Mercuric Chloride induced Oxidative Stress, Genotoxicity, Haematological Changes and Histopathological Alterations in Fish *Channa Punctatus* (Bloch, 1793).

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Abstract

The current study was strived to investigate the adverse effects of Mercuric chloride (HgCl_2) overload in the fish *Channa punctatus*. Two sub-lethal test concentrations of HgCl_2 viz., 1/20th and 1/10th of 96 h LC_{50} i.e., 0.03 mg/L and 0.07 mg/L, respectively, were used for exposure. Blood, liver and kidney tissues of the control and exposed specimens were sampled at intervals of 15, 30 and 45 days to assess alterations in oxidative stress, genotoxicity haematological parameters and histopathology. Significant changes in Hb%, RBC count, WBC count, antioxidant enzyme activity viz., super oxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione reductase (GR) were recorded. Micronuclei (MN) induction, nuclear abnormalities (NA) and histopathological alterations were also observed in the exposed fish. Significant ($p < 0.05$) increase in activities of SOD, CAT, GSH, GR and after 45 d decrease in the level of GSH and GR suggest an undermined anti-oxidative defence system in the fish exposed to HgCl_2 . Histological examination of liver and kidney showed serious tissue injury and histological alterations. Significant increase in MN and NA frequencies reveal the DNA damage in erythrocytes of fish and haematological changes show the toxicological potential of HgCl_2 . The observed genotoxicity, biochemical, haematological and histological changes in the present study provide the most extensive insight of HgCl_2 stress in *Channa punctatus*.

1.introduction

Heavy metals are tainting the aquatic habitats and very badly affecting the aquatic organisms. Worldwide, heavy metal contamination is turning into the most genuine condition issue to the environment (Pushkar et al., 2019). Various reports have affirmed that heavy metals and other poisonous compounds are majorly responsible for the degradation of health of many rivers in India (Javed, 2015; M. Kumar et al., 2019; Siddiqui & Pandey, 2019). Mercury sullying in the environment presents genuine medical problem to humans and to flora and fauna in water bodies (Dash & Das, 2015).

Mercury (Hg) is a highly hazardous pollutant exceedingly bio accumulative and displays a chemical speciation in the environment majorly in three forms elemental, inorganic (iHg) and organic, all of which are toxic (Rice et al., 2014). However, the principal natural and anthropogenic emissions of mercury exist as inorganic form i.e., Mercuric (II) chloride <https://www.epa.gov/mercury/basic-information-about-mercury>. Although exceedingly toxic, this odourless, colourless substance is utilized in various kind of applications viz., agriculture, medicine, chemical industries etc.

<https://www.britannica.com/science/mercury-chemical-element/Principal-compounds>. Mercuric II chloride gets entry into water or soil from the weathering of rocks that contain mercury, release of water containing mercury from factories or water treatment plants and from incineration of municipal waste (for example, in thermometers, electrical switches, fluorescent light bulbs, or batteries that have been thrown away) <https://www.atsdr.cdc.gov/PHS/PHS.asp?id=112&tid=24>. Mercury chloride has caused an increase in various types of tumours in mice and rats. The EPA has determined that mercury chloride is a potential human carcinogen. Harmful effects of mercury that can affect the foetus include brain damage,

intellectual disability, coordination problems, blindness, seizures and speech disorders. Children with mercury poisoning can develop nervous and digestive problems as well as kidney damage (Friis, 2012).

With concern to mercury pollution, aquatic habitats are highly affected zone in which mercury is considered as major threat for aquatic organisms. The target organ for mercury is the nervous system but it can impair any organ (*Mercury Contamination of Aquatic Ecosystems*, 2018). In fishes it has been documented that mercury affects various organs viz., the brain (Morcillo et al., 2017; Pandey, 2012; Rice et al., 2014) muscles (Kimáková et al., 2018; Łuczyńska et al., 2016) immune systems and immune response (Minoia et al., 2009) and the reproductive system (Crump & Trudeau, 2009a). It has also been observed that mercury is very dangerous and highly toxic chemical in the environment which can cause structural degeneracy of cells, imbalance in antioxidant system, variations of energy metabolism, disturbance in calcium homeostasis and induction of genotoxicity (Berg et al., 2010; Berntssen et al., 2003; Yadav & Trivedi, 2009a, 2009b). Any chemical form of mercury has the potential to disrupt proteins, inactivate enzymes and cause critical changes in the physiological activities of any tissue with which it may come in contact in adequate concentration (Crump & Trudeau, 2009b; Gonzalez et al., 2005; K. V. Sastry and Sharma, 1980; K. V. Sastry & Rao, 1981; Suhendrayatna et al., 2019; Vasanthi et al., 2019a). The liver and kidney are the primary organs responsible for the regulation of metabolic pathways, maintenance of body equilibrium and detoxification (Awasthi et al., 2018; Kumar et al., 2019; Ratn et al., 2018) therefore, mercury can affect these organs severely and impair their functional role by interfering with key physiological and metabolic processes. Assessment of different biomarkers in blood is an imperative and sensitive index, they play a crucial role in toxicological studies, the obtained experimental data help in clinical correlation (Olayode et al., 2020; Perveen et al., 2019).

