral responses of *Heteropneustes fossilis* (Linn.) exposed to Diazinon. Research & Reviews: A Journal of Toxicology 8(1): 9-13.

Saha, S.; Saha, N.C. & Mukherjee, D. 2018b. Acute Toxicity and Behavioral Alterations of Oligochaete Worm, Branchiura sowerbyi Exposed to Diazinon. Research & Reviews: A Journal of Life Sciences 8: 1-5.

Saha, S.; Saha, S.; Mistri, A. & Saha, N.C. 2024. Antioxidant enzyme activity and pathophysiological consequences in the sludge worm *Tubifex tubifex* under acute and sub-lethal exposures to the fungicide Tilt®. Pesticide Biochemistry and Physiology 198: 105738.

Samajdar, I. & Mandal, D.K. 2015. Acute toxicity and impact of an organophosphate pesticide, chlorpyrifos on some haematological parameters of an Indian minor carp, *Labeo bata* (Hamilton 1822). International Journal of Environmental Sciences 6(1): 106-113.

Samiec, P.S.; Drews-Botsch, C.; Flagg, E.W.; Kurtz J.C.; Sternberg, Jr, P.; Reed, R.L. & Jones, D.P. 1998. Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes. Free Radical Biology and Medicine 24(5): 699-704.

Sayeed, I.; Parvez, S.; Pandey, S.; Bin-Hafeez, B.; Haque, R. & Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicology and Environmental Safety 56(2): 295-301.

Sharbidre, A.A.; Metkari, V. & Patode, P. 2011a. Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, Poecilia reticulata. Pesticide Biochemistry and Physiology 101(2): 132-141.

Sharbidre, A.A.; Metkari, V. & Patode, P. 2011b. Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia reticulata*. Pesticide Biochemistry and Physiology 101(2):132-141.

Sharma, P.; Garai, P.; Banerjee, P.; Saha, S.; Chukwuka, A.V.; Chatterjee, S.; Saha, N.C. & Faggio, C. 2023. Behavioral toxicity, histopathological alterations and oxidative stress in *Tubifex tubifex* exposed to aromatic carboxylic acids-acetic acid and benzoic acid: A comparative time-dependent toxicity assessment. Science of The Total Environment 876: 162739.

Sharma, Y.; Bashir, S.; Irshad, M.; Gupta, S.D. & Dogra, T.D. 2005. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicology 206(1): 49-57.

Sies, H. 1999. Glutathione and its role in cellular functions. Free Radical Biology and Medicine 27(9-10): 916-921.

Silva, K.C.C.; Assis, C.R.D.; Oliveira, V.M.; Carvalho Jr, L.B. & Bezerra, R.S. 2013. Kinetic and physicochemical properties of brain acetylcholinesterase from the peacock bass (*Cichla ocellaris*) and in vitro effect of pesticides and metal ions. Aquatic Toxicology 126: 191-197.

Sledge, D.; Yen, J.; Morton, T.; Dishaw, L.; Petro, A.; Donerly, S.; Linney, E. & Levin, E.D. 2011. Critical duration of exposure for developmental chlorpyrifos-induced neurobehavioral toxicity. Neurotoxicology and Teratology 33(6): 742-751.

Stalin, S.I. & Das, S. 2012. Biochemical changes in certain tissues of Cirrhina mrigala (Hamilton) (Cyprinidae: Cyprniformes) exposed to fenthion. International Journal of Environmental Sciences 2(3): 1268-1277.

Sun, F. & Chen, H.-S. 2008. Monitoring of pesticide chlorpyrifos residue in farmed fish: Investigation of possible sources. Chemosphere 71(10): 1866-1869.

Thompson, C.M.; Frick, J.A.; Natke, B.C. & Hansen, L.K. 1989. Preparation, analysis and anticholinesterase properties of O, O-dimethyl phosphorothioate isomerides. Chemical Research in Toxicology 2(6): 386-391.

Topal, A.; Şişecioğlu, M.; Atamanalp, M.; Işık, A. & Yılmaz, B. 2016. The in vitro and in vivo effects of chlorpyrifos on acetylcholinesterase activity of rainbow trout brain. Journal of Applied Animal Research 44(1): 243-247.

