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Research Article

Toxicological manifestations in gills, liver, kidney and muscles of *Channa punctatus* exposed to mercuric chloride

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Abstract
Aquatic regimes are exposed to a variety of pollutants that are mainly released by anthropogenic activities. Mercuric chloride ($HgCl_2$) pose serious hazards to freshwater fish resource for its toxicity and long persistence. It is also a threat to humans who consume fish as a food resource. This study aimed to determine the consequences of acute exposure to $HgCl_2$ in the freshwater food fish *Channa punctatus* (Bloch, 1973). The acute study of 96 hours was composed of three groups (in triplicates), having ten fish in each group which includes group I (control), group II (0.112 mg/l of $HgCl_2$) and group III (0.224 mg/l of $HgCl_2$). Results showed induction in reactive oxygen species (ROS) level in erythrocytes of group III (22159 ± 258.036). The biomarkers of oxidative stress, glutathione reduced (GSH) and lipid peroxidase (LPO) showed significant ($p < 0.05$) decrement and increment in their activity, respectively, in gills, liver, kidney and muscle tissues of the fish treated with $HgCl_2$. Further, micronuclei (MN) and nuclear abnormalities (NAs) were formed in the erythrocytes of the fish of groups II and III, revealing DNA damage, hence showing genotoxicity. Histopathological studies in sample tissues of $HgCl_2$ treated group demonstrated irreversible tissue injuries and anomalies. Thus, the findings from the study demonstrate that biological stress is induced in fish because of acute exposure to $HgCl_2$, leading to health impairment

Keywords: Histopathological anomalies, Mercuric chloride, Micronuclei, Nuclear abnormalities, Oxidative stress

INTRODUCTION

Heavy metals and other toxic compounds deteriorate water quality in many rivers in India (Kumar *et al*., 2020; Kumar *et al*., 2015; Siddiqui and Pandey, 2019). Among heavy metals, mercury (Hg) is a highly hazardous pollutant present globally in both freshwater and marine water (Visha *et al*., 2018), and found to exist in three toxic forms, namely elemental, inorganic (iHg) and organic https://www.epa.gov/mercury/basic-information-about-mercury. In the environment, mercury is released by both natural and anthropogenic sources https://www.epa.gov/international-cooperation/mercury-emissions-global-context. Organic mercury in the environment is of critical concern because of its highest toxicity (Ren *et al*., 2020).

The toxicity caused by HgCl2 is related to the production of intracellular reactive oxygen species (ROS), which initiate oxidative stress and cellular damage (Trivedi *et al*., 2022). Estimation of oxidative stress parameters such as GSH and LPO reflects ill effects of mercury toxicity (Trivedi *et al*., 2022; Ren *et al*., 2020).

Oxidative stress can induce genotoxicity caused due exposure to toxic compounds in various organisms (Trivedi *et al*., 2022; Kumar *et al*., 2022a; Kumar *et al*., 2022b). Heavy metals are found to cause histological damage in fish. The study of histological anomalies in the gill, liver, kidney and muscle helps to assess the effects of pollutant present in the aquatic ecosystem (Balali-Mood *et al*., 2021; Garai *et al*., 2021).

Many studies have documented the toxic effects of mercury in aquatic organisms; however, insufficient relevant data is present on mercury-induced oxidative stress, genotoxicity and histopathological manifestations in fish; hence, it must be investigated further (Borges *et al*., 2022; Trivedi *et al*., 2022). Thus, in this study, a freshwater food fish with high nutritional and low-cost value, *Channa punctatus* was taken as a test organism to investigate the effects on the health of fish exposed to $HgCl_2$.

MATERIALS AND METHODS

For the study, toxicant $HgCl_2$ of the analytical grade of S.D. Fine-chem Ltd., Mumbai, India, was obtained from a local retailer and the test fish *C.punctatus* (30 ± 3.0 g; 14.5 ± 1.0 cm) were procured from the river Gomti, Dubbaga, Lucknow (Uttar Pradesh). Primary treatment of fish was done to free them from any dermal infection (Awasthi et al., 2019). Subsequently, fish were acclimatized to laboratory conditions and were accordingly fed with the commercial food Optimum, manufactured by Perfect Companion Group Co Ltd, Thailand (American Public Health Association (APHA, 2017) and the Organization for Economic Co-operation and Development (OECD, 2019).