The mechanism of HgCl_2 toxicity is related with the generation of intracellular reactive oxygen species (ROS), which can initiate cellular damage (Ahmad & Mahmood, 2019; Livingstone, 2001). Cappello et al., (2016) documented that oxidative stress is a key pathway to initiate mercury toxicity in fish. Evaluation of activities of important antioxidant enzymes, viz., SOD, CAT, GSH and GR clearly reflect the expanse of oxidative stress (Kumari et al., 2014). Unfortunately, relatively little is known about the hematological changes, genotoxicity and histopathological alterations under chronic exposure of HgCl_2 . To get illustration of above-mentioned parameters under the stress of HgCl_2 different markers were studied. MN assay is one of the commonly used test for the evaluation of DNA damage induced by different aquatic pollutants in fish (Andrianopoulos et al., 2006; Dwivedi et al., 2017; Mahboob et al., 2013; Shukla & Trivedi, 2019). DNA damage can best be shown by MN induction and, thus, it can be use efficiently to have sight of genotoxicity. Some studies have also mentioned, in addition to MN, the presence of other nuclear abnormalities, such as blebbed, lobed, binucleated, and notched nuclei, indicating that these abnormalities need to be seen similar to MN induction, and, thus, must be considered as potential indicators of genotoxicity (Abara et al., 2016; Anbumani & Mohankumar, 2011; Ansari et al., 2009; Çavaş & Ergene-Gözükara, 2005a; Nirchio et al., 2019; Shahjahan et al., 2020). The hematological parameters viz., RBC count, WBC count and Hb% have been used as potential indicator of stress induced by various pollutants in fish (Ates et al., 2008; Bujjamma & Padmavathi, 2018; Kumar et al., 2011). Heavy metals

cause histological damage in the fish with an inflammatory defensive reaction (Álvarez-Muñoz et al., 2009; Berntssen et al., 2003; Macirella et al., 2016; Mela et al., 2007). It is peremptory that histological alterations are the markers of pollutants in the complete health of organism in the ecosystem (Velkova-Jordanoska & Kostoski, 2005). The study of histological alterations in organs viz., liver and kidney of fish is precise way to assess the effects of aquatic pollutants in experimental studies.

Many studies have documented different mercury concentrations given through food and aqueous medium using both short and long term exposures; various biological parameters been implemented to detect the impact of this toxic heavy metal on fish liver and kidney viz., quantification of genes expression, proteomic, transcriptome analysis, DNA microarray, biochemical analysis (Bleau et al., 1996; de Jesus et al., 2011; Gonzalez et al., 2005; Mela et al., 2014; Yong Ung et al., 2010) but very little amount of information is present on oxidative stress, genotoxicity, hematological and histopathological alterations (Berntssen et al., 2003; M. Mela et al., 2007; Yadav & Trivedi, 2009a). Ample of data is available on the toxicity of methyl mercury (MeHg) (Pal et al., 2012; Pandey, 2012; Souza-Araujo et al., 2016; Suhendrayatna et al., 2019; Vasanthi et al., 2019) but the effects of HgCl_2 have been considerably neglected despite the fact that HgCl_2 primarily gets accumulated in the liver and kidney of fishes (Peressutti et al., 2008; Régine et al., 2006). This study is conducted to fill this gap and investigate different parameters viz., oxidative stress, genotoxicity, histopathological and hematological alterations induced by HgCl_2 in liver and kidney of fish *Channa punctatus*.

2. Materials And Methods

2.1. Test chemical

HgCl_2 manufactured by S.D Fine-chem Ltd., Mumbai, India was purchased from a local dealer. Analytical grade chemical was used in the study.

2.2. 96 h-LC₅₀ of HgCl_2 for fish, *Channa punctatus*

The standard acute toxicity bioassay under OECD guidelines for fish acute bioassays (OECD203, 92/69/EC, method C1) and the standard methods of APHA, (2012) were used to find out 96 h LC₅₀ of HgCl_2 for *C. punctatus*. To ascertain approximate toxic range of the test chemical, ten healthy, equal sized and well acclimatized fish (30 ± 3.0 g; 14.5 ± 1.0 cm) were exposed to definite concentrations of HgCl_2 on a logarithmic scale viz., 0.1, 1.0, 10, 100 and 1000 mg/L up to 96 h in a semi static bio assay system. Fishes were loaded at the rate 4 g fish per litre of water (Burress, 1975). The toxicity range of HgCl_2 on the basis of mortality was found between 0.1 and 1.0 mg/L. After ascertaining approximate toxic range, ten distinct logarithmic concentrations viz., 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/L were selected to determine the LC₅₀ value of HgCl_2 . The percentage fatality of fish was noted up to 96 h of exposure period. Experiments were repeated three times to check its reproducibility. Probit analysis method (Finney, 1971) was used to determine 96 h LC₅₀ values of HgCl_2 .