Tuzmen, N.; Candan, N.; Kaya, E. & Demiryas, N. 2008. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. Cell Biochemistry and Function 26(1): 119-124.

Van der Oost, R.; Beyer, J. & Vermeulen, N.P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13(2): 57-149.

Van der Wel, H. & Welling, W. 1989. Inhibition of acetylcholinesterase in guppies (*Poecilia reticulata*) by chlorpyrifos at sublethal concentrations: methodological aspects. Ecotoxicology and Environmental Safety 17(2): 205-215.

Varga, S.I. & Matkovics, B. 1997. Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 117(1) :83-88.

Winston, G.W. & Di Giulio, R.T. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Journal of Aquatic Toxicology 19(2): 137-161.

Xing, H.; Li, S.; Wang, Z.; Gao, X.; Xu, S. & Wang, X. 2012a. Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. Chemosphere 88(4): 377-383.

Xing, H.; Li, S.; Wang, Z.; Gao, X.; Xu, S. & Wang, X. 2012b. Histopathological changes and antioxidant

response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. Chemosphere 88(4):377-383.

Xu, W.A.N.G.; Jilong, L.I.; Houjuan, X.I.N.G. & Shiwen, X.U. 2011. Review of toxicology of atrazine and chlorpyrifos on fish. Journal of Northeast Agricultural University 18(4): 88-92.

Yamano, T. & Morita, S. 1995. Effects of pesticides on isolated rat hepatocytes, mitochondria, and microsomes II. Archives of Environmental Contamination and Toxicology 28:1-7.

Yen, J.; S. Donerly, E.D. Levin, and E. A. Linney. 2011. Differential acetylcholinesterase inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish. Neurotoxicology and Teratology 33(6): 735-741.

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مقاله کامل

پاسخ‌های استرس اکسیداتیو القایی کلرپیریفوس و تغییر فعالیت استیل کولین استراز در اندام بویایی ماهی *Labeo bata*

ایشیتا سمجدار[۱]، شاباجیت ساها*[۲]، دیپاک کومار مندال[۳]

[۱]گروه جانورشناسی، کالج رایدیگی، جنوب پاراگاناس، بنگال غربی، هند.
[۲]آزمایشگاه تحقیقات بوم‌سم‌شناسی، گروه جانورشناسی، دانشگاه بردوان، بنگال غربی، هند..
[۳]گروه جانورشناسی، ویسوا-بهاراتی، شانتینیکتان، بنگال غربی، هند.

چکیده: سمیت تحت کشنده کلرپیریفوس بر شاخص‌های استرس اکسیداتیو انتخابی (مالون دی‌آلدئید (MDA)، سوپراکسید دیسموتاز (SOD)، کاتالاز (CAT)، گلوتاتیون پراکسیداز (GPx)، گلوتاتیون S-ترانسفراز (GST) و گلوتاتیون احیا شده (GSH) و فعالیت استیل کولین استراز (AChE) در اندام بویایی *Labeo bata* در معرض سمیت کشنده مورد بررسی قرار گرفت. کلرپیریفوس (CPF) در معرض در غلظت‌های از پیش تعیین‌شده (۲۱/۹۳ و ۳۶/۵۴ میکروگرم در لیتر) تجویز شد و در روزهای ۱۵ و ۳۰ دوره‌های آزمایشی مورد بررسی قرار گرفت. گروه‌های مورد بررسی مقادیر قابل‌توجهی بالاتر ($P<۰/۰۵$) MDA، SOD، CAT، GST و GPx در ماهی‌های در معرض CPF نسبت به گروه شاهد نشان دادند. AChE و GSH در ماهی مواجهه یافته به‌طور قابل توجهی کمتر بود. به‌طور کلی، نشانگرهای زیستی نشان می‌دهد که قرار گرفتن در معرض CPF قادر به ایجاد اثرات استرسی خاص در سطوح بیوشیمیایی و فیزیولوژیکی است که بر سلامت کلی و طول عمر چنین حیواناتی تأثیر منفی می‌گذارد.

کلمات‌کلیدی: سمیت تحت کشنده، کلرپیریفوس، شاخص‌های استرس اکسیداتیو، *Labeo bata*.