Evaluation of Genotoxic And Biochemical Stress Response To Mercuric Chloride In Erythrocytes of Freshwater Fish Channa Punctatus

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Abstract

Experiments were conducted to evaluate health of fish, *Channa punctatus* inhabiting mercuric chloride. Acute toxicity bioassays were performed to calculate 96h LC$_{50}$ value and it was found to be 1.38mg/L. Chronic toxicity assay to investigate genotoxic effects on the erythrocytes of fish by comet assay and micronucleus assay along with alterations in blood biochemistry were evaluated. Results showed concentration and duration dependent significant DNA damage as observed by comet assay and micronucleus assay. The frequency of nuclear aberrations along with appearance of micronuclei were observed after 30 and 60 days of exposure. The blood biochemistry was studied by recording changes in levels of various biochemical parameters in blood serum and results showed the significant (p < 0.05) variations among levels of biochemical constituents such as Glucose, Lipids, Proteins, Bilirubin, Urea, Creatinine, Cholesterol, ALP, Albumin, SGOT, SGPT and Total Glycerides. The results indicated the stress response of specimen towards toxicant. The present study highly recommends the use of genotoxicity and blood biochemical analysis as the useful biomarker to assess toxicity in the aquatic water and hence to safeguards the surrounding ecosystem.

Introduction

Aquatic ecosystems are crucial for the survival and existence of environment. The indiscriminate release of pollutants due to anthropogenic activities including agricultural run-off, industrial discharge and other domestic wastes causes accumulation of toxicants and hence deteriorates quality of aquatic environment and possess threat to biological organisms including humans. Aquatic contaminants cause genetic variations that leads to cancer and mutations (Ahmad et al. 2011). Heavy metals are non-biodegradable and persistent in nature, they had tendency to gets accumulated in living bodies (Javed et al. 2017). Mercury is the non-essential metal that gets released in the surrounding environment due to natural (earth's crust) and anthropogenic (fossil fuels combustion and mining) processes. According to WHO, occupational exposure, dental amalgam, intake of contaminated fish, are responsible for mercury exposure to humans and mercury is ranked third in category of most toxic elements and mercury poisoning causes cardiovascular, haematological, cellular, renal, pulmonary, neurological, immunological, reproductive and embryonic toxicity (Rice et al. 2014; Bernhoft. 2012). To evaluate the effects of water borne contaminants, fishes are most authentic experimental model because they are sensitive to surrounding environment and as being the first recipient to toxicant, they are able to accumulate and metabolize these contaminants in their bodies similar to higher vertebrates (Ali et al. 2020). *Channa punctatus* is available throughout the year, easy to maintain under laboratory conditions, has high commercial importance and ease of non-invasive blood collection makes this fish an excellent model for toxicological studies (Talukdar et al. 2016; Kumar et al. 2010; Javed et al. 2017). Blood is the most important and abundant body fluid that transports nutrients and oxygen to lungs and tissues of body and serves as an ideal medium for toxicological analysis. The physiological condition of body can be regulated through physiology of blood. To evaluate aquatic toxicity, one bioassay is not much sufficient to identify hypothesis as each test had few shortcomings and error rates, so genotoxicity studies consisting of comet assay and micronucleus assay along with serum biochemistry are considered as promising and sensible methods to evaluate the effects of toxicants and are regarded as analytical and authentic biomarker for toxicological studies. Thus, the present study aimed to evaluate lethal concentration
of mercuric chloride and its genotoxic effects after chronic exposure in the freshwater fish, *Channa punctatus*. The recovery potential of fish was also evaluated along with toxicity studies.

**Materials And Methods**

**2.1 Chemical**

Mercuric chloride (AR/ACS) was purchased from LOBA chemie (Laboratory reagent and pure chemicals) company, Maharashtra, India (CAS No. 7487-94-7). It has molecular weight of 271.52g/mol, boiling point is 304°C, melting point 276°C and density is 5.43g/cm³. It is odourless, white crystalline solid. Chemicals used for the experimental work were purchased from Himedia Research Laboratory, Mumbai, India.

**2.2 Test animals and experimental conditions**

The freshwater fish, *Channa punctatus* commonly known as snakehead fish weighing 14 ± 2 g and length 13 ± 2 cm were procured from local fish market and acclimatized in 100L capacity plastic aquarium (60x30x60 cm) for 30 days under laboratory conditions with 12 h light and dark cycle. In order to avoid the accumulation of waste and excessive ammonia production, water of aquarium was changed daily and fishes were fed with egg white. The physicochemical analysis of water was done by using portable water analysing kit (HACH DR 1900) and values were found within range given by APHA and EPA as shown in Table 1. The ethical approval for this work is not needed as *Channa punctatus* is a food fish.

**2.3 Administration of test item**

The feeding to fish were stopped one day before conduct of test. The test item (mercuric chloride) was dissolved in water and stock solution (10 mg/L) was prepared. Eight-test concentrations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 and 3.2 mg/L) were prepared from stock solution and each concentration was carefully mixed to water in individual plastic tanks.

**2.4 Determination of Acute toxicity**

The acute toxicity bioassays were performed for the duration of 96 h and all the test groups were carefully observed and any dead fish found were removed immediately from the tanks. The toxicity tests were conducted in triplicates (n = 10; and total fish = 270) and at the same time control group was run by using a tap water. The toxicity tests were performed in accordance with the standard methods given in the manual of APHA/AWWA/WEF (2005). Fishes were carefully and slowly released in the experimental tanks having different concentrations of mercuric chloride. The 96h LC₅₀ values with 95% confidence limits were calculated and regression equation was prepared by using EPA computer probit analysis software (version 1.5) as recommended by Finney (1980).

**2.5 Fish behaviour**

The fish from control tank and experimental tank were keenly observed for behavioural changes daily for 20 min after every 1, 6, 24, 48, 72 and 96h for each test concentration (Rice et al. 1997). The behavioural responses such as swimming pattern, mucous secretion, equilibrium, air gulps, schooling, mucous production, nervous control, excitability, loosening of scales, lethargy, discoulouration, opaque eyes, changes
in pigmentation and any other change in activity of fish during the experiment were keenly observed throughout the acute exposure period. Fish behaviour is regarded as potential biomarker to aquatic stress.

2.6 Chronic toxicity tests

Chronic, static, non-renewal toxicity bioassay were conducted with three sub-lethal concentrations of mercuric chloride (1/30th, 1/20th, and 1/10th of LC$_{50}$ value) i.e. 0.046, 0.069 and 0.138 mg/L. Fish were divided into four groups, one control (n = 9; total fish = 36) and three treatment groups (n = 9; total fish = 108) for the duration of 15, 30, 60 days and 60 days recovery. Mercuric chloride solutions of different concentrations were prepared and gently mixed in the experimental tanks containing 100L of tap water. The control group was run simultaneously by using the tap water. After the completion of 60 days exposure, fish from all treated groups were removed carefully and transferred to plastic tanks for recovery for 60 days. Water was changed every day and individuals were fed with food during this period.

