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Zn^{2+} induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, *Channa punctatus* (Bloch, 1793)

Arun Ratn[a], Rajesh Prasad[a], Yashika Awasthi[a], Manoj Kumar[a], Abha Misra[b], Sunil P. Trivedi[a,*]

[a] Environmental Toxicology & Bioremediation Laboratory, Department of Zoology, University of Lucknow, Lucknow-226007, India
[b] Department of Zoology, Arya Mahilla P.G. College, (M.J.P. Rohilkhand University), Shahjahanpur-242001

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ABSTRACT

Zn^{2+} is essential for normal physiological functioning of all organisms in small quantities, but when its concentration enhances in surrounding environment it acts as a toxicant to organisms. Common sources of Zn^{2+} pollution are electroplating, alloying, mining, and allied industrial operations. The present study aims to assess the biochemical, histopathological and genotoxicological implications under Zn^{2+} intoxication along with its accumulation patterns in prime biotransformation sites-liver and kidney, of a bottom feeder fish, *Channa punctatus*. Fish were chronically exposed to two different concentrations of Zn^{2+} *i.e.,* 5 mg/L (permissible limit, T1) and 10 mg/L (twice the permissible limit, T2). Simultaneous control was maintained. A significant ($p < 0.05$) increment in Zn^{2+} bioaccumulation, antioxidant enzymes activities of SOD, CAT and GR and induction in micronuclei frequencies along with the significant ($p < 0.05$) decrement in total protein and GSH were observed in all the exposed groups after 28 d. Altered biochemical parameters coupled with enhanced induction in micronuclei and accumulation of Zn^{2+} in liver and kidney of fish can be regarded as sensitive biomarkers of Zn^{2+} induced toxicological manifestations and thus, they may be effectively utilized for reliable ecotoxicological biomonitoring of aquatic regimes polluted with Zn^{2+}.

1. Introduction

Zinc (Zn^{2+}) is one of the earliest known heavy metals. It becomes toxic to aquatic fauna when available in higher concentrations (Wood, 2011). Zn^{2+} enters into the aquatic environment as a result of anthropogenic activities *viz.,* electroplating, alloying, mining, ceramics, and allied industrial operations, mostly through industrial effluents. Elevated concentration of Zn^{2+} deteriorates aquatic flora and fauna. Fishes, which are at the top of the trophic level in aquatic food chain, quickly reflect physiological discomforts under xenobiotic stress generated by Zn^{2+}. Respiratory distress, either due to gill damage or heavy mucous accumulation is one of the apparent symptoms in fishes acutely exposed to Zn^{2+}. This badly affects fishes as well as to those who consume them directly or indirectly through food chain (Ganesan and Karuppasamy, 2015). Besides dermatological problems and immunological abnormalities, Zn^{2+} also causes reduced growth, loss of appetite and mortality in fish exposed to its higher concentrations (Gioda et al., 2007). Though Zn^{2+} is an active element of many enzymes for several metabolic reactions, it becomes toxic when present in higher concentrations (Lushchak, 2011; Kozlowski et al., 2009). Higher uptake of Zn^{2+} in chronically exposed fishes can lead to various biochemical and genotoxicological abnormalities (Atli and Canli, 2007). Chronic exposure of Zn^{2+} is quite likely to lead its bioaccumulation in liver and kidney, primary sites of biotransformation in fishes concerned with detoxification and excretion respectively (Avci et al., 2005). Several studies have worked out adverse effects of Zn^{2+} accumulation in various tissues of fishes exposed to higher concentration of Zn^{2+} (Kumar et al., 2015; Kawade and Khillare, 2012). Further, Zn^{2+} mediated ROS and metabolic reactive biomolecules persuade lesions *viz.*, vacuolization, pyknosis, necrosis, hypertrophy and inflammation in liver while cavity reduction in renal tubule (CRRT), glomerulus degenerative changes (GDC), vacuolization and necrosis in kidney of the test fish.