2.3. Experimental setup

The *Channa punctatus* has been selected for this study. Healthy specimens (30 ± 5.0 g; 15 ± 1.0 cm) of fish, acquired through fishermen from aquatic habitats of Lucknow (Longitude $26^{\circ} 55'$ N, Latitude $80^{\circ} 59'$ E), Uttar Pradesh, India. Fish were transported to the laboratory and treatment of 0.05% KMnO_4 solution for 2–5 min was given, to avoid external infections if any (Awasthi et al., 2018). Fish were acclimatized to laboratory conditions for 15 days in 160 L properly ventilated glass aquaria ($100 \times 40 \times 40$ cm³) loaded with 100 L of 15 days aged tap water (APHA, 2012). During acclimatization period commercial aquarium food pellets (Perfect Companion Group Company Limited, Thailand) were given to fish. Fish were kept without feeding one day before the starting of experiment. (OECD, 2019). Total 45 fish were erratically divided into three groups each having 15 fish, group I served as control, group II – 0.03 mg/L of HgCl_2 (96 h-LC₅₀/20), group III – 0.07 mg/L (96 h-LC₅₀/10) of HgCl_2 . Every group was maintained in triplicate in separate aquaria. No fish were found dead across the experiment. Sequentially, aquaria were emptied every day to avoid accumulation of waste and excretory materials (Palermo et al., 2015). After each exposure period (15, 30 and 45) three fish from each replicate were insentient by using 0.1% (v/w) diethyl ether and their blood was draw through a heparinized syringe and stored in EDTA (1.8 mg/mL) coated vials for the evaluation of MN, NA and haematological parameters. After sacrificing the fish, liver and kidney samples were collected for the estimation of the HgCl_2 induced damage, viz., oxidative stress and histopathological alterations.

2.4. Genotoxicity measurement: Micronucleus test and Nuclear abnormalities.

Micronuclei frequencies were estimated by following methods of Kumar, 2012; Schmid, 1975 and methodologies practised earlier in our laboratory (Awasthi et al., 2018; Yadav & Trivedi, 2009a). Nuclear abnormalities were identified according to (Anbumani & Mohankumar, 2011; Shahjahan et al., 2020).

2.5. Haematological analysis

The total erythrocytes and leucocytes counts were done by Neubauer haemocytometer (Shah and Altindag, 2004). Hayem's fluid was used to dilute (1:200) blood (Mishra et al., 1977) for erythrocytes and Turks fluid was used to dilute the blood (1:20) for leucocytes. Diluted blood was loaded on haemocytometer chamber then erythrocytes and leucocytes were counted. Expressed as $10^3/\text{mm}^3$ (Wintrobe 1967).

Hemoglobin percentage (Hb%) was calculated by Sahli method (Godkar and godkar, 2003). Hb% was expressed as g/dL.

2.6. Histopathology of liver and kidney

The liver and kidney tissues were cleaned with 0.9% NaCl solution to remove impurities. Fixation of tissues was done in Bouin's fluid for 48 h and after that washing was done twice a day with 70% ethanol for four to five days to remove extra Bouin's fluid. Tissues were dehydrated in graded series of ethanol,

cleaned with xylol and later inserted in paraffin wax to get blocks. Blocks were stored overnight in 4°C. Microtome (YSI062 Yorco Precision Rotary Microtome, India) was used for sectioning (1mm) of tissues. Sections were placed on hot plate for flattening and stained with hematoxylin for one minute and eosin was used to counterstaining for two minutes. After proper processing sections were mounted in DPX. The pictures were obtained using oil immersion microscope (Nikon Corporation K 12,432) with 10/40X magnification of objective lens.

2.7. Assessment of oxidative stress

Evaluation of oxidative stress biomarkers, viz., CAT, GR and SOD was done by following the methodologies of Aebi, 1984; Carlberg and Mannervik, 1985; Kakkar et al., 1984 respectively and previously performed methods in our laboratory (Ratn et al., 2018).

Glutathione (GSH) was estimated by following methodology of Moron et al., 1979 with some modifications. GSH was measured by its reaction with DTNB (5,5'-dithio 2-nitrobenzoic acid) (Ellman's reaction) and produces a compound that absorbs light at 412 nm. 1 mL of T.E buffer (1 M Tris HCl and 0.01 M EDTA) was mixed with 50 µl 0.01M DTNB and 100 µL of tissue homogenate was added and incubated at room temperature for 10 minutes and the absorbance was taken at 412 nm in a UV-VIS spectrophotometer (Shimadzu, UV-1800 pharma spec). The blank consisted of distilled water instead of tissue homogenate.

2.8. Statistical analysis

Each group was executed with three replicates and all were presented as mean ± standard error mean (S.E.M.). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to test the significance ($p < 0.05$) of each result in blood, liver and kidney tissue samples of all experimental groups. All data were evaluated by SPSS software (version 20.0, SPSS Company, Chicago, USA).

