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Studies on Induction of Nuclear Abnormalities in Peripheral Blood Erythrocytes of Fish Exposed to Copper

Safina Kousar[1,*], Muhammad Javed[2]

[1] GC Women University, Department of Zoology, Faisalabad. 38040, Pakistan.
[2] University of Agriculture, Department of Zoology, Fisheries and Wildlife, Faisalabad.

* Corresponding Author: Tel.: +92.0331 8769437; Fax: +92.0331 8769437;
E-mail: safina_137@yahoo.com

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Abstract

A wide variety of chemicals especially metals contaminate the aquatic ecosystem and stimulate a variety of toxicity mechanisms, such as oxidative damage to proteins, DNA and lipids. Very limited efforts have been made to investigate the genotoxic effects of metals in fish. Therefore, the present study was conducted to determine copper induced genotoxic damage in peripheral blood erythrocytes of 150-day old four freshwater fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* by using micronucleus test. For this purpose, 96-hr LC_{50} and lethal concentration of copper for all the four fish species were determined. Each fish species was exposed to four different sub-lethal concentrations (17, 25, 33, 50% of 96-hr LC_{50}) of copper, separately, for a period of 30 days. Results showed exposure concentration dependent increases in the frequencies of micronuclei and other nuclear abnormalities. Findings of the experiment also confirmed that the micronucleus test acts as a useful tool in determining the potential genotoxicity of metals in erythrocytes of various fish species. Among four fish species, *Cirrhina mrigala* showed significantly higher frequency of micronuclei with the mean value of 16.98±11.70%, followed by that of *Ctenopharyngodon idella*, *Catla catla* and *Labeo rohita*. However, frequency of other nuclear abnormalities followed the order: *Labeo rohita* > *Cirrhina mrigala* > *Ctenopharyngodon idella* > *Catla catla*.

Keywords: Acute toxicity, metals, major carps, frequency of Micronuclei and other nuclear abnormalities, sub-lethal concentration.

Introduction

Genotoxic compounds represent major ecological challenges because they may lead to unusual disorders that could be transmitted to the next generations (Haldsrud and Krokje, 2009). Copper, found in all natural waters and sediments, act as an essential trace metal for living organisms. However, its elevated levels are reported to cause higher genotoxic damage in exposed organisms by inducing oxidative stress and generation of reactive oxygen species (Gabbianelli *et al*., 2003).

Genotoxic potential of metals can be successfully monitored in freshwater environments by the application of reliable techniques, such as Micronucleus test used for evaluating DNA damage, on exposed sentinel species (Frenzilli *et al*., 2009; Bolognesi and Hayashi, 2011). Micronucleus test has been commonly used for the estimation of biological impacts of water pollutants on genotoxic damage in fish (Ergene *et al*., 2007). Micronuclei are very small fragments of chromatin material which are developed from broken section of chromosome or from the chromosomes that could not be incorporated into daughter nuclei (Fagr *et al*., 2008). Various metallic ions act as valuable genotoxins at particular concentrations just because of their ability to bind to thiol groups and induce instability in the spindle formation in the cells (Patra *et al*., 2004). Thus, micronucleus test ensures continuous and effective evaluation of metallic pollution level in aquatic environments (Obiakor *et al*., 2012).

In aquatic ecosystem, fish species are the creatures that take higher place in food chain and are significant food sources for human beings. In addition to that in aquatic ecosystem, the fish could not escape from detrimental effects of various toxicants. This is the reason due to which fish are generally used as indicators of metallic ion pollution in aquatic habitats (Agah *et al*., 2009). As indicator of genotoxicity, the formation of micronuclei in peripheral blood erythrocytes of fish was evaluated during this experiment. Moreover, selection of peripheral blood erythrocytes of fish as a target cell to investigate genotoxic damage was based on the important role of blood in movement of toxic substances absorbed

through skin, gills and digestive tract of various fish species. Keeping in view the economic importance of carps in Pakistan, four fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* were selected to determine the genotoxic effects of copper on fish blood. However, the main objective of present study was to assess species specific as well as concentration dependent genotoxic effects of copper on fish.