Acute bioassay guidelines of OECD (2019) and standard techniques elaborated in APHA (2017) were used to estimate 96hr LC_{50} of $HgCl_2$ for *C. punctatus.* The LC_{50} value was determined by using Trimmed Spearman-Karber software (Hamilton *et al.*, 1978). To check the reproducibility, experiments were repeated three times. The experimental exposure period was for 96hr to state the changes in fish health under $HgCl_2$ exposure. In total three groups were designed (maintained in triplicate) in which fish of group I were under control conditions, while fish were exposed to 0.112 mg/l of $HgCl_2$ (96 h-LC_{50}/10) in group II and to 0.224 mg/l (96 h-LC_{50}/5) of $HgCl_2$ in group III. No mortality was witnessed throughout the experiment. After the stipulated exposure period, one fish from each aquarium was euthanized to collect samples of blood and tissue (gills, liver, kidney and muscles) for further examination (Ratn *et al.,* 2018).

Generation of ROS in blood cells was measured under a fluorescence Nikon Corporation K 12432 microscope by using a fluorescent dye 2´, 7´-dichlorodihydrofl uorescein diacetate (20 µM, DCFH-DA; Sigma Aldrich, USA) and microphotographs were analyzed by using the Image J software (version 1.50, USA)(Awasthi *et al.,* 2019). Activities of biomarkers of oxidative stress such as, GSH were evaluated by following the methodology of Ellman (1959) and the method of Buege and Aust (1978) was used to assess LPO. Genotoxicity in terms of MN and NA was measured and described by following the procedures of Anbumani and Mohankumar (2011), Kumar (2012), Schmid (1975), and Shahjahan *et al.* (2020).The MN frequency was calculated as follows:

MN% =Number of cells containing micronuclei/Total number of cells counted x 100 Eq. 1

For studying histological variations, tissues were fixed by the methods followed by Ratn *et al.* (2018). Nikon Corporation K 12,432, an oil immersion microscope, was used to collect and observe the images of fixed tissues and ImageJ software (ImageJ bundled with 64-bit Java 1.8.0_172) was used to analyze them. The data were subjected to a One-way analysis of variance (ANOVA) with Tukey's post hoc to analyze them for significance ($p < 0.05$). SPSS software (Version 20.0, SPSS Company, Chicago, USA) was used for the statistical analysis.

Under the provisions of the Committee for control and supervision of experiments on animals (CPCSEA), the Government of India, an Institutional Animal Ethics Committee (IAEC) vide registration no. 1861/GO/Re/S/16/CPCSEA already exists in the University of Lucknow, Lucknow. The approved protocol mentioned in CPCSEA was followed for maintenance and experiment.

RESULTS AND DISCUSSION

The present study showed that the 96hr LC_{50} value of $HgCl_2$ for *C. punctatus* calculated was1.12 mg/l. As per U.S. Food and Drug Administration (FDA) the maximum allowed mercury concentration in fish per serving per week is 0.46 µg/g (https://www.fda.gov). A significant ($p < 0.05$) increment was observed in ROS level of fish in both the $HgCl_2$ exposed group II (0.112 mg/l of $HgCl_2$) and III(0.224 mg/l of $HgCl_2$)as compared to group I (control) after 96hr of exposure period in this study (Fig. 1). The highest generated ROS was recorded in group III(96 h-LC_{50}/5), that showed increment in corrected total cell fluorescence (CTCF) value from 9348.66 ± 372.84 in group I (control) to 22159 ± 258.036 in group III ($1/5^{th}$ of 96 h-LC50) in blood cells of the test fish (Fig. 1). Earlier studies have also recorded that mercury exposure induces ROS which disturbs the antioxidant system of the body and leads to oxidative stress as reported earlier (Trivedi *et al.,* 2022; Vieira *et al.,* 2021; Hayati *et al.,* 2019).

Non-enzymatic biomarker of oxidative stress GSH, plays an important role in the intracellular antioxidant

system by not allowing entry of mercury within the tissue, binding of mercury to the cellular molecules and scavenging ROS and antioxidant enzyme substrates (Jan *et al.,* 2015, Li *et al.,* 2021). In the present study, the activity of GSH in $HgCl_2$ exposed group II and group III decreased significantly ($p < 0.05$) compared to the control showing that $HgCl_2$ deactivates the production of GSH in the gills, liver, kidney and muscle of the fish. The highest reduction was recorded in group III compared to the control group I (Fig. 2a). This decreased level of GSH in tissues upon exposure to $HgCl_2$ in *C. punctatus* agrees with the study of Trivedi *et al.,* 2022, Li *et. al.,* 2020; Zhang *et al.,* 2016; Monterio *et al.,* 2010 who reported reduced GSH level in *C. punctatus, Ctenopharyngodon idella,* zebrafish and *Brycon amazonicus* exposed to mercuric chloride, respectively, suggesting binding of GSH to mercury.