3. Results

3.1 LC₅₀ of Mercuric chloride (HgCl₂)

The 96 h-LC₅₀ value of HgCl₂ to *C. punctatus* was estimated to be 0.78 mg/L with 95% lower and upper confidence limits of 0.85 mg/L and 0.72 mg/L respectively. For sub-lethal treatments, fish were exposed to 1/20th of 96 h-LC₅₀ (0.03 mg/L), and 1/10th of 96 h-LC₅₀ (0.07 mg/L) of HgCl₂

3.2 Physicochemical parameter

In the test medium i.e., 7 d aged tap water significant physio-chemical parameters (pH, temperature (°C), dissolved oxygen (DO), alkalinity and hardness) were recorded before and after completion of 15, 30 and 45 d of exposure periods, both in control and treated groups (Table 1). All values were found well within the prescribed limits for survival of the fishes (APHA, 2012).

3.3 Evaluation of Genotoxicity

3.3.1 Micronuclei

MN frequencies increased significantly ($p < 0.05$) in erythrocytes of *C. punctatus* after exposure to sub-lethal test concentrations of mercuric chloride (96 h-LC₅₀/20; 0.03 mg/L, 96 h-LC₅₀/10; 0.07 mg/L). MN frequency was reported in increasing trend (Fig. 1). The frequencies of MN of group I were recorded as 0.14 ± 0.02 , 0.26 ± 0.01 and 0.33 ± 0.03 after the exposure periods 15, 30 and 45 days respectively. In Group II, treated with 96 h-LC₅₀/20; 0.06 mg/L were estimated as 0.63 ± 0.07 , 1.19 ± 0.11 , 1.69 ± 0.10 after exposure periods of 15, 30 and 45 days respectively. In comparison to control, the frequencies of MN in group III, treated with 96 h-LC₅₀/10; 0.12 mg/L were recorded as 0.97 ± 0.12 , 1.62 ± 0.07 , 2.22 ± 0.17 after the exposure periods of 15, 30 and 45 days respectively. A significant ($p < 0.05$) increase in micronuclei frequencies were observed in Group III, after every exposure period in comparison to control. The maximum frequency (2.22 ± 0.17) of micronuclei was recorded in Group III after 45 d of exposure period. Significant ($p < 0.05$) differences in micronuclei frequencies were also observed among all successive exposure periods 15, 30 and 45 d in different groups (Fig. 1).

3.3.2 Nuclear Abnormalities

The nuclear abnormality (NA) analysis in erythrocytes of *C. punctatus* showed a significantly ($p < 0.05$) higher frequency of alterations including nuclear bud, notched, lobed, blebbed, bifurcated, condensed, karyorrhectic nuclei after exposure to sub-lethal test concentrations of mercuric chloride (96 h-LC₅₀/20; 0.06 mg/L, 96 h-LC₅₀/10; 0.12 mg/L). NA frequency was reported in increasing trend (Fig. 2A). The frequencies of NA in group I were recorded as 0.11 ± 0.01 , 0.21 ± 0.01 and 0.26 ± 0.01 after the exposure periods 15, 30 and 45 days respectively. In Group II, treated with 96 h-LC₅₀/20; 0.03 mg/L it was recorded as 0.21 ± 0.01 , 0.72 ± 0.11 and 1.35 ± 0.07 after exposure periods of 15, 30 and 45 days respectively. In comparison to control, the frequencies of NA in group III, treated with 96 h-LC₅₀/10; 0.07 mg/L were recorded as 0.26 ± 0.01 , 1.18 ± 0.10 and 1.81 ± 0.08 after the exposure periods of 15, 30 and 45 days respectively. A significant ($p < 0.05$) increase in nuclear abnormalities were observed in Group III, after every exposure period in comparison to Group I. The maximum frequency (1.81 ± 0.08) of NA was recorded in Group III after 45 d of exposure period. Different types of nuclear abnormalities are shown in Fig. 2 (B).

3.3 Haematological examination

Haematological parameters viz., RBCs count, WBCs count and Hb% of *C. punctatus* exposed to sub-lethal concentration of HgCl₂ at different intervals of exposure (15, 30 and 45 d) period were calculated in this study. The blood values of *C. punctatus*, namely the RBC, and Hb% were decreased, whereas the WBC level increased significantly ($p < 0.05$) in Group II and Group III as compared to Group I (control) after 15, 30 and 45 d of exposure period in dose and time dependent manner.

The results showed that RBC count of *C. punctatus* exposed to sub lethal level of HgCl_2 decreased significantly from $1.65 \pm 0.02 \text{ } 10/\text{mm}^3$ recorded at control to $1.57 \pm 0.02 \text{ } 10/\text{mm}^3$ (Group II) and $1.50 \pm 0.03 \text{ } 10/\text{mm}^3$ (Group III) after 45 d of exposure. The values were statistically significant at ($p < 0.05$).