Materials and Methods

Phase I:

The experiment was conducted in the laboratories of Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. For this project 150-day old four fish species were collected from the Fish Seed Hatchery, Faisalabad and transported to the wet laboratory with proper care. Fish fingerlings of all the four species were acclimatized to laboratory conditions for two weeks prior to experiments. During this period fish fingerlings were fed to satiation on feed (34% digestible protein and 3.00 Kcal/g digestible energy) twice daily. Remains of feed and excretory waste were siphoned off daily to avoid stress on the fish. Analytical grade copper chloride ($CuCl_2$: Merck) was used to test its effects on selected fish species. Glass aquaria (60 liter water capacity) were used to carry out acute toxicity tests with four fish species, separately. The aquaria were thoroughly rinsed and filled with 50 liter dechlorinated tap water. Ten fish of each species were placed in aquarium, separately, for each test concentration of metallic ions. Metal concentration of water was started from zero with an increment of 0.05 and 5mgL^{-1} for low and high concentration, respectively. The average weight of fish under experiment is given in the Table 1.

To avoid instant stress to the fish, the metallic ion concentration of each test media was increased gradually to achieve the 50% test concentration within 3 hours and full toxicant concentration in 6 hours. Test media were supplied with constant air through capillary system fitted with an air pump. Temperature (30C^{o}), hardness (300 mgL^{-1}) and pH (7.5) were maintained throughout the experimental period. The fish were not fed during acute toxicity trials. The concentration of metal was started from zero up to that concentration at which 50% and 100% fish mortality occurred during 96 hours test duration. During 96-hr toxicity tests, the observations on fish mortality were made after every 2 hours. The dead fish were immediately removed from the medium. No mortality was recorded in the control fish species placed in clean metal free water. At the end of each test, water samples were taken and analyzed for the desired metal concentration by following the methods described in SMEWW (1989). The analyzed concentrations of copper in the test media coincided quite satisfactorily with the desired concentrations. The acute toxicity tests for each fish species were conducted in three replications for each test concentration. However, their arithmetic means were expressed in the results.

Phase II:

In order to study the genotoxic effects of metals on fish, experiments were conducted with 150-day old fish species in glass aquaria under controlled laboratory conditions. After acclimatization all the four fish species were exposed to four different sub-lethal concentrations viz. 17%, 25%, 33% and 50% of 96-hr LC_{50} of copper in 50 liter glass aquaria, separately, for 30 days. Fish, 10 for each sub-lethal concentration, were kept in glass aquaria with three replications for each treatment.

Fish grown in metal free tap water used as negative control. During present investigation cyclophosphamide (Sigma) is used as positive control treatment at a rate of 20µgg^{-1} body weight. It is an alkylating agent and thus has clastogenic effects on various animal species (Cavas and Ergen., 2005). Cyclophosphamide has been approved to cause significant genotoxic effects in *O. niloticus* (Ozkan *et al*., 2011). Continuous aeration was supplied to all fish aquaria through capillary system. All toxicity trials were conducted under constant water hardness (300 mgl^{-1}), pH (7.5) and temperature (30^{o}C). Fish were fed to satiation with feed (34% DP and 3.00Kcal/g DE) twice daily throughout the experimental period. The test media were replaced weekly and toxicant concentration was maintained up to required level.

Micronucleus Test:

After 30-day exposure of copper, blood samples taken from caudal vein of fish were directly smeared on slides and air-dried. Smears were subsequently

Table 1. The average weight of fish under experiment

Fish Species	Average Weight (g)
Labeo rohita	14.47±0.43
Cirrhina mrigala	11.28±0.67
Catla catla	19.66±0.24
Ctenopharyngodon idella	10.58±0.33

fixed in methanol for 10 minutes and stained with the 10% Giemsa solution (Wright's stain powder 300mg + Giemsa stain powder 30mg + Absolute methyl alcohol 100ml) for 8 minutes (Barsiene *et al*., 2004). Duplicate slides were prepared and examined from each copper exposed fish species, separately, for scoring nuclear changes and micronucleus frequencies under oil emersion (100 X) lens.