High amounts of polyunsaturated fatty acid residues are found in aquatic organisms, acting as a substrate for oxidation (Collodel *et al.,* 2022). The result of the present study recorded a significant increment ($p > 0.05$) in the activity of LPO in the gills, liver, kidney and muscle tissue of the fish in treated group II and group III. The highest activity was recorded in group III compared to group I (control) (Fig. 2b). The increased LPO level in vital tissues of the fish exposed to $HgCl_2$ in the present study agrees with the previous data demonstrating imbalance between the ROS production and

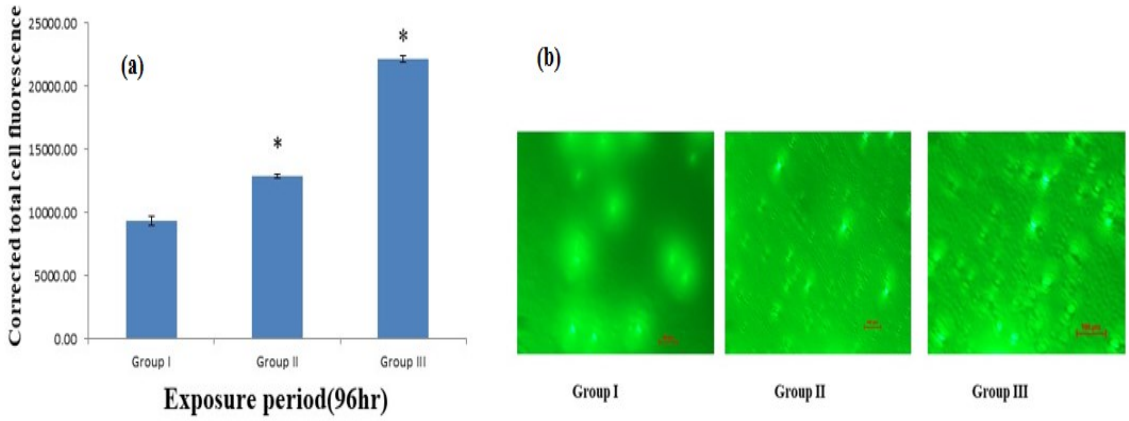

Fig. 1. ***(a)*** *Graph shows a significant ($p < 0.05$) increase in ROS represented as corrected total cell fluorescence (CTCF) in groups (II and III) exposed to toxicant $HgCl_2$ as compared to the control (Group I),* ***(b)*** *Microphotographic images showing ROS induction in erythrocytes of Clarias punctatus of groups II and III after exposure period as compared to the control (group I)*