2.7 Alkaline single cell gel electrophoresis (SCGE)/ comet assay

Comet assay was performed in accordance to methodology of Ahuja and Saran (1999) with slight modifications. Thirty microliters of blood was taken through cardiac puncture and diluted with 980µL of phosphate buffer saline (PBS). The slides having layer of 1% (w/v) normal melting point agarose (NMPA) was dried by incubating at 37°C for 24h. A second layering was performed by using 80µL of 0.5% of low melting point agarose (LMPA) and 20µL of diluted blood was mixed in it. The slides were carefully covered with cover slips and placed at 4°C for 10min and then these slides were incubated in electrophoretic buffer (electrophoresis buffer was prepared by adding 3mL of ethylene diamine tetra acetic acid (EDTA) and 18 mL of NaOH solution in ddH$_2$O, the final volume was prepared to 100mL at pH 13). The electrophoresis was performed at 300Ma and 0.55V/cm for 25min. After this, the slides were placed in neutralization buffer for 15min. These slides were dried overnight and next day stained with ethidium bromide (stock solution of ethidium bromide was prepared by dissolving 10g of ethidium bromide in 50 mL of double distilled water. The working solution was prepared by taking 1mL from stock solution and mixed with 9mL of double distilled water) and processed slides were analysed under a fluorescence microscope (Nikon eclipse E 200) at the excitation filter of 515-560nm with the barrier filter of 590nm at the magnification of 40x. The parameters considered for the quantification of DNA damage were tail length (TL), % tail DNA and Olive tail moment (OTM) calculated by the CASPLAB software. The degree of DNA damage was analysed by mean ± standard error.

2.8 Blood micronucleus

The whole blood was drawn by cardiac puncture with heparinized syringe and thin smear of blood was prepared on the clean glass slide. The prepared smear was air dried and fixation was done by dipping slide in methanol for 15 minutes, air dried and stored for staining. The working Giemsa stain were prepared by adding 10 mL of Giemsa solution to 40 mL of double distilled water. The slides were stained for 30 minutes and then washed in double distilled water and were air dried. To detect the frequencies of binucleated (BN), micronucleated (MN) cells and nuclear abnormalities including chained, echinocytic, blebbed, lobed, notched, clumped, sphaerocytic, bi-nucleated, vacuolated, necrotic, irregular and multi-nucleated erythrocytes, 1000
cells were scanned at 100X using light microscope (Olympus-X-31) and photographed with Nikon D 3100 digital camera.

2.9 Identification criteria for micronuclei and aberrations

(i) The small circular or ovoid non-refractile chromatin bodies that showed the same staining pattern like the main nucleus were regarded as MN (Al-Sabti and Metcafe 1995). The size of MN must be smaller than the one-third of the main nuclei. (ii) There should be clear separation of MN from the main nuclei. (iii) MN should have same colour and are on same plane of focus. The cells that are having two nuclei with approximately same sizes were taken as binucleates (Cavas et al. 2005). Nuclear abnormalities (NA) were classified according to Carrasco et al. 1990. Notched nuclei were regarded as nuclei with vacuoles and are having appreciable depth into a nucleus and does not contain nuclear material. Blebbed nuclei are referred to as small evaginations of the nuclear membrane that contains euchromatin. Lobed nuclei consist of evaginations larger than blebbed nuclei and contains several lobes.

2.10 Blood biochemistry

For the biochemical examination of blood after the completion of respective exposure durations and recovery period, 0.5–0.75 mL of blood was withdrawn by cardiac puncture with the help of heparinized syringe from each animal. The blood was collected in microcentrifuge tubes (1.5 mL) and centrifuged at 3000rpm for 5 min and separated serum around 350 µL was transferred to new microcentrifuge tube for the analysis of biochemical parameters through fully automatic C71 Clinical Chemistry Analyser (BeneSphera™, Avantor, India). The serum (300 µL) and required reagents were placed in the sample and reagent trays of the auto-analyser. The parameters for each program were defined as per protocol for each analyte, that were previously calibrated and standardized for accuracy of experiment. The biochemical parameters analysed were triglyceride (TG), total protein (TP), glucose (Glu), creatinine (CREAT), urea, total bilirubin (T BILI), alanine phosphatase (ALP), total cholesterol (TC), Serum glutamic – oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) using fully automatic clinical chemistry analyser.

2.11 Statistical analysis

To calculate LC$_{50}$ value, Probit analysis program (version 1.5) was used. All the results were represented as mean ± S.E using SPSS version 16. Significant correlated values between various parameters were further tested for significance by applying paired t-test (SPSS, version 16). Comet analysis, micronuclei and total nuclear aberrations and serum biochemistry were performed in triplicates and values were expressed as mean ± S.E. The data was subjected to analysis of variance (one way ANOVA) and statistical differences were determined by Tukey's post hoc test by using SPSS, version 16.

Results

3.1 Acute Toxicity Bioassays
Acute toxicity bioassays were done to calculate LC$_{50}$ value of mercuric chloride for fish, *Channa punctatus* and to study behaviour of mercuric chloride exposed fish.

### Table 1

| Physicochemical Parameters | Values (Mean ± SD) |
|----------------------------|--------------------|
| Dissolved oxygen           | 3.50 ± 0.05mg/L    |
| Temperature                | 27.7 ± 1.00 °C     |
| Electrical conductivity    | 585.3 ± 2.52uS/cm  |
| Salinity                   | 0.25 ± 0.05%       |
| pH                         | 7.05 ± 1.00        |
| Total dissolved solids     | 286.7 ± 25.17mg/L  |
| Alkalinity                 | 410.33 12.71 mg/L  |

#### 3.2 LC$_{50}$ value

96h LC$_{50}$ value with 95% confidence limits of mercuric chloride to *Channa punctatus* was calculated by probit analysis and it was found to be 1.38 mg/L (1.20–1.55).

The regression equation of the expected probit (Y) and log concentration (X) is $Y = a + bx = 34.55X - 4.571$. There observed increase in percent mortality with respect to increase in toxicant concentration and duration of exposure, and hence linear and positive relationship was observed between log concentration and empirical probability.