Scoring of Cells with Micronuclei and Other Nuclear Abnormalities:

Blind scoring of micronuclei and other nuclear abnormalities were performed on coded slides. A total of 2,000 erythrocytes (1000/slide) with intact cellular and nuclear membranes were examined for each fish specimen. The frequency of micronuclei and other nuclear abnormalities including bi-nucleated cells, dumbbell shaped nuclei, blebbed, notched and de-shaped nuclei were evaluated (per 1,000 cells) by scoring at a 1,000X magnification using 50i bright-field microscope (Figure 1).

For scoring of micronuclei, a criterion devised by Fenech *et al*. (2003) was adopted. According to these criteria the diameter of micronuclei should be less than one third of main nucleus. Micronuclei should be separated or marginally overlap with main nucleus as long as there is clear identification of nuclear boundary. Micronuclei should have similar staining as the main nucleus. Micronuclei should be on same plane of focus as the main nuclei. Frequency of micronuclei was calculated by using the following formula:

$$\text{Micronucleus Frequency (\%)} = \frac{\text{Number of cells with micronuclei}}{\text{Total number of cells counted}} \times 100$$

Statistical Analysis:

MINITAB computer program, based on Probit method, was used to statistically analyze the fish mortality data. The 96-hr LC_{50} and lethal concentrations of copper for four fish species were determined, separately, by using the Probit analysis method (Hamilton *et al*., 1977). Data obtained from this experiment was analyzed statistically by using MICROSTAT package of computer by following Steel *et al*., (1996). Mean values of 96-hr LC_{50} and lethal concentrations for each fish species were obtained at 95% confidence intervals. Data were statistically analyzed by using Factorial design (RCBD). Normality of distribution of data was assessed through Analysis of Variance (ANOVA). To compare the frequency of micronuclei and other nuclear abnormalities between control and treated fish groups, the non-parametric Mann-Whitney U-test was performed (Steel *et al*., 1996). The relationships among various parameters were also established by using regression analyses.

Results

Acute Toxicity Tests:

Acute toxicity tests showed significant ($P<0.05$) variations in the tolerance limits, in-terms of 96-hr LC_{50} and lethal concentration, of four fish species

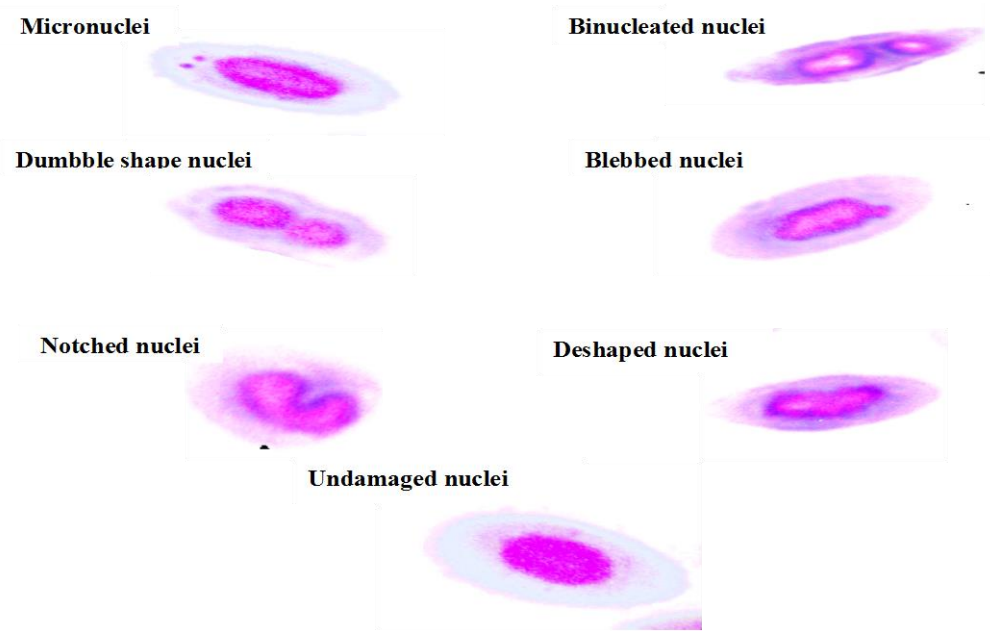

Figure 1. Micronuclei and other nuclear abnormalities observed in the peripheral erythrocytes of fish due to metals.