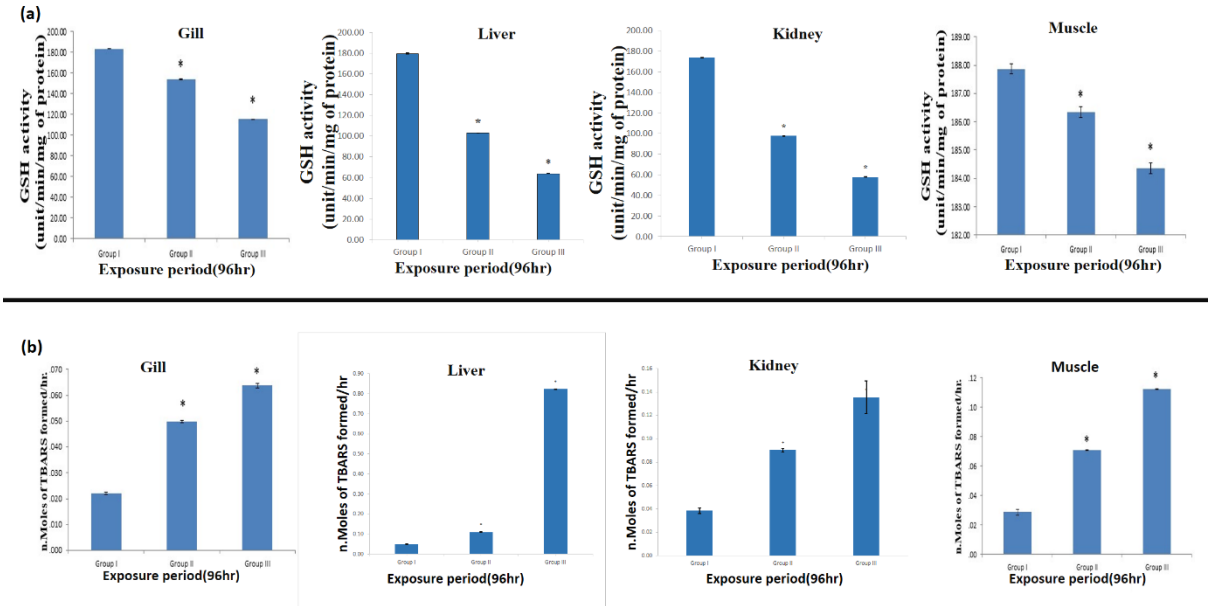

Fig. 2. *Activity of oxidative stress parameters viz., GSH and LPO in control (group I), 0.112 mg/L of $HgCl_2$ (96 h-LC_{50}/10) (group II) and 0.224 mg/L (96 h-LC_{50}/5) of $HgCl_2$ (group III) in gill, liver, kidney and muscle tissue* ***(a)*** *and* ***(b)****, respectively, for 96hr of exposure period in fish Clarias punctatus*