### Table 2

Acute toxicity (96h LC$_{50}$), regression equation and 95 confidence limits of mercuric chloride to *Channa punctatus*

| Model Organism     | 96h LC$_0$ (mg/L) | 96h LC$_{50}$ (mg/L) | 96h LC$_{100}$ (mg/L) | Fiducial Limits | Chi square | Regression Equation | $R^2$ |
|--------------------|-------------------|----------------------|----------------------|------------------|------------|---------------------|-------|
| *Channa punctatus* | 0.4               | 1.38                 | 3.2                  | 1.20–1.55        | 15.32      | 34.55X - 4.571      | 0.951 |

#### 3.3 Fish Behaviour

Results demonstrated that fish exposed to different concentrations of mercuric chloride showed apparent changes in the behaviour as compared to control fish. The control group showed normal schooling behaviour, attentive to slight disturbances, showed active feeding, normal operculum activity, pigmentation and well- synchronized body movements and this group of fish were taken as standards during the whole duration of experimentation because this group of fish does not show notable alterations. Table 3 reveals the
various behavioural alterations observed in *Channa punctatus* after treatment with different concentrations of mercuric chloride for 96 hours. The alterations in fish behaviour were recorded after 1, 6, 24, 48, 72 and 96h of exposure.

### Table 3
Impact of mercuric chloride on behavior of *Channa punctatus* for 96 hours.

| Parameters                  | Control | Conc. 1 (0.4 mg/L) | Conc. 2 (0.8 mg/L) | Conc. 3 (1.2 mg/L) | Conc. 4 (1.6 mg/L) | Conc. 5 (2.0 mg/L) | Conc. 6 (2.4 mg/L) | Conc. 7 (2.8 mg/L) | Conc. 8 (3.2 mg/L) |
|-----------------------------|---------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Hyperactivity               | -       | -                  | +                  | ++                 | +++                | +++                | +++                | +++                | +++                |
| Rate of Operculum activity  | +       | +                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |
| Rate of swimming            | +       | +                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |
| Loss of balance             | -       | -                  | +                  | ++                 | +++                | +++                | +++                | +++                | +++                |
| Loss of Pigmentation        | -       | -                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |
| Loosening of scales         | -       | -                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |
| Mucus production            | -       | -                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |
| Opaque eyes                 | -       | -                  | +                  | ++                 | +++                | +++                | +++                | +++                | +++                |
| Hyperventilation            | -       | -                  | ++                 | ++                 | +++                | +++                | +++                | +++                | +++                |

Note: Symbol (-) ---> None (+)---> Mild, (++)---> Moderate, (+++)--> Strong

### 3.4 Chronic Bioassays
Chronic tests were conducted on *Channa punctatus* using three sub-lethal concentrations of mercuric chloride i.e. 0.046, 0.069 and 0.138 mg/L for 15, 30 and 60 days to study its effects on fish biology.

### 3.5 DNA damage
The DNA damage measured as Comet Tail length (TL), Olive tail moment (OTM) and Tail intensity (DNA %) in the erythrocytes of the control and exposure group are shown in Fig. 2 and Table 4. The DNA strand breaks after exposure to three sub-lethal concentrations in fish, *C.punctatus* are shown in Fig. 3, Fig. 4, Fig. 5 and Fig. 6. The fish specimens exposed to various concentrations of mercuric chloride exhibited significantly higher level of DNA damage (p < 0.05) in their blood cells than the control groups. The DNA damage was found to be duration and concentration dependent, with the highest damage at the sub-lethal concentration 3(0.138mg/L) for 60 days exposure, followed by sub-lethal concentration 3 (0.138mg/L) for 30 days
exposure and sub-lethal concentration 3 (0.138 mg/L) for 15 days exposure. The highest DNA damage in all the parameters were observed at the highest concentration for the 30 and 60 day exposure, while the lowest DNA damage was observed at the sub-lethal concentration 1 (0.046 mg/L) for 15 days exposure in all groups.
Table 4
DNA damage in terms of Tail DNA (%), Olive Tail moment and Tail length (TL) in erythrocytes of *Channa punctatus* exposed to mercuric chloride

| Tail DNA (%) | Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|--------------|------------|---------|---------|---------|----------|---------|
| Concentrations ↓ |            |         |         |         |          |         |
| Control      |            | 13.17 ± 0.58<sup>aP</sup> | 16.88 ± 0.32<sup>aQ</sup> | 18.93 ± 0.91<sup>aR</sup> | 15.90 ± 0.53<sup>bQ</sup> | 30.477** |
| 0.046mg/L    |            | 17.14 ± 0.56<sup>bPQ</sup> | 17.56 ± 0.52<sup>aPQ</sup> | 19.45 ± 0.68<sup>aQ</sup> | 16.54 ± 0.56<sup>aP</sup> | 4.631* |
| 0.069mg/L    |            | 18.26 ± 0.31<sup>bcp</sup> | 18.77 ± 0.48<sup>aP</sup> | 23.83 ± 0.37<sup>bQ</sup> | 17.79 ± 0.46<sup>aP</sup> | 46.896** |
| 0.138mg/L    |            | 19.69 ± 0.42<sup>cP</sup> | 23.08 ± 0.47<sup>bQ</sup> | 28.89 ± 0.41<sup>cR</sup> | 20.64 ± 0.41<sup>aP</sup> | 92.710** |
| F Value      |            | 34.059** | 37.330** | 106.009** | 18.222** |

| Olive Tail Moment (OTM) | Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|------------------------|------------|---------|---------|---------|----------|---------|
| Concentrations ↓       |            |         |         |         |          |         |
| Control                |            | 2.16 ± 0.19<sup>aP</sup> | 3.77 ± 0.33<sup>aQ</sup> | 2.83 ± 0.01<sup>aPQ</sup> | 2.63 ± 0.18<sup>aP</sup> |
| 0.046mg/L              |            | 3.15 ± 0.62<sup>aP</sup> | 4.05 ± 0.08<sup>aP</sup> | 7.75 ± 0.69<sup>bQ</sup> | 4.12 ± 0.14<sup>bP</sup> | 10.309<sup>NS</sup> |
| 0.069mg/L              |            | 3.19 ± 0.38<sup>aP</sup> | 4.19 ± 0.01<sup>aQ</sup> | 9.34 ± 0.10<sup>bR</sup> | 4.07 ± 0.04<sup>bPQ</sup> | 18.741** |
| 0.138mg/L              |            | 4.06 ± 0.47<sup>aP</sup> | 6.47 ± 0.08<sup>bQ</sup> | 12.79 ± 0.23<sup>cR</sup> | 5.94 ± 0.36<sup>cQ</sup> | 196.551** |
| F Value                |            | 3.066<sup>NS</sup> | 50.843** | 128.324** | 39.398** | 139.952** |

| Tail Length (TL) | Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|------------------|------------|---------|---------|---------|----------|---------|
| Concentrations ↓ |            |         |         |         |          |         |
| Control          |            | 15.77 ± 0.69<sup>aP</sup> | 13.67 ± 0.39<sup>aPQ</sup> | 15.34 ± 0.61<sup>aQ</sup> | 12.28 ± 0.12<sup>aP</sup> | 10.220 |
| 0.046mg/L       |            | 16.48 ± 0.39<sup>aP</sup> | 18.34 ± 0.91<sup>bP</sup> | 22.23 ± 0.57<sup>bQ</sup> | 16.96 ± 0.29<sup>bP</sup> | 19.477 |
| 0.069mg/L       |            | 16.88 ± 0.35<sup>aP</sup> | 21.94 ± 0.39<sup>cQ</sup> | 27.19 ± 0.35<sup>cR</sup> | 20.82 ± 0.39<sup>cQ</sup> | 131.699 |
| 0.138mg/L       |            | 18.98 ± 0.34<sup>bP</sup> | 22.44 ± 0.60<sup>cQ</sup> | 28.92 ± 0.22<sup>cR</sup> | 20.77 ± 0.28<sup>cQ</sup> | 124.720 |
| F Value          |            | 8.875** | 45.513** | 170.833** | 200.927** |