Desai et al. (2002) has also elucidated the adverse effects of Zn^{2+} on the cytoplasmic proteins of skeleton cells in fishes. Further, it has also been reported that prolonged exposure of Zn^{2+} generates oxidative stress in fishes by overproduction of reactive oxygen species (ROS) through impairment of equilibrium dynamics of normal oxidative metabolism (Lushchak, 2011; Qu et al., 2014). Heavy metal induced oxidative stress and consequent damage of biomolecules, *viz*, DNA and

* Corresponding author.
E-mail addresses: trivedi_sp@lkouniv.ac.in, sat060523@gmail.com (S.P. Trivedi).

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protein leading to physiological perturbations in fishes is already well documented (Yadav and Trivedi, 2009; Dwivedi et al., 2015; Palermo et al., 2015). Despite the fact that higher concentration of Zn^{2+} are inevitable in aquatic environment on account of established and upcoming industrial operations, only scanty information is available regarding biochemical and genotoxicological anomalies in fish subjected to higher concentrations and chronic exposure of Zn^{2+}. Fishes loaded with extra amount of Zn^{2+} are undesirable for human consumption.

To understand the eco-genotoxicological mechanisms of Zn^{2+} mediated biochemical, molecular and histopathological responses in liver and kidney of *Channa punctatus*, we have assessed (i) bioaccumulation of Zn^{2+} in liver and kidney (ii) the level of ROS and total protein (iii) oxidative stress in terms of GSH, SOD, CAT and GR (iv) DNA damage as MN induction and (v) histological lesions in Zn^{2+} exposed *Channa punctatus* (family Channidae, order Perciformes, Class Actinopterygii), a bottom dwelling and widely distributed fish.

It is presumed that our findings will be useful to uncover the mechanistic approaches associated with Zn^{2+} manifested toxicological perturbations by assessing the specific molecular biomarkers in fish. Thus, indices so generated shall be helpful in management of aquaculture practices in metal infested water besides safeguarding human health.

2. Materials and methods

2.1. Acclimatization of fish and experimental design

Channa punctatus (35 ± 3.0 g; 14.5 ± 1.0 cm), a widely distributed fish throughout the South East Asia including greater part of Indian subcontinent and available throughout the year were procured from lentic habitats of Lucknow, India. They were brought to the laboratory in wide mouthed plastic containers avoiding stress and injuries as far as possible. Prior to the acclimatization, fish were treated with 0.05% $KMnO_4$ for 5 min to remove dermal infections, if any. Fishes were loaded in glass aquaria having 7 d aged tap water at the rate 4 g fish per litre of water (Burress, 1975). Subsequently, fish specimens were acclimatized for 10 d in 100 L well aerated glass aquaria (100 × 40 × 40 cm^3), containing 40 L aged tap water under laboratory conditions (hardness 75.6 as $CaCO_3$ mg/L, alkalinity 75.85 as $CaCO_3$ mg/L, dissolved oxygen (DO) 6.81 mg/L, chemical oxygen demand (COD) 64.5 mg/L, total dissolved solids (TDS) 238.58 mg/L, pH 7.3 and temperature 27 ± 2 °C). During acclimatization, fish were fed twice a day with boiled egg pieces, minced goat liver and commercial aquarium food pallets (Perfect Companion Group Company Limited, Thailand). Fishes were maintained in laboratory throughout the experimental period following standard protocols (Apha et al., 2012). A total number of 90 acclimatized fish specimens were randomly divided into three groups (one control, 'C' and two treatment groups, T1 and T2). Each group has three replicates having 10 fishes. For T1, exposure concentrations was 5 mg/L, the maximum permissible limit (PL) of Zn^{2+} in the inland surface water as prescribed by the Central Pollution Control Board (CPCB), New Delhi, India. In T2 fishes were exposed to two times the concentration of T1. For all the three sets, T1, T2 and C fishes were maintained in aged tap water for 7, 14, 21 and 28 d of exposure periods. They were fed twice a day after each 7 d interval and aquaria water with desirable concentration of Zn^{2+} was renewed. Food residues, metabolic and excretory wastes were renewed on daily basis (Palermo et al., 2015). After stipulated exposure periods, Zn^{2+} exposed test fish were anaesthetized with 0.01% diethyl ether prior to their sacrifice for the assessment of ROS level, induction of micronuclei (MN) in erythrocytes, accumulation of Zn^{2+}, GSH level, activities of SOD, CAT, and GR in liver and kidney tissues and to study histopathological changes in both tissues.