Exposure of HgCl_2 has increased the white blood cells count significantly ($p < 0.05$) from $58.40 \pm 0.23 \text{ } 10/\text{mm}^3$ (Group I) to $60.33 \pm 0.09 \text{ } 10/\text{mm}^3$ (Group II) and $75.53 \pm 0.22 \text{ } 10^3/\text{mm}^3$ (Group III) at 45 d of exposure period.

Haemoglobin level was significantly ($p < 0.05$) decreased in different exposure concentrations from $8.10 \pm 0.06 \text{ g/dL}$ (Group I) to $6.53 \pm 0.15 \text{ g/dL}$ (Group II) and $4.53 \pm 0.07 \text{ g/dL}$ (Group III) after 45 d of exposure period.

3.4 Histopathological alterations in liver and kidney

Mercuric chloride shows remarkable histological alterations in liver and kidney of the test fish *Channa punctatus* after 45 d of exposure period.

The histology of kidney shows glomerulus and renal tubules. The recurrent exposure of HgCl_2 alter the components of kidney tissue and results in Compact glomerulus (CG), Cavity reduction in renal tubule (CRRT), Hypertrophy (Hy), Necrosis (N), and Vacuolization (V). In Fig. 3, Group I represent the histology of the kidney of the unexposed group. The Group II and III represent the section of exposed group with a reduction in tubular lumens, compact glomerulus and vacuolization in a dose dependant manner. Hypertrophy and necrosis were found only in Group III. However, the histopathological alterations were more definite in group III as shown in Fig. 3. Table 2 shows histological findings in kidney.

The liver histology exhibits of polygonal cells arranged in liver parenchyma with rounded nuclei known as hepatocytes; blood vessels and bile ducts. Histological findings of liver tissue of fish *Channa punctatus* for control and exposed groups, after 45 d were represented in Fig. 3. Control group shows no damage in the liver tissue. In Group II and III, the vacuolization, pyknosis and inflammation were recorded in increasing trend in a dose dependant manner; whereas the maximum alterations were observed in Group III, demonstrated by necrosis and inflammation. Table 2 describes the observed histological changes in liver tissue.

3.5 Oxidative stress estimation in Liver

Activities of antioxidant enzymes to evaluate the mechanisms of HgCl_2 tolerance in *Channa punctatus* were measured. The effect of HgCl_2 on the activity of SOD, CAT, GSH and GR in liver tissue samples is depicted in Fig. 4.

The activities of SOD and CAT in liver tissue of fish in Group II (1/20th 96h-LC₅₀) were not found significant in comparison to Group I after 15 d of exposure period. However, a significantly ($p < 0.05$) high induction in activities of SOD and CAT was observed in Group III (1/10th 96h-LC₅₀), after all the exposure

periods in comparison to Group I. The maximum elevation in activities of SOD and CAT was recorded as 11.85 ± 0.08 and 45.49 ± 0.05 in Group III after 30 d of exposure period. Figure 4 displays a significant ($p < 0.05$) difference in SOD and CAT activities among all successive exposure periods.

The activities of GSH and GR also showed a significant ($p < 0.05$) induction under HgCl_2 stress in Group II and Group III after 15 and 30 d of exposure periods as compared to Group I. The highest activities were recorded as 230.76 ± 0.39 and 0.49 ± 0.00 in Group III after 30 d of exposure period. However, a significant ($p < 0.05$) reduction in activities of GSH and GR was recorded as 135.29 ± 0.37 and 0.19 ± 0.00 in Group III as compared to control after 45 d of exposure period as the exposure time extended.

3.6 Oxidative stress estimation in kidney

In kidney tissue SOD and CAT activities were found to be significantly ($p < 0.05$) increased in Group III (1/10th 96h- LC_{50}), after every exposure period in comparison to Group I. the highest induction in activities of SOD and CAT was recorded as 8.29 ± 0.19 and 37.10 ± 0.40 in Group III after 30 d of exposure period. After 15 d of exposure period in Group II (1/20th 96h- LC_{50}) the activities of SOD and CAT in kidney tissue of fish were not found significant in comparison to Group I.

Further, activities of GSH and GR also increased significantly ($p < 0.05$) in Group II and Group III after 15 and 30 d of exposure periods as compared to Group I. The highest activities were recorded as 223.33 ± 1.11 and 0.45 ± 0.00 in Group III after 30 d of exposure period. However, as the exposure time extended up to 45 days activities of GSH and GR were reduced significantly ($p < 0.05$) and recorded as 116.6 ± 0.07 and 0.07 ± 0.00 in Group III as compared to control.

4. Discussion

This study demonstrates that long term exposure to HgCl_2 in fish *Channa punctatus* triggers the oxidative stress, genotoxicity, haematological and histological impairments in blood, liver and kidney tissues. Relatively small amount of information about effects of long term exposure to HgCl_2 is available in literature (Aragão et al., 2018).