The averages followed by different superscripts (a-d) show variation among concentrations and different superscripts (P-R) show variation among duration statistically, NS denotes Non-Significant; * denotes P <
0.05% and **denotes P < 0.01%

### 3.6 Blood aberrations and Micronucleated (MN) studies

Significant changes in the frequencies of MN and aberrations parameters in erythrocytes were recorded in the present study on exposure to mercuric chloride for durations 15, 30 and 60 days. The results of erythrocytes micronucleus and aberrations with fish *C. punctatus* are summarized in Fig. 7 and Fig. 8. The values of MN and aberrations frequencies ranged from 0.87 ± 0.44 to 1.23 ± 0.02 and 11.00 ± 0.58 to 13.00 ± 0.58 in the erythrocytes of control fish respectively. As compared to control, the exposed fish showed elevated frequencies in erythrocytes in both concentration and duration dependent manner that varies from 2.13 ± 0.23 (0.046mg/L, 15 days) to 5.01 ± 0.04 (0.138mg/L, 60 days) and ranged from 15.20 ± 0.58 (0.046mg/L, 15 days) to 23.27 ± 0.59 (0.138mg/L, 60 days) in case of MN and aberrations respectively. Highly significant values (p < 0.01) were obtained for all the test concentrations and exposure durations when compared to control group as shown in Table 5 (% MN frequencies) and Table 6 (% aberrations frequency).

In the recovery fish, the frequencies of MN and aberrations showed decrease as significant values (p < 0.01) were obtained when compared with exposed fish for 60 days (Table 5 and Table 6). The frequencies ranged from 2.03 ± 0.04 to 3.64 ± 0.22 (MN) and 17.33 ± 0.67 to 20.53 ± 0.33 (aberrations) in recovered erythrocytes as compared to values of 1.23 ± 0.03 to 5.01 ± 0.04 (MN) and 13.00 ± 0.58 to 23.27 ± 0.59 (aberrations) observed for exposed fish in response to toxicant for 60 days.

| Table 5  | MN frequencies in erythrocytes of *Channa punctatus* exposed to mercuric chloride |
|----------|----------------------------------------------------------------------------------|
| **MN frequencies (%)** | **Duration →** | 15 days | 30 days | 60 days | Recovery | F Value |
| **Concentrations ↓** | | | | | (60 days) | |
| Control | 0.87 ± 0.44^aP | 1.18 ± 0.09^aP | 1.23 ± 0.03^aP | 1.14 ± 0.08^aP | 0.577NS | |
| 0.046mg/L | 2.13 ± 0.23^bP | 2.18 ± 0.01^bP | 3.22 ± 0.02^bQ | 2.03 ± 0.04^bP | 23.385** | |
| 0.069mg/L | 3.14 ± 0.18^bcP | 3.26 ± 0.12^cP | 4.21 ± 0.10^cQ | 2.80 ± 0.17^cP | 16.924** | |
| 0.138mg/L | 3.93 ± 0.08^dP | 4.07 ± 0.01^dP | 5.01 ± 0.04^dQ | 3.64 ± 0.22^dP | 25.314** | |
| **F Value** | 25.076** | 267.079** | 833.909** | 52.615** |

The averages followed by different superscripts (a-d) show variation among concentrations and different superscripts (P-Q) show variation among duration statistically, NS denotes Non-Significant; * denotes P < 0.05% and **denotes P < 0.01%
Table 6
Aberration frequencies in erythrocytes of *Channa punctatus* exposed to mercuric chloride

| Aberration frequencies (%) | Duration → | 15 days | 30 days | 60 days | Recovery (60 days) | F Value |
|----------------------------|------------|---------|---------|---------|-------------------|---------|
| Concentrations↓            |            |         |         |         |                   |         |
| Control                    | 11.00 ± 0.58<sup>aP</sup> | 12.00 ± 0.58<sup>aP</sup> | 13.00 ± 0.58<sup>aP</sup> | 10.67 ± 0.67<sup>aP</sup> | 3.077<sup>NS</sup> |
| 0.046mg/L                  | 15.20 ± 0.58<sup>bP</sup> | 19.00 ± 0.58<sup>bQR</sup> | 20.87 ± 0.35<sup>bR</sup> | 17.33 ± 0.67<sup>bPQ</sup> | 18.844** |
| 0.069mg/L                  | 18.47 ± 0.81<sup>cP</sup> | 21.40 ± 0.31<sup>cQ</sup> | 21.93 ± 0.18<sup>bQ</sup> | 20.40 ± 0.31<sup>cPQ</sup> | 10.658** |
| 0.138mg/L                  | 20.50 ± 0.46<sup>cP</sup> | 22.87 ± 0.35<sup>cQ</sup> | 23.27 ± 0.59<sup>cQ</sup> | 20.53 ± 0.33<sup>cP</sup> | 11.018** |
| F Value                    | 44.867**   | 104.889** | 101.496** | 78.108** |

The averages followed by similar superscripts (a-c) do not show variation among concentrations and similar superscripts (P-R) do not show variation among duration statistically, NS denotes Non-Significant; * denotes *P* < 0.05% and **denotes *P* < 0.01%

### 3.7 Serum biochemistry

Table 7 and Fig. 9 demonstrates the biochemical findings of the exposure of three sub-lethal concentrations of mercuric chloride to fish, *C. punctatus* for 15, 30 and 60 days. The comparison between control groups and exposed groups showed significant (*p* < 0.05) difference in all biochemical parameters except in the glucose content for exposure of 15 days and creatinine content during 15 and 30 days exposure. In comparison to control group, there occurs variations among the treated groups at various sub-lethal concentrations of mercuric chloride.
Table 7
Comparison of biochemical parameters in blood serum of *Channa punctatus* exposed to mercuric chloride for 15, 30 and 60 days