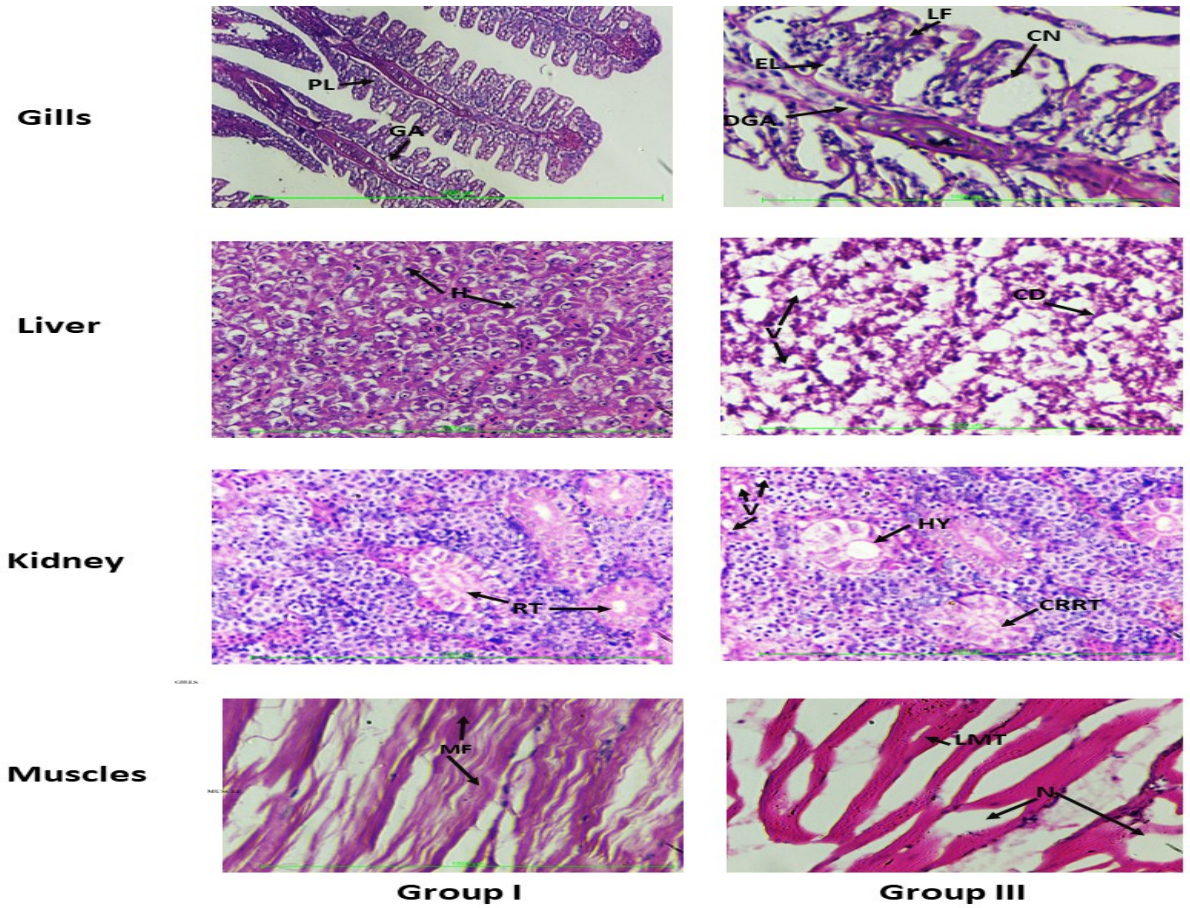

Fig. 3. *Microphotographs showing the histopathological anomalies in $HgCl_2$ exposed fish after 96hr. Control group I shows primary lamella (PL) and gill arches (GA) in gills, hepatocytes (H) in liver, renal tubules (RT) in kidney and muscle fibres (MF) in muscles tissues of C.punctatus. Treated group III shows lamellar fusion (LF), destruction of gill arches (DGA), epithelial lifting (EL) and cellular necrosis (CN) in gills, vacuolization (V) and cytoplasmic degeneration (CD) in liver, cavity reduced in renal tubule (CRRT), hypertrophy (HY) and vacuolization (V) in kidney and lesion of muscle tissue (LMT) and necrosis (N) in muscle tissues of C.punctatus.*

antioxidant defense system in fish due to physiological and oxidative stress, induced by mercuric chloride exposure (Monteiro *et al*., 2010; Li *et al.,* 2020).

The clastogenic and aneugenic events forming MN because of the exposure to toxicants such as dichlorvos, phorate, chromium trioxide, doxorubicin, arsenic trioxide and copper sulphate pentahydrate have been documented adequately (Trivedi *et al.,* 2021a; Trivedi *et al.,* 2021b; Awasthi *et al.,* 2019; Chondrou *et al.,* 2018; Yadav and Trivedi, 2009).

In the present study, MN frequencies significantly ($p < 0.05$) increased in erythrocytes of *C. punctatus* in fish exposed to $HgCl_2$ after 96 hr exposure period. The highest frequency was recorded in group III as compared to group I (control) (Fig 4 a). Nuclear abnormalities (NAs) witnessed in group III were notched, bilobed and bifurcated nuclei (Fig 4 b). Results in the study, showing induction in MN and NA frequencies in *C. punctatus*, revealed DNA damage because of overproduced ROS due to the exposure to $HgCl_2$ which concord with the studies of Braham *et al.,* 2017 and Ansari *et al.,* 2008 documented that exposure to genotoxic agents induces NAs in the erythrocytes of *Ameiurus nebulosus, Catostomus commersonii, Micropterus salmoides, M.dolomieu and C. punctata*.

Histopathology is a clinical tool used to study abrasions on biological tissues (de Oliveira Ribeiro *et al.,* 2002). In the present study, the histopathological alterations were observed in $HgCl_2$ exposed gills, liver, kidney and muscles of fish of treated group II (96 h-LC_{50}/10) and III (Fig.3) after the 96hr of the exposure period while fish in group I showed no histopathological alterations. The changes found in the gill tissues of the fish in treated group III included lamellar fusion (LF), destruction of gills arches (DGA), epithelial lifting (EL) and cellular necrosis (CN). Similar gill alterations have also been reported in fish exposed to heavy metals such as lead, copper, mercury, chromium etc., as reported by Sultana *et al.* (2016) and Shahid *et al.* (2022) in *Labeo rohita* and *Oreochromis niloticus*, respectively. Liver and