The haematological parameters viz., Hb %, RBC and WBC counts may apply to confirm different forms of toxicants because of its strong connection with the environment and often used to identify the physiological status of animals (Kumar et al., 2011). To evaluate the toxicity of HgCl_2 in aquatic organisms the genotoxicity, histopathological and biochemical parameters may be utilized as potential biomarkers (Jyotsana et al., 2014; Nagarajan Nagarani et al., 2009; Turkez & Dirican, 2012; Yadav & Trivedi, 2009a; Zhang et al., 2016a).

The decrease in Hb % shows that the power of fish to supply oxygen to every tissue is confined and this will ultimately lead to slow physical movement (Nussey et al., 1995). Reduction in RBCs is the most common haematological parameter in toxicological investigations that are detected in present research. Decrease in Hb % and RBC level can reveal an anaemic situation (Kulkeaw & Sugiyama, 2012). This study is in accordance with Panigrahi & Misra, (1978) who documented that mercury reduces Hb % and red

blood cell count in the fish *Anabas scandens*. Similarly, RBCs count and Hb % was declined in fish *Tinca tinca* exposed to lead and mercuric chloride (Shah and Altindag, 2004). The decrease in RBCs counts in the present study could have caused due to inhibition of RBCs production by the mercury chloride (Setiyowati et al., 2019). Likewise, Li et al., (2011) documented a declination in total amount of RBC in the blood of *Oncorhynchus mykiss* when exposed to verapamil, a cardiovascular medicine. Mainly, the decrement in RBC counts in fish could mark the anaemic condition of the fish under stress due to environmental pollution (Li et al., 2011). Many studies have documented that heavy metal toxicity decreases the RBCs count. (Al-Asgah et al., 2015; Kumar et al., 2011; Kumar and Banerjee, 2016; Vinodhini and Narayanan., 2009).

WBCs are engaged in the regulation of immunological work in various organisms and increment in WBC count in treated fish shows a common immune response to mercury chloride (Hedayati & Ghaffari, 2013; Mohamed, 2014). WBCs count may also arises due to the protection mechanism against adverse effect of the xenobiotics on immune system which liberates lymphocytes from lympho myeloid tissue (Ates et al., 2008). Various studies also recorded increased WBC count due to heavy metal exposure (Abdel-Warith et al., 2020; Bujjamma & Padmavathi, 2018; Singh et al., 2008)

In natural habitats, high concentration of mercury in the liver and kidneys of aquatic organisms were observed (Baby et al., 2011; Friis, 2012; Suhendrayatna et al., 2019; Źarski et al., 2017). HgCl_2 strain is considered harmful to the most organisms including fishes because it causes ROS production (Ibrahim, 2015). Consequently, excessive production of ROS leads to oxidative stress in liver and kidney tissues by disturbing the activities of antioxidants which damage the defence processes leading to oxidative damage of lipid membrane, cellular protein, nucleic acid, tissue degeneration and DNA damage (Sharma et al., 2012). Anti-oxidative defence system in every organism comprises the enzymatic scavengers to mitigate overproduction of ROS and fix the impairments caused by them (Birben et al., 2012). Two most common enzymes, liable for removal of ROS are SOD and CAT. Increased superoxide radicals scavenge by SOD enzyme and forms hydrogen per oxide (H_2O_2) which is further converted to water by the action of CAT enzyme (Matés, 2000). The present study shows increment in SOD and CAT levels in time dependent manner in the liver and kidney tissues. This can be concluded that excess HgCl_2 increases the activity of SOD and CAT because the SOD–CAT system provides the first line defence system against toxicants in the body. In a similar study Zhang et al., (2016) found that exposure to HgCl_2 induces the activities of SOD and CAT in zebrafish embryos larvae. Jyotsana et al., (2014) in her study on effects of HgCl_2 on the antioxidant system in zebra fish showed an increase in catalase activity. Nagarani et al., (2011) also documented that HgCl_2 increased oxidative stress in marine fish *Terapon jarbua* during coastal aquaculture practices.

Glutathione, as a transporter of mercury and an antioxidant, plays three crucial roles in defending the body from mercury toxicity. GSH do not allow mercury to bind to cellular proteins and prevents damage to both enzymes and tissues (Jan et al., 2015). Entry of mercury in tissue cells conversion of mercury in intracellular toxin is also prevented by Glutathione-mercury complex (Drisko, 2018). According to reports

published HgCl_2 at low concentration decreases mitochondrial GSH and increases H_2O_2 formation in rat kidney mitochondria under conditions of damage electron transport chain (Sener et al., 2003). Besides, increase of GSH content may be related to protection against oxidative challenge (Lushchak, 2012). Organisms living in aquatic habitats have high content of GSH that may provide the first line of defence against the influence of toxic heavy metals (Kurutas, 2016) which may be the reason for observed induction in GSH activity.