| Glucose | Duration → | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|---------|------------|---------|---------|---------|---------------------|---------|
|         | Concentrations ↓ |         |         |         |                     |         |
| Control | 40.88 ± 0.27<sup>aP</sup> | 41.41 ± 0.01<sup>aP</sup> | 43.04 ± 0.29<sup>cQ</sup> | 41.00 ± 0.29<sup>aP</sup> | 16.322** |
| 0.046mg/L | 42.78 ± 0.12<sup>bP</sup> | 44.84 ± 0.44<sup>bQ</sup> | 48.72 ± 0.31<sup>dR</sup> | 42.10 ± 0.34<sup>aP</sup> | 85.739** |
| 0.069mg/L | 43.92 ± 0.28<sup>cQ</sup> | 51.83 ± 0.29<sup>cR</sup> | 41.24 ± 0.58<sup>bP</sup> | 44.40 ± 0.02<sup>bQ</sup> | 167.326** |
| 0.138mg/L | 45.41 ± 0.03<sup>dP</sup> | 51.53 ± 0.71<sup>cR</sup> | 36.94 ± 0.30<sup>aQ</sup> | 50.75 ± 0.31<sup>cR</sup> | 258.434** |
| F Value | 86.941** | 135.030** | 159.665** | 257.471** |

| Total Cholesterol | Duration → | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|-------------------|------------|---------|---------|---------|---------------------|---------|
|                   | Concentrations ↓ |         |         |         |                     |         |
| Control | 83.86 ± 0.89<sup>aP</sup> | 84.18 ± 1.17<sup>aP</sup> | 85.20 ± 0.58<sup>aP</sup> | 82.57 ± 0.31<sup>aP</sup> | 1.803<sup>NS</sup> |
| 0.046mg/L | 85.57 ± 0.34<sup>aP</sup> | 86.62 ± 0.35<sup>aP</sup> | 90.23 ± 0.55<sup>bQ</sup> | 86.97 ± 0.37<sup>bP</sup> | 24.094** |
| 0.069mg/L | 90.53 ± 0.86<sup>bP</sup> | 92.53 ± 0.66<sup>bP</sup> | 93.87 ± 0.89<sup>cP</sup> | 93.45 ± 0.52<sup>cP</sup> | 3.900* |
| 0.138mg/L | 92.54 ± 1.46<sup>bP</sup> | 92.75 ± 0.45<sup>bP</sup> | 98.21 ± 0.47<sup>cQ</sup> | 94.75 ± 0.29<sup>cPQ</sup> | 10.410** |
| F Value | 17.583** | 34.456** | 73.016** | 217.643** |

| Creatinine | Duration → | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------|------------|---------|---------|---------|---------------------|---------|
|            | Concentrations ↓ |         |         |         |                     |         |
| Control | 0.90 ± 0.01<sup>aP</sup> | 0.96 ± 0.02<sup>aPQ</sup> | 0.98 ± 0.01<sup>aQ</sup> | 0.94 ± 0.01<sup>aPQ</sup> | 5.281<sup>NS</sup> |
| 0.046mg/L | 0.95 ± 0.01<sup>aP</sup> | 0.98 ± 0.01<sup>aP</sup> | 1.15 ± 0.02<sup>aR</sup> | 1.09 ± 0.01<sup>bQ</sup> | 86.272** |
| 0.069mg/L | 1.16 ± 0.01<sup>bP</sup> | 1.49 ± 0.01<sup>bQ</sup> | 1.78 ± 0.01<sup>bS</sup> | 1.64 ± 0.03<sup>cR</sup> | 268.993** |
| 0.138mg/L | 1.37 ± 0.03<sup>cP</sup> | 1.52 ± 0.01<sup>bP</sup> | 2.04 ± 0.07<sup>cQ</sup> | 1.87 ± 0.04<sup>cQ</sup> | 51.592** |
| F Value | 167.875** | 455.211** | 175.301** | 380.614** |
### Glucose

| Concentrations  | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------------|---------|---------|---------|--------------------|---------|
| Control          | 20.41 ± 0.30\textsuperscript{aPQ} | 20.42 ± 0.32\textsuperscript{aP} | 21.43 ± 0.33\textsuperscript{aP} | 20.11 ± 0.02\textsuperscript{aP} | 4.503\textsuperscript{NS} |
| 0.046mg/L        | 22.65 ± 0.31\textsuperscript{aPQ} | 25.61 ± 0.32\textsuperscript{bPQ} | 29.48 ± 1.19\textsuperscript{bP} | 25.67 ± 0.39\textsuperscript{bP} | 17.820\textsuperscript{**} |
| 0.069mg/L        | 25.21 ± 0.06\textsuperscript{cQ} | 27.06 ± 0.27\textsuperscript{bcR} | 37.67 ± 0.88\textsuperscript{cR} | 31.99 ± 0.87\textsuperscript{cR} | 76.781\textsuperscript{**} |
| 0.138mg/L        | 26.21 ± 0.06\textsuperscript{dP} | 24.63 ± 0.82\textsuperscript{cQ} | 39.74 ± 0.18\textsuperscript{cQ} | 30.70 ± 0.92\textsuperscript{cQ} | 117.915\textsuperscript{**} |
| **F Value**      | 142.428\textsuperscript{**} | 34.177\textsuperscript{**} | 120.337\textsuperscript{**} | 66.180\textsuperscript{**} |         |

### Urea

| Concentrations  | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------------|---------|---------|---------|--------------------|---------|
| Control          | 63.57 ± 0.87\textsuperscript{aP} | 61.45 ± 0.66\textsuperscript{aP} | 61.45 ± 0.66\textsuperscript{aP} | 60.92 ± 0.29\textsuperscript{aP} | 3.183\textsuperscript{NS} |
| 0.046mg/L        | 69.91 ± 0.87\textsuperscript{bPQ} | 71.02 ± 0.83\textsuperscript{bP} | 71.99 ± 0.84\textsuperscript{bP} | 68.64 ± 0.18\textsuperscript{bP} | 3.836\textsuperscript{*} |
| 0.069mg/L        | 77.31 ± 0.59\textsuperscript{cQ} | 80.68 ± 1.25\textsuperscript{cR} | 79.28 ± 0.16\textsuperscript{cQR} | 70.58 ± 0.32\textsuperscript{cP} | 39.049\textsuperscript{**} |
| 0.138mg/L        | 82.34 ± 0.57\textsuperscript{dP} | 90.81 ± 0.82\textsuperscript{dQ} | 92.39 ± 1.19\textsuperscript{dQ} | 89.75 ± 0.25\textsuperscript{dQ} | 30.988\textsuperscript{**} |
| **F Value**      | 122.969\textsuperscript{**} | 185.248\textsuperscript{**} | 261.346\textsuperscript{**} | 2106\textsuperscript{**} |         |