2.2. Test chemical

Emplura grade test chemical, $ZnSO_4{\cdot}7H_2O$, of Merck Specialties Private Limited, Mumbai, procured through a local dealer of Lucknow.

2.3. Measurement of Zn^{2+} accumulation in liver and kidney

After every exposure period, liver and kidney were taken out from each sample of all the three replicates of T1, T2 and C. They were washed with phosphate buffer saline (PBS) and dried in hot air oven at 100 °C. 500 mg of dried tissue was then transferred in glass bottles (20 mL) containing nitric acid and perchloric acid (10:1 v/v) for autolysis and digestion. Glass bottles containing samples were heated at 100 °C on hot plate until a clear solution was obtained. Prior to Zn^{2+} quantification, digested samples were diluted with double distilled water. After every exposure period, Zn^{2+} concentration (in μg g^{-1} of tissue dry weight) was measured in liver and kidney samples by Atomic Absorption Spectrophotometer (Shimadzu AA-7000F). During the estimation of Zn^{2+} concentration, the operating parameters for the Zn^{2+} element were set as 2.0 L min^{-1} acetylene, 17.0 L min^{-1} air, 213.9 nm wavelength and 0.7 nm slit width as recommended by the manufacturer.

2.4. Measurement of intracellular ROS level

Zn^{2+} induced ROS generation in blood cells was assessed using a fluorescent dye 2′, 7′-dichlorodihydrofluorescein (20 μM, DCFH-DA; Sigma Aldrich, USA). Briefly, the whole blood was incubated with DCFH-DA for 30 min. DCFH-DA, a non-polar compound, enters into the blood cells where it was converted into a non-fluorescent impermeable polar compound, H_2DCF, in presence of intracellular ROS. Following incubation, the slides were prepared using blood containing DCFH-DA. After drying the slides in dark, the intracellular fluorescence was measured using a fluorescent microscope (Nikon Corporation K 12432) at excitation and emission wavelength of 485 nm and 528 nm, respectively with 40/100× magnification of objectives. Quantification of fluorescence intensity was measured using Image J software (version 1.50, USA) and ROS data were expressed as the fold changes of exposed groups against the control. The fluorescence intensity of DCF dye was directly proportional to the concentration of intracellular ROS produced in the blood cells.

2.5. Assessment of biochemical parameters in Zn^{2+} exposed liver and kidney of fish, Channa punctatus

2.5.1. Preparation of cell lysate

Liver and kidney tissues were quickly removed, weighed and washed with phosphate buffered saline (PBS), stored at −20 °C for biochemical analyses after completion of the exposure periods. Simultaneously, both tissues were homogenised in homogenisation buffer (HB) in a proportion of 1:10 (w/v). After tissue homogenisation, cell suspension was collected in 10 mL falcon tubes and centrifuged at 3000 rpm for 10 min at 4 °C. The pellet obtained was dissolved in HB and again centrifuged at 3000 rpm for 10 min at 4 °C after two times washing. Finally, pellet was suspended in 500 μL lysis buffer. 10 μL each of Phenylmethylsulfonyl fluoride (PMSF) and Dithiothreitol (DTT) were also added in the cell suspension. The whole suspension was incubated for 30 min at 4 °C. It was again centrifuged at 12,000 rpm for 15 min at 4 °C and then post mitochondrial supernatant or cell lysate was collected and further used for analysis of protein content, GSH and antioxidant enzymes.

2.5.2. Total protein estimation

Total protein contents in tissue homogenate of liver and kidney of fish, *C. punctatus,* were determined at 700 nm in spectrophotometer (Shimadzu, UV-1800 pharma spec), according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.