against copper exposure (Table 2). Among four fish species, *Labeo rohita* was appeared significantly ($P<0.05$) least sensitive to copper, followed by that of *Ctenopharyngodon idella*, *Cirrhina mrigala* and *Catla catla* with mean 96-hr LC_{50} values of 33.41±0.03, 21.36±0.42, 17.17±0.24 and 16.85±0.20 mgL^{-1}, respectively (Table 2).

Frequency of Micronuclei and other Nuclear Abnormalities in Fish Blood Erythrocytes:

In *Labeo rohita*, the frequency of micronuclei in peripheral blood erythrocytes were significantly higher with a mean value of 28.59±0.11 % at 50% copper LC_{50}, followed by that of 20.64±0.10, 14.29±0.35, 13.32±0.31, 10.50±0.11 and 0.50±0.18% observed due to 33%, positive control, 25%, 17% LC_{50} and negative control treatments, respectively. Frequency of all other nuclear abnormalities including binucleated cells, dumble shape, blebbed, notched and deshaped nuclei were significantly higher at 50% copper LC_{50} exposure while the same remained significantly lower due to negative control (Table 3).

In *Cirrhina mrigala*, copper at 50% LC_{50} exposure caused significantly higher mean micronuclei frequency of 35.05±0.88% while it was significantly lowest (0.24±0.12%) due to negative control. In peripheral blood erythrocytes of *Cirrhina mrigala*, the frequency of all other nuclear abnormalities, except notched nuclei, was significantly higher due to 50% copper LC_{50} exposure, while these were significantly lower due to negative control treatment (Table 3).

Copper exposure to *Catla catla* at 50% LC_{50} induced significantly higher mean micronuclei frequency of 26.13±0.36%, than positive control (24.88±0.48%). However, the frequency of micronuclei varied significantly among different treatments that followed the order: 50% > positive control > 33% > 25% > 17 % copper LC_{50} > negative control. Frequency of other nuclear abnormalities viz. binucleated erythrocytes, dumble shapped, blebbed, notched and deshaped nuclei were significantly higher due to exposure of 50% copper LC_{50} (Table 3).

In peripheral blood erythrocytes of *Ctenopharyngodon idella*, the exposure of copper at 50% LC_{50} caused significantly higher induction of micronuclei, than positive control treatment with the mean values of 23.66±0.47 and 13.99±0.22%, respectively. However, frequency of all other nuclear abnormalities, except blebbed nuclei, was significantly higher for positive control fish (Table 3).

Table 4 shows the mean frequency (at all sub-lethal concentrations of metal) of micronuclei and other nuclear abnormalities induced in peripheral blood erythrocytes of four freshwater fish species due to copper exposure. Among four fish species, *Cirrhina mrigala* showed significantly higher frequency of micronuclei with the mean value of 16.98±11.70%, followed by that of *Ctenopharyngodon idella*, *Catla catla* and *Labeo rohita*. However, *Labeo rohita* and *Catla catla* showed statistically ($P<0.05$) similar frequency of micronuclei. Mean frequency of all other nuclear abnormalities was significantly higher in peripheral blood erythrocytes of *Labeo rohita*, followed by that of *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Catla catla* with the mean values of 18.00±10.02, 16.73±11.13, 14.95±9.35 and 13.65±12.92%, respectively.

Micronuclei frequency determined in peripheral blood erythrocytes of all the four fish species showed significantly higher dependence on metal concentration (Table 5). The partial regression coefficients for *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* were positive and highly significant at $P<0.01$. In addition to that, frequency of other nuclear abnormalities showed significantly higher dependence on copper concentration. Moreover, the high value of R^2 for each fish species predicts significantly high reliability of these regression models (Table 5).