### SGOT

| Concentrations  | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------------|---------|---------|---------|--------------------|---------|
| Control          | 2.10 ± 0.01\textsuperscript{dP} | 2.14 ± 0.02\textsuperscript{dP} | 2.14 ± 0.04\textsuperscript{cP} | 2.15 ± 0.00\textsuperscript{cP} | 0.802\textsuperscript{NS} |
| 0.046mg/L        | 2.00 ± 0.01\textsuperscript{cQ} | 1.28 ± 0.02\textsuperscript{aP} | 1.22 ± 0.04\textsuperscript{bP} | 1.29 ± 0.01\textsuperscript{bP} | 304.466\textsuperscript{**} |
| 0.069mg/L        | 1.81 ± 0.02\textsuperscript{bR} | 1.25 ± 0.04\textsuperscript{aQ} | 1.13 ± 0.02\textsuperscript{abPQ} | 1.08 ± 0.03\textsuperscript{aP} | 131.716\textsuperscript{**} |
| 0.138mg/L        | 1.63 ± 0.01\textsuperscript{aR} | 1.17 ± 0.01\textsuperscript{aQ} | 1.04 ± 0.02\textsuperscript{aP} | 1.07 ± 0.03\textsuperscript{aP} | 5.618\textsuperscript{*} |
| **F Value**      | 283.524\textsuperscript{**} | 339.388\textsuperscript{**} | 289.361\textsuperscript{**} | 392.583\textsuperscript{**} |         |
## Glucose

### SGPT

| Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|------------|---------|---------|---------|----------|---------|
| Concentrations ↓ |         |         |         | (60 Days) |         |
| Control     | 30.00 ± 0.36<sup>aP</sup> | 30.00 ± 0.37<sup>aP</sup> | 30.28 ± 0.03<sup>aP</sup> | 30.34 ± 0.03<sup>aP</sup> | 0.493<sup>NS</sup> |
| 0.046mg/L   | 34.09 ± 1.22<sup>bP</sup> | 34.88 ± 1.05<sup>bP</sup> | 34.99 ± 0.01<sup>bP</sup> | 33.02 ± 0.31<sup>bP</sup> | 1.231<sup>NS</sup> |
| 0.069mg/L   | 35.70 ± 0.35<sup>bcP</sup> | 39.18 ± 1.09<sup>cQ</sup> | 39.23 ± 0.40<sup>cQ</sup> | 35.48 ± 0.03<sup>cP</sup> | 11.761** |
| 0.138mg/L   | 38.39 ± 0.54<sup>dp</sup> | 40.89 ± 0.25<sup>cQ</sup> | 41.04 ± 0.41<sup>dQ</sup> | 36.72 ± 0.17<sup>dP</sup> | 31.405** |
| F Value     | 24.239** | 37.743** | 275.378 | 251.089   |         |

### Total Glycerides

| Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|------------|---------|---------|---------|----------|---------|
| Concentrations ↓ |         |         |         | (60 Days) |         |
| Control     | 45.47 ± 0.36<sup>aPQ</sup> | 45.86 ± 0.01<sup>aQ</sup> | 45.19 ± 0.32<sup>aPQ</sup> | 44.71 ± 0.01<sup>aP</sup> | 4.075* |
| 0.046mg/L   | 49.89 ± 0.02<sup>bQ</sup> | 51.74 ± 0.08<sup>bR</sup> | 52.93 ± 0.27<sup>bS</sup> | 49.13 ± 0.09<sup>bP</sup> | 135.670** |
| 0.069mg/L   | 51.91 ± 0.29<sup>cp</sup> | 55.89 ± 0.33<sup>cQ</sup> | 59.15 ± 0.39<sup>cS</sup> | 53.79 ± 0.31<sup>cQ</sup> | 85.030** |
| 0.138mg/L   | 53.89 ± 0.26<sup>dp</sup> | 56.88 ± 0.33<sup>cQ</sup> | 60.76 ± 0.19<sup>dR</sup> | 54.59 ± 0.23<sup>cP</sup> | 140.706** |
| F Value     | 181.459** | 438.334** | 536.092** | 542.911** |         |

### Bilirubin

| Duration → | 15 days | 30 days | 60 days | Recovery | F Value |
|------------|---------|---------|---------|----------|---------|
| Concentrations ↓ |         |         |         | (60 Days) |         |
| Control     | 0.41 ± 0.00<sup>aQ</sup> | 0.42 ± 0.00<sup>aQ</sup> | 0.42 ± 0.01<sup>aQ</sup> | 0.39 ± 0.00<sup>aP</sup> | 13.500** |
| 0.046mg/L   | 0.45 ± 0.00<sup>bP</sup> | 0.49 ± 0.00<sup>bQ</sup> | 0.51 ± 0.00<sup>bR</sup> | 0.49 ± 0.00<sup>bQ</sup> | 57.000** |
| 0.069mg/L   | 0.49 ± 0.00<sup>CP</sup> | 0.54 ± 0.01<sup>cQ</sup> | 0.54 ± 0.01<sup>bQ</sup> | 0.59 ± 0.00<sup>cR</sup> | 32.750** |
| 0.138mg/L   | 0.51 ± 0.01<sup>CP</sup> | 0.73 ± 0.01<sup>dR</sup> | 0.70 ± 0.01<sup>cR</sup> | 0.62 ± 0.01<sup>dQ</sup> | 165.714** |
| F Value     | 131.278** | 541.417** | 214.536** | 666.667** |         |

### ALP
**Glucose**

| Duration → | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------|---------|---------|---------|---------------------|---------|
| Concentrations ↓ |         |         |         |                     |         |
| Control    | 3.24 ± 0.02<sup>aP</sup> | 3.24 ± 0.04<sup>aP</sup> | 3.11 ± 0.08<sup>aP</sup> | 3.21 ± 0.00<sup>aP</sup> | 1.971<sup>NS</sup> |
| 0.046mg/L  | 4.20 ± 0.01<sup>bQ</sup>  | 4.38 ± 0.09<sup>bQ</sup>  | 4.78 ± 0.07<sup>bR</sup>  | 3.81 ± 0.05<sup>bP</sup>  | 43.483** |
| 0.069mg/L  | 4.82 ± 0.09<sup>cQ</sup>  | 5.09 ± 0.06<sup>cR</sup>  | 5.25 ± 0.02<sup>cR</sup>  | 3.94 ± 0.03<sup>bP</sup>  | 109.945** |
| 0.138mg/L  | 5.37 ± 0.03<sup>dQ</sup>  | 5.63 ± 0.01<sup>dS</sup>  | 5.79 ± 0.05<sup>dS</sup>  | 4.03 ± 0.04<sup>CP</sup>  | 615.756** |
| F Value    | 358.387** | 349.006** | 394.157** | 133.125**           |         |