Estimation of GR activity is also helpful in finding out more oxidative stress caused in the presence of HgCl_2 . This enzyme provides more GSH by reduction of GSSG (stored) in the body for mitigation of oxidative stress in the body efficiently. Oxidative stress increases the GR activity due to exposure of heavy metals and other pollutants in fish liver and other fish tissues (Stephensen et al., 2002). Mercury enhances the GR activity in catfish liver indicates that GR belongs to the defence system protecting the organism against oxidative stress, as also suggested by Tekman et al., (2008)

On the other hand, chronic exposure of liver and kidney tissues to HgCl_2 caused a decrement in GSH and GR level. This decreased GSH and GR level may be due to a direct binding of Hg to the GSH or due to an increased oxidation of the thiol group of GSH which would not be reconverted to GSH by GR activity (Ercal et al., 2005). GSH and GR depletion appears to increase the risk of oxidative stress due to alleviated cell protection ability. The findings of this study reveal that HgCl_2 induces oxidative stress in liver and kidney tissues which are in accordance with the study of Monteiro et al., (2010) who have documented the adverse effects of HgCl_2 on the antioxidant defence mechanism of tropical fresh water fish, *Brycon amazonicus*.

Metal catalyzation leads to the formation of ROS capable of damaging DNA. Once the oxidative stress exceeds the defence mechanism of antioxidant system, it leads to DNA damage, which is one of the most common causes of genotoxicity (Awasthi et al., 2019). In this study, frequencies of MN and NA are recorded to justify this. This study shows the induction in MN and NA frequencies which directly reflects DNA damage. The mechanisms of MN formation, which can be related to clastogenic and aneugenic events and their significance are well documented (Chondrou et al., 2018; Decordier et al., 2011; Schmid, 1975; Ueda et al., 1992). Ample numbers of investigations are there which have measured induction of MN by HgCl_2 for genotoxicity measurement in fishes (Nagarajan Nagarani et al., 2009; Nirchio et al., 2019; Qureshi, Yaqoob, 2013; Yadav & Trivedi, 2009) On the other hand, very less studies are available for the utilization of other nuclear anomalies in erythrocytes for the assessment of DNA damage in aquatic organisms (De Souza Azevedo et al., 2012). Over past few years, the concurrent analyses of NA and MN frequencies in blood have received consideration in genotoxic studies. However, the mechanisms responsible for formation of NAs is due to know. Further, various studies have documented that NAs can be induced in response to exposure to genotoxic agents (Braham et al., 2017; De Souza Azevedo et al., 2012; Guilherme et al., 2008; Hoshina et al., 2008; Nirchio et al., 2019). This study provides a proof that NAs may arise in the erythrocytes of fish, *Channa punctatus* due to oxidative stress induced by HgCl_2 . Similarly, Ansari et al., (2008) have documented that oxidative stress is responsible for the induction of

NAs (Lobed nuclei, blebbed nuclei, bilobed nuclei and binucleated nuclei) in erythrocytes of fish *Channa punctatus* on the exposure of deltamethrin. Çavaş and Ergene-Gözükara, (2005) consider that the exposure to vestiges of petroleum induced lobed and blebbed nuclear abnormalities in fishes. This study shows highly significant and positive relationship with HgCl₂ concentration and frequency of micronuclei and other nuclear abnormalities.

Histopathological examination of two major organs responsible for accumulation and elimination of toxicants in fishes, liver and kidney was done to further evaluate the toxic potential of HgCl₂. Ample number of data is present regarding metal induced histopathological changes in Piscean species (Álvarez-Muñoz et al., 2009; Hampel et al., 2008; Jabeen et al., 2019; Padrilah et al., 2018). Similarly, Awasthi et al., (2019) have also documented the histopathological alterations as the end-point marker for assessment of toxic potential of heavy metal in liver tissues of fish, *Channa punctatus*. Our results show histopathological impairments viz., increase in vacuolization (V), pyknosis (Py), necrosis (N), inflammation (In) and hypertrophy (Hy) in liver and the cavity reduction in renal tubules (CRRT), glomerulus degenerative changes (GDC), vacuolization (V) and necrosis (N) in kidney of exposed fishes for both groups (Group II and Group III). Hosseini et al., (2018) have also reported hepatic and renal histopathological damage in rats intoxicated with HgCl₂. Similar histopathological changes were observed in liver of fish *Danio rerio* exposed to HgCl₂ (Macirella et al., 2016). Reduction in renal tubules and tubular lumens in kidney (Mishra & Mohanty, (2009) were also reported in fishes exposed to heavy metals. Velma & Tchounwou, (2011) have also documented similar histopathological lesions in tissues of gold fish induced by chromium.

Conclusion

Until now, knowledge about oxidative stress induced by HgCl₂ and consequent molecular damage in fish tissues is still not well elucidated. Outcomes of this study explains an extensive profile of HgCl₂ induced eco-toxicological manifestations in a fish of wider availability. Major outcome of the present investigations includes HgCl₂ induced haematological changes, damaged antioxidant defence system in two major organs (liver and kidney) in fishes which leads to increment in MN and NA frequencies, and resulting histo-pathological changes in both the vital organs. Our results give valuable data for a broader understanding of the effects of HgCl₂ on fish liver and kidney supporting the strong relationship between genotoxicity and biochemical biomarkers. Further studies are needed to understand the dangerous effects of heavy metals on hepatic and renal tissue in order to have a more comprehensive overview of the impact on aquatic biota.