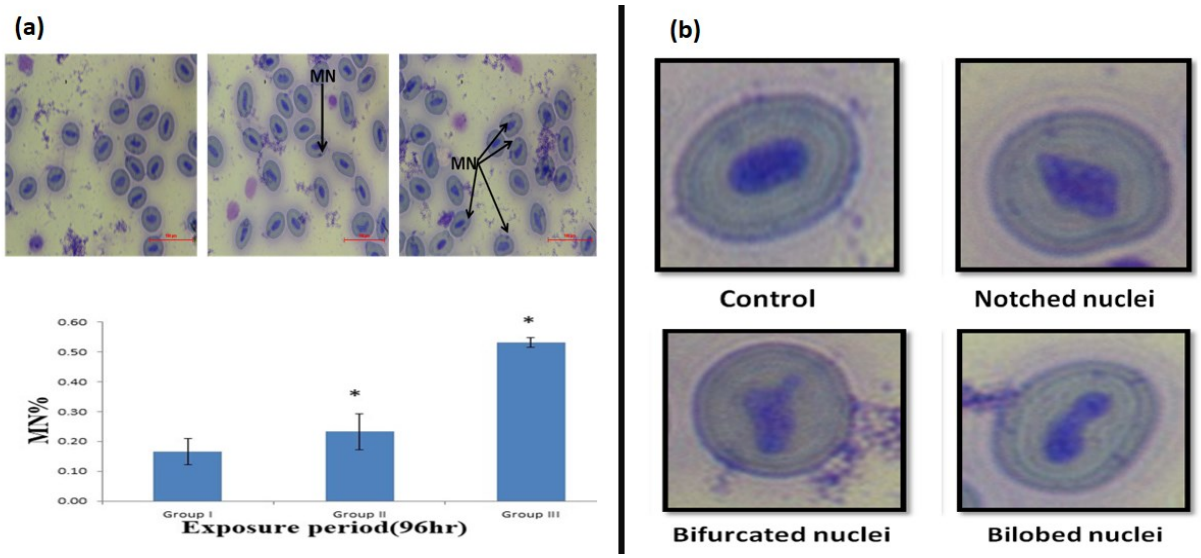

Fig. 4. ***(a)*** *Variations in MN frequency in fish erythrocytes of groups II and III as compared to group I after the exposure period of 96hr.****(b)*** *Nuclear abnormalities (NAs) viz., notched nuclei, bifurcated nuclei and bilobed nuclei observed in erythrocytes of group III exposed to $HgCl_2$ in Clarias punctatus*

kidney are two major organs responsible for the accumulation and elimination of toxicants in aquatic organisms. In the present study, anomalies witnessed in the liver tissue of the fish in treated group III included vacuolization (V) and cytoplasmic degeneration (CD) while in kidney tissue of fish in treated group III showed reduced cavity in renal tubule (CRRT), hypertrophy (Hy) and vacuolization (V). Earlier studies have also documented similar histopathological alterations in the liver and kidney of fish *C. punctatus* and *Salminus franciscanus* when exposed to toxicants such as chromium, dichlorvos, mercuric chloride, lead etc. (Awasthi *et al.* 2019; Savassi *et al.,* 2020; Trivedi *et al.,* 2021a; Trivedi *et al.,* 2022). Similarly, a study in rats intoxicated with $HgCl_2$ demonstrates hepatic and renal histopathological alterations (Shahid *et al.,* 2022). Histological study in the muscle tissue of the *C. punctatus* exposed to $HgCl_2$ in group III demonstrated lesion of muscle tissue (LMT) and necrosis (N) compared to the control in the present study. Alterations observed in muscle tissue accord with the findings having similar alterations in the muscle tissue of the fish *Labeo rohito, Cyprinus carpio communis, Tilapia zilli* and *Solea vulgaris* exposed to various toxicants such as contaminated drainage water, lead nitrate, cadmium chloride, arsenic, cadmium etc. (Kaur *et al.,* 2018; Patnaik *et al.,* 2011; Fatma 2009).

Conclusion

Toxicants affect various organs of aquatic organisms differentially upon acute and chronic exposure. As the effect of acute exposure to $HgCl_2$ on various organs of fish is yet to be established, the present study demonstrates that acute exposure to $HgCl_2$ affects the health of fish residing at the higher trophic level. The significant ($p < 0.05$) increase in ROS, MN%, LPO and significant ($p < 0.05$) decrease in GSH contents are evident even the acute exposure of low concentration (0.112 mg/l and 0.224 mg/l) of $HgCl_2$ prompts oxidative stress and toxicological manifestations in *C. punctatus.* This reveals acute exposure to $HgCl_2$ in aquatic habitats that reduces the growth and development of aquatic organisms, thereby decreasing aquaculture production and negatively impacting the ecological cycle. Therefore, it is further required to monitor and establish the impact of acute exposure to $HgCl_2$ in fish and to take necessary actions to mitigate the release of $HgCl_2$ in the environment to ensure sustainable and improved aquatic habitats.

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Conflict of interest

The authors declare that they have no conflict of interest.

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