**Total Proteins**

| Duration → | 15 days | 30 days | 60 days | Recovery (60 Days) | F Value |
|------------|---------|---------|---------|---------------------|---------|
| Concentrations ↓ |         |         |         |                     |         |
| Control    | 4.89 ± 0.33<sup>bP</sup> | 4.85 ± 0.32<sup>CP</sup> | 4.85 ± 0.32<sup>CP</sup> | 4.23 ± 0.01<sup>CP</sup> | 1.300<sup>NS</sup> |
| 0.046mg/L  | 4.85 ± 0.32<sup>bR</sup>  | 4.08 ± 0.04<sup>cQ</sup>  | 3.32 ± 0.01<sup>bP</sup>  | 2.94 ± 0.03<sup>bP</sup>  | 27.233** |
| 0.069mg/L  | 3.14 ± 0.06<sup>aPQ</sup> | 3.22 ± 0.06<sup>bQ</sup>  | 2.57 ± 0.14<sup>bPQ</sup> | 2.48 ± 0.28<sup>bP</sup>  | 5.635*  |
| 0.138mg/L  | 2.89 ± 0.26<sup>aR</sup>  | 2.34 ± 0.10<sup>aPQ</sup> | 1.35 ± 0.01<sup>AP</sup>  | 1.68 ± 0.12<sup>aPQ</sup> | 19.817** |
| F Value    | 16.522** | 39.845** | 71.329** | 48.207**           |         |

The averages followed by different superscripts (a-d) show variation among concentrations and different superscripts (P-S) show variation among duration statistically, NS denotes Non-Significant; * denotes P < 0.05% and **denotes P < 0.01%

**Discussion**

There are various studies reported in literature that determines the acute toxicity (LC<sub>50</sub>) on fish in response to various pollutants. The acute toxicity of mercuric chloride for *Channa punctatus* was found to be 1.21mg/L and 0.81 mg/L (Gupta 2005; Maheshwari 2017). Similarly, acute toxicity of mercuric chloride for *Percocyprispungi*, *Clarias batrachus* and *Oreochromis mossambicus* was found to be 0.327 mg/L, 1.85ppm and 0.58ppm (Yuan et al. 2017; Manorama and Pundkar. 2018; Vasanthi et al. 2019). There occur variations among LC<sub>50</sub> values because of difference in laboratory conditions, genetic variations of test organisms, environmental factors, mode of administration of toxicant and sensitivity of test species to broad range of environmental pollutant.

Fish serves as an early bioindicator to detect aquatic pollution as fish are the one of the most vital aquatic organisms in an aquatic ecosystem because they play multiple roles in the trophic web and responds to even
low concentrations of toxicants and answers indirectly in terms of feeding on contaminated aquatic living organisms. Fish are the best indicator of heavy metal pollution in an ecosystem as their metabolic state changes in response to toxicants in surrounding medium (Tasneem and Yashmeen 2018).

Nucleic acid content marks the identity of an organism and is an index of the amount of protein synthesis. The exposure of genotoxic compounds leads to an increase in the activity of the DNase that digests the DNA of an organism and hence disturbs the central dogma of the cell of an organism (Tasneem et al. 2018). The physiology of fish in terms of growth and development depends on the ratio of DNA content and it serves as an important biochemical parameter. The growth of cell and protein synthesis are dependent on the DNA content of fish. There occur drastic changes in quality of DNA, when fish exposed to heavy metal loaded contaminated water (Tasneem and Yashmeen 2018). Genotoxicity leads to carcinogenesis (Malacarne et al. 2021).

There occur variations among the results of the comet assay from one study to another due to differences in the type of species, weight and the size of fish and also due to different environmental factors such as dissolved oxygen, salinity and temperature. Heavy metals are the positively charged compounds so they get directly bound to negatively charged DNA molecules and cause mutagenesis. The comet assay has an edge over other genotoxic techniques such as chromosome aberrations and sister chromatids exchange, as the cells used for comet assay need not to be mitotically active (Nagarani et al. 2012).

In the present study, there observed, concentration and duration dependent increase in DNA damage as tail length, %tail DNA and olive tail moment (OTM) in the fish, *Channa punctatus* in response to mercuric chloride. Similar observations were studied by previous workers too. The results on DNA damage in present study are in agreement with the findings of Matsumoto et al (2003) that showed the significant DNA damage in erythrocytes of *Oreochromis niloticus* when exposed to chromium. In another study, *Channa punctatus* when exposed to malathion showed significant DNA damage (Kumar et al. 2010). DNA damage observed in present study could be due to DNA single strand, DNA double strand breaks, DNA adducts formations, DNA-protein or DNA-DNA cross-links as proposed by (Kushwaha et al. 2012). The DNA damage was reported in *Lates calcarifer* in response to heavy metal, Cadmium and Mercury. There occurs significant (*p* < 0.01) increase in OTM in response to increase in duration to heavy metals. As per another theory, it was due to the formation of reactive oxygen species that leads to lipid peroxidation and increase in genotoxicity in the living system. The release of reactive oxygen species due to respiratory burst, by haemocytes are favourable to innate immune response. During the favourable environment, there is a balance between production and destruction of reactive species but in response to genotoxic compounds, this balance gets disrupted and causes DNA damage (Senthamilselvan et al. 2012).

The interaction of genotoxic compounds with DNA molecules leads to the formation of alkaline labile adducts that contributes to the DNA strand breakage through enzymatic removal of damaged nucleotides (Ahmed et al., 2017). It was reported that even low concentration of mercuric chloride leads to DNA damage in human hepatic cell line and this damage was produced through non-apoptotic mechanism (Bucio et al. 1999; Ozer et al. 2000).
Nuclear abnormalities including fragmented nucleus, vacuolated nucleus, binucleated and nuclear bud, along with appearance of micronucleus are taken as authentic indicators of cytogenotoxicity. These cytogenetic abnormalities are caused due to the clastogenic effects of toxicants and it might cause gene amplification and chromosomal detachment. There occurs vacuole formation in blood cells, which is due to the unequal distribution of haemoglobin and swelling of cells were caused because of necrosis of cellular membrane (Sharma and Chadha 2020). A significant decrease was observed in DNA damage in both genotoxic assays after 60 days of recovery, that suggests that there is possibility of full turnover of fish cells. Similar findings were observed by Marques et al. (2011) where DNA damage returned to control after 24hours on cessation of exposure. Another study on Trout exposed to vineyard pesticide showed decline in DNA damage during recovery period (Bony et al. 2008).