Declarations

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Compliance with Ethical Standards

Under the provisions of committee for the purpose of control and supervision of experiments on animals (CPCSEA), 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. We have followed the protocols mentioned therein CPCSEA for maintenance and experiment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Physicochemical parameters of test medium

Physicochemical parameters	Groups	Exposure periods (In days)			
		0	15	30	45
pH	Group I	7.1	7.1	6.9	7
	Group II	7.1	7.2	7.2	7.3
	Group III	7	7.3	7.4	7.4
Temperature (°C)	Group I	26.2	26.3	26.2	27.3
	Group II	26.5	26.7	27.2	28.5
	Group III	27.2	27.9	28.6	29.3
Dissolved oxygen (mg/L)	Group I	7.19	7.06	7.04	6.91
	Group II	7.09	7.05	7.06	6.82
	Group III	7.14	7.1	6.8	6.9
Hardness (mg/L)	Group I	72.78	73.2	74.28	75.38
	Group II	72.84	73.64	74.47	76.26
	Group III	73.64	74.54	75.67	76.89
Alkalinity (mg/L)	Group I	79.8	79.7	79.6	78.2
	Group II	78.6	78.7	76	75.2
	Group III	79.3	78.4	76.3	75.1

Table 2. The statistical analysis of histological alterations induced by Mercuric chloride in liver and kidney of *C. punctatus*.

Parameters	Group I	Group II	Group III
Liver			
Vacuolization (V)	-	2x	5x
Inflammation (In)	-	-	2x
Pyknotic nuclei (PN)	-	1x	4x
Necrosis (N)	-	1x	3x
Kidney			
Cavity reduction in renal tubule (CRRT)	-	2x	5x
Compact Glomerulus (CG)			
Necrosis (N)	-	2x	5x
Vacuolization (V)	-	-	3x
Hypertrophy (Hy)	-	3x	5x
	-	-	3x

None-, mild x, moderate xx, strong xxx

Figures

Figure 1

Micronuclei frequency induced by Mercuric chloride in groups (Group II-96 h-LC50/20; 0.03 mg/L and Group III- 96 h-LC50/10; 0.07 mg/L) as compared to control group (Group I) for 15, 30 and 45 d of exposure periods. (Mean $\pm$ SD, n=3 fishes of three replicates of each group). (*represent the significant ($p < 0.05$) difference from control). Micrographs of micronuclei at 100X magnification.

Figure 2

(A) Nuclear abnormalities frequency induced by Mercuric chloride in groups (Group II-96 h-LC50/20; 0.03 mg/L and Group III- 96 h-LC50/10; 0.07 mg/L) as compared to control group for 15, 30 and 45 d of exposure periods. (B) Microphotograph showing Nuclear abnormalities in fish erythrocytes at 100X magnification. (Mean $\pm$ SD, n=3 fishes of three replicates of each group). (*represent the significant ($p < 0.05$) difference from control).

Figure 3

Microphotographs of Group I (control) fish showing compact hepatocytes (H) and well-spaced sinusoids (S) in liver, normal renal tubules (RT) and glomerulus (G) in kidney. Group II and Group III show vacuolization (V), pyknotic nuclei (PN), inflammation (In) and necrosis (N) in liver. Similarly, kidney show cavity reduction in renal tubule (CRRT), compact glomerulus (CG), necrosis (N) and Hypertrophy (Hy) in a dose dependant manner. (Group I, Control; Group II, 1/20th of 96h-LC50 concentration of Mercuric chloride; Group III 1/10th of 96h-LC50 concentration of Mercuric chloride).

Figure 4

SOD, CAT, GSH and GR activity in control (Group I) and Mercuric chloride treated groups (Group II-1/20th of 96h-LC50 concentration of Mercuric chloride; Group III-1/10th of 96h-LC50 concentration of Mercuric chloride) in liver tissue of fish *C. Punctatus* for 15, 30 and 45 d of exposure periods. (Mean $\pm$ SD, n=3 fishes of three replicates of each group). (*represent the significant ($p < 0.05$) difference from control).

Figure 5

SOD, CAT, GSH and GR activity in control (Group I) and Mercuric chloride treated groups (Group II-1/20th 96h-LC50 concentration of Mercuric chloride; Group III-1/10th 96h-LC50 concentration of Mercuric chloride) in kidney tissue of fish *C. punctatus* for 15, 30 and 45 d of exposure periods. (Mean $\pm$ SD, n=3 fishes of three replicates of each group). (*represent the significant (p < 0.05) difference from control).

Figure 6

Schematic diagram to depict the plausible mechanism of HgCl₂ induced haematological changes, disbalance in antioxidant system and consequent oxidative stress triggering the induction of micronuclei and nuclear anomalies and histopathological changes in the liver and kidney of fish, *C. punctatus*.