Discussion

During this experiment, the acute toxicity of copper, in-terms of 96-hr LC_{50} and lethal concentration, to *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* was determined at constant water temperature (30°C), hardness (300 mgL^{-1}) and pH (7.5). It is observed during present investigation that the toxicity of copper to all the four fish species varied significantly ($P<0.05$). The variation in tolerance limits of four fish species for copper is attributed to their physiological differences and species-specificity to interact against

Table 2. Acute toxicity of copper observed for the fish

Fish Species	Mean 96-hr LC_{50} (mgL^{-1})	95% Confidence Interval (mgL^{-1})	Mean 96-hr Lethal Concentration (mgL^{-1})	95% Confidence Interval (mgL^{-1})
Labeo rohita	33.41±0.03 a	31.19 - 35.18	45.53±0.04 a	42.76 - 50.28
Cirrhina mrigala	17.17±0.24 c	15.88 - 18.17	24.33±0.12 c	22.73 - 27.11
Catla catla	16.85±0.20 d	15.64 - 17.83	23.83±0.05 d	22.24 - 26.55
Ctenopharyngodon idella	21.36±0.42 b	19.72 - 22.61	31.61±0.26 b	29.49 - 35.19

CI = Confidence Interval; Means with similar letters in a single column are statistically non-significant at $P<0.05$.

Table 3. Frequency (%±SD) of micronuclei and other nuclear abnormalities in the peripheral blood erythrocytes of fish exposed to various concentrations of copper

	Negative control	Positive control	17% of LC_{50}	25% of LC_{50}	33% of LC_{50}	50% of LC_{50}
Labeo rohita						
Micronuclei frequency (%)	0.50±0.18 f	14.29±0.35c	10.50±0.11 e	13.32±0.31d	20.64±0.10 b	28.59±0.11a
Binucleated cells(%)	0.15±0.00 e	2.98±0.00 b	0.84±0.01 d	2.30±0.03 c	2.40±0.04 c	3.25±0.01 a
Cells with dumble shape nucleus (%)	0.55±0.00 f	4.56±0.18 b	2.24±0.03 e	2.95±0.06 d	3.52±0.09 c	6.21±0.05 a
Cells with blebbed nucleus (%)	0.25±0.07 f	4.17±0.09 b	1.60±0.12 e	2.30±0.03 d	2.94±0.03 c	4.85±0.12 a
Cells with notched nucleus (%)	0.35±0.10 f	7.34±0.22 b	4.78±0.19 e	5.30±0.04 d	5.93±0.06 c	9.13±0.02 a
Deshaped cells	0.60±0.17 f	6.05±0.15 b	3.38±0.22 e	4.61±0.10 d	5.10±0.12 c	7.28±0.11 a
Cirrhina mrigala						
Micronuclei frequency (%)	0.24±0.12 f	13.57±0.33d	12.21±0.54 e	17.26±0.06 c	23.54±0.21 b	35.05±0.88 a
Binucleated cells (%)	0.24±0.02 f	1.35±0.05 c	0.83±0.03 d	0.75±0.07 e	6.36±0.28 b	7.77±0.38 a
Cells with dumble shape nucleus (%)	0.82±0.02 f	2.75±0.03 d	1.67±0.02 e	2.99±0.05 c	5.26±0.21 b	6.69±0.29 a
Cells with blebbed nucleus (%)	0.14±0.04 f	1.96±0.06 e	3.19±0.07 c	2.19±0.12 d	4.88±0.08 b	6.78±0.17 a
Cells with notched nucleus (%)	0.82±0.04 f	1.68±0.04 d	2.21±0.01 b	2.39±0.02 a	1.44±0.16 e	2.16±0.03 c
Deshaped cells (%)	0.10±0.01 f	3.64±0.03 e	4.95±0.03 d	6.93±0.10 c	7.03±0.09 b	10.42±0.2 a
Catla catla						
Micronuclei frequency (%)	0.89±0.06 f	24.88±0.48 b	1.15±0.10 e	14.14±0.04 d	21.60±0.16c	26.13±0.36 a
Binucleated cells (%)	0.35±0.01 f	1.37±0.03 c	0.45±0.03 e	1.19±0.112 d	3.75±0.08 b	6.38±0.11 a
Cells with dumble shape nucleus (%)	0.10±0.01 f	3.14±0.04 c	0.95±0.05 e	1.99±0.05 d	5.25±0.03 b	8.96±0.10 a
Cells with blebbed nucleus (%)	0.20±0.02 f	4.22±0.12 b	0.35±0.02 e	1.05±0.02 d	3.10±0.10 c	7.13±0.03 a
Cells with notched nucleus (%)	0.15±0.10 f	2.16±0.03 c	0.65±0.04 e	1.99±0.19 d	4.20±0.07 b	7.72±0.24 a
Deshaped cells (%)	0.10±0.04 e	3.78±0.07 b	0.75±0.07 d	1.34±0.04 c	3.70±0.06 b	5.44±0.09 a
Ctenopharyngodon idella						
Micronuclei frequency (%)	1.97±0.39 f	13.99±0.22d	13.07±0.26 e	17.16±0.66c	21.81±0.53 b	23.66±0.47 a
Binucleated cells (%)	0.10±0.01 f	3.47±0.26 a	0.38±0.16 e	1.46±0.20 c	0.96±0.15 d	1.71±0.21 b
Cells with dumble shape nucleus (%)	0.20±0.03 f	5.33±0.16 a	2.06±0.09 e	2.73±0.19 d	4.14±0.10 c	5.12±0.11 b
Cells with blebbed nucleus (%)	0.79±0.03 e	4.30±0.11 b	1.05±0.02 d	3.51±0.10 c	4.33±0.06 b	5.26±0.18 a
Cells with notched nucleus (%)	0.30±0.02 f	7.53±0.22 a	0.81±0.01 e	3.22±0.02 d	3.95±0.03 c	4.60±0.06 b
Deshaped cells (%)	0.49±0.11 f	5.67±0.26 a	2.06±0.17 e	3.80±0.17 d	4.81±0.21 c	5.55±0.17 b