The changes in the levels of various biochemical constituents in fish in response to environmental toxicants were reported by various workers. Jagadeshwarhu and Devi (2018) studied the effect of sub-lethal concentrations of copper (1/16th, 1/12th, 1/8th and 1/4th of 96h LC$_{50}$ on the glucose, total lipids and glycogen in blood, liver and muscles of fish, Oreochromis mossambicus. There occurs concentration and time dependent increase in muscle, liver and blood glucose and decrease in lipid and glycogen levels. Mohiseni et al. (2016) studied the biochemical changes in the common carp in response to Lead and Cadmium exposure. It was observed that as compared to control, the level of plasma glucose were elevated throughout the exposure duration of lead, while in case of chromium exposure, plasma glucose levels increases in the beginning but after prolonged exposure, there occurs decline in its level till it gets depleted. The reason for this could be decline of energy reserves to handle the stress caused by heavy metal accumulation and this could be due to improper gluconeogenesis (Javed et al. 2017). A significant decrease was observed in glycogen content in liver in the freshwater fish, Channa punctatus in response to exposure to polluted water by waste of thermal power plant (Javed and Usmani. 2015). The increase in glucose content in response to heavy metals exposure is due to the enhanced glycogenolysis that results in the formation of more glucose to meet energy demand during stressful conditions (Javed et al. 2015). The alterations in glucose level might be due to renal injury, lack of nutrition and liver damage. During the environmental stress, glycogen level gets depleted and this leads to increase in glucose content. The glucose synthesis from non-carbohydrate sources such as amino acids and extra hepatic proteins, also leads to elevation in glucose levels (Thangamalathi et al. 2016). The decrease in protein content is due to the metabolic utilization of the ketoacids for the synthesis of glucose via gluconeogenesis pathway. The possible reason could be the formation of heat shock proteins that leads to heavy metal induced apoptosis (Sobha et al. 2007). The plasma protein contains albumins and this also causes decrease in albumin level. The necrosis of liver leads to liver damage and results in the increase in ALT, SGOT and SGPT levels in the blood and kidney and liver damage elevates cholesterol content in blood. The liver dysfunctioning shows hypothyroidism that results in increase in levels of total glycerides (Mohiseni et al. 2016). Lipids are the reserve source of energy. Mercuric chloride affects lipid metabolism of the fish and hence decrease in activity of NADPH and Glucose-6-phosphate dehydrogenase were observed and there occur high energy demands to survive in toxic environment (Bhilave et al. 2008).

The high concentrations of mercuric chloride showed increase in content of nitrogenous compounds and hence increases creatinine, bilirubin and uric acid in the blood due to that there occurs disruption to the
functioning of kidney and gills (Mutlu et al. 2015).

MN and nuclear aberrations are formed during the process of cell division. There occurs change in their expression at different times after damage to DNA, and it depends on the mechanism of induction and cell cycle kinetics. MN assay mainly targets interphase cells regardless of its karyotype. So, this assay is widely used as biomarker in environmental biomonitoring studies and this assay is successfully applied to study genotoxicity in fish (Bolognesi and Hayashi 2011; Kaur and Dua 2016). Mercury is regarded as potent mutagen. Its toxicity on tubulin, the structural subunit of the cellular microtubules that plays a vital role in spindle fibres formation and cytoplasmic organization, interferes to tubulin polymerization that causes contraction of chromosomes at metaphase stage of cell division, and leads to delayed division of centromere and reduction in anaphasic movement (Rocha et al. 2009).

Conclusion

Based on the results of the present study, it was concluded that mercuric chloride is toxic heavy metal that leads to acute and chronic toxicological alterations in the freshwater fish C. punctatus. The DNA damage studied by comet assay and micronucleus assay indicates the genotoxicity of this compound and act as a potent biomarker for environmental toxicity studies. The biochemical alterations suggest the oxidative stress in the body of specimen and acts as a potent biomarker to surrounding environment.

Declarations

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Ethical Approval: This work deals with the freshwater fish, Channa punctatus and this is a food fish and do not come under the preview of animal ethics committee in India.

Consent to Publish: All the authors agreed to publish this work in ESPR.

Conflict of Interest: The authors declare that there is no conflict of interest.

Consent to Participate: Not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Author contributions: H.K.G and A.D designed study, H.K.G and K.P. performed the experiments and statistical analysis, A.D, H.K.G and K.P drafted the manuscript. All the authors have read and approved the final manuscript.

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**Figures**
Figure 1

Scatter plot representing percentage mortality in Channa punctatus with respect to various concentrations of Mercuric chloride.

\[ y = 34.554x - 4.5714 \]

\[ R^2 = 0.9513 \]
Figure 2

Graphs showing DNA damage in erythrocytes in terms of tail DNA (%) (a-b); tail length (c-d); olive tail moment (e-f) in fish *C. punctatus* subjected to mercuric chloride for 15, 30 & 60 days along with comparison of recovered and treated fish for 60 days. Values are mean±S.E. (vertical bars); mean followed by different superscripts (a-c) show variation among concentrations and different superscripts (P-R) show variation among duration statistically at α= 5%
Figure 3

Erythrocytes from Channa punctatus on exposure to Mercuric chloride for 15 days. a: Control; b: Conc.1 (0.046 mg/L), c: Conc. 2 (0.069 mg/L) and d: Conc. 3 (0.138 mg/L)
Figure 4

Erythrocytes from Channa punctatus on exposure to Mercuric chloride for 30 days. a: Control; b: Conc. 1 (0.046 mg/L), c: Conc. 2 (0.069 mg/L) and d: Conc. 3 (0.138 mg/L)
Figure 5

Erthrocytes from Channa punctatus on exposure to Mercuric chloride for 60 days. a: Control; b: Conc. 1 (0.046 mg/L), c: Conc. 2 (0.069 mg/L) and d: Conc. 3 (0.138 mg/L)
Figure 6
Erthrocytes from Channa punctatus on exposure to Mercuric chloride after Recovery of 60 days. a: Control; b: Conc.1 (0.046 mg/L), c: Conc. 2 (0.069 mg/L) and d: Conc. 3 (0.138 mg/L)
Figure 7

Graphs showing erythrocytes (a,b) MN & (c,d) aberrations frequencies in fish C. punctatus subjected to mercuric chloride for 15, 30 & 60 days along with comparison of recovered and treated fish for 60 days. Values are mean±S.E. (vertical bars); mean followed by different superscripts (a-c) show variation among concentrations and different superscripts (P-R) show variation among duration statistically at α= 5%
Figure 8

Erythrocytic nucleo-cellular abnormalities in the blood of Channa punctatus exposed to mercuric chloride. Abbreviations No- Normal cell, Cl- Clumped cell, Mn- Micronuclei, Sp- Spindle cell, Sh- Spherocyte, Ne- Necrotic cell, Ir- Irregular cell, Not- Notched cell, De- Degmacyte, Bn- Binucleated, Nu- Nucleoids. The statistically significant values (p<0.05; p<0.01) of MN and aberrations in erythrocytes on all the exposure concentration and durations showed the genotoxic and cytotoxic nature of mercuric chloride.
Figure 9

Graphs showing comparison of glucose (a,b), cholesterol (c,d), creatinine (e,f), urea (g,h), SGOT (i,j), albumin (k,l), SGPT (m,n), Total glycerides (o,p), bilirubin (q,r), ALP (s,t) and Total protein (u,v) in fish Channa punctatus subjected to mercuric chloride for 15, 30 & 60 days along with comparison of recovered and treated fish for 60 days. Values are mean±S.E. (vertical bars); mean followed by different superscripts (a-d) show variation among concentrations and different superscripts (P-S) show variation among duration statistically at α = 5%