Means with similar letters in a single row are statistically non-significant at P<0.05.

Table 4. Induction of micronuclei and other nuclear abnormalities (mean±SD) in peripheral erythrocytes of various fish species exposed to copper

	Fish Species			
Frequency (%)	*Labeo rohita*	*Cirrhina mrigala*	*Catla catla*	*Ctenopharyngodon idella*
Micronuclei	14.64±9.48 c	16.98±11.70 a	14.80±11.46 c	15.28±7.75 b
Other nuclear abnormalities	18.00±10.02 a	16.73±11.13 b	13.65±12.92 d	14.95±9.35 c

Means with similar letters in a single row are statistically non-significant at P<0.05.

Table 5: Relationships between exposure concentrations of copper and frequency of nuclear abnormalities induced in peripheral erythrocytes of fish

	Fish Species	Regression Equation	SE	r/MR	R^2
Micronuclei frequency (%)	*Labeo rohita*	3.88+1.57** (Concentration)	0.066	0.991	0.982
	Cirrhina mrigala	1.06+3.85** (Concentration)	0.311	0.969	0.939
	Catla catla	15.11+6.02**(Concentration)	0.029	0.992	0.984
	Ctenopharyngodon idella	1.48+2.08** (Concentration)	0.249	0.935	0.874
Other nuclear abnormalities (%)	*Labeo rohita*	0.65+1.69** (Concentration)	0.075	0.990	0.980
	Cirrhina mrigala	0.44+4.02** (Concentration)	0.046	0.999	0.998
	Catla catla	6.33+4.19** (Concentration)	0.559	0.922	0.850
	Ctenopharyngodon idella	9.08+1.47** (Concentration)	0.169	0.940	0.883

SE: Standard Error; r: Multiple Regression Coefficient; R^2: Coefficient of Determination; ** Highly Significant at P<0.01

heavy metals (Svecevicius, 2010) or /and due to differences in the rate of synthesis of metallothionein protein (Hollis *et al*., 2001). Metallothionein works as metal-chelating agent that plays an important role in the regulation, detoxification and depuration of metals from the body of aquatic organisms, through oxygen free radical scavenging actions and metal binding (Kalpaxis *et al*., 2003). Results obtained from this experiment was in accordance with Javid *et al*. (2007), Azmat *et al*. (2012) and Yaqub and Javed (2012). They reported variability in the sensitivity of different species of Indian major carps towards metals toxicity.

During this study it was observed that exposure to copper caused significant increase in the frequency of micronuclei and other nuclear abnormalities in peripheral erythrocytes of *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*. Jiraungkoorskul *et al*. (2007) previously demonstrated the induction of micronuclei and other nuclear abnormalities in the erythrocytes of three fish species viz. *Oreochromis niloticus*, *Poronotus triacanthus* and *Puntius altus* at 25% of 96-hr LC_{50} of lead, copper and cadmium and reported significant increase in micronuclei frequency and frequency of other nuclear abnormalities (notched, blebbed, lobed and binucleated cells) in fish erythrocytes after 48-hr exposure. Induction of micronuclei and other nuclear abnormalities in the erythrocytes of *C. carpio* due to copper exposure was also reported by Zhu *et al*. (2004). Guner *et al*. (2011) reported significantly increased frequency of nuclear abnormalities in the erythrocytes of *G. affinis* due to copper and cadmium toxicity. The findings obtained from this experiment reinforced the evidences of copper induced genotoxic action in fish. Fish can respond to mutagens present in aquatic habitat even at very low concentration. These mutagens may cause micronuclei formation in cells. However, repairement process in fish is very slow as compared to other animals. Thus fish might be used as model species to check the presence of toxic chemicals in aquatic environment (Mahboob *et al*., 2013).

Results of present investigation showed concentration dependent increase in the frequency of micronuclei and other nuclear abnormalities, observed in peripheral erythrocytes of all the four fish species. These findings are similar to the Ahmed *et al*. (2011) who also observed concentration dependent increase in micronuclei frequency in the erythrocytes of *Oreochromis mossambicus* due to arsenic. Moreover, in *Channa punctatus*, the frequency of micronuclei was found to be significantly higher due to arsenic exposure while it was lower in control fish (Patowary *et al*., 2012).

Among the four fish species, *Cirrhina mrigala* was significantly more sensitive to copper while *Labeo rohita* was significantly least sensitive. Micronucleus test showed inter-species variability in micronuclei frequency as reported by Buschini *et al*. (2004), Kim and Hyun (2006), Barbosa *et al*. (2010) and Kumar *et al*. (2010). These variations may occur due to difference in metabolic indices of different fish species, DNA repairement ability as well as cell proliferation in tissues. Genotoxic damage mainly depends on the type of pollutant involved and fish species exposed to that pollutant (Ali *et al*., 2008). During the present investigation, frequency of micronuclei and other nuclear abnormalities showed highly significant and positive relationship with copper concentration. These results are in conformity with Summak *et al*. (2010) who also reported significantly positive correlation (r=0.980) between metal (Al, Cu, Ni, Pb, Zn, Cd) concentrations and frequency of nuclear abnormalities in *Oreochromis niloticus*. Similarly Ergene *et al*. (2007) also reported significant linear relationship between metal (Cu, Cd, Ni, Pb) concentration and frequency of micronuclei and other nuclear abnormalities in erythrocytes of mullet and catfish.

From this study it is concluded that copper is highly toxic metal causing genotoxicity to the fish. It is responsible for concentration dependent increase in the frequency of micronuclei and other nuclear abnormalities in peripheral blood erythrocytes of all the four fish species under study. In addition to that result also revealed the species specific genotoxic behavior of copper. Based on the genotoxic nature of copper it can also be used as biomarker to assess the pollution level in aquatic environment.

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