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Original Research Article

Assessment of DNA damage in peripheral blood erythrocytes of fish exposed to arsenic under laboratory conditions

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A B S T R A C T

A growing interest in the environmental genotoxicity studies has led to the development of several tests for detection of genotoxic substances in the aquatic environment. Metals gained attention because of their harmful effects on the physiology of aquatic organisms especially fish. This study was conducted to determine the genotoxic effects of arsenic in peripheral blood erythrocytes of four freshwater fish species (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*) by using Comet assay. All these fish species are of economic importance and also has great food value. In order to access the genotoxic effects of metal, fish fingerlings were exposed to four different sub-lethal concentrations of (17, 25, 33 and 50%) of arsenic for a period of 30 days. All the four fish species showed concentration dependent increase in the extent of DNA damage with significantly higher damage at 50% of arsenic LC_{50} exposure. However, DNA damage was significantly lower due to negative control treatment. All the four fish species showed variable response to genotoxic effects of metals. Among four fish species, *Cirrhina mrigala* showed significantly higher percentage of damaged cells, genetic damage index and cumulative tail length of comets with the mean values of 86.67±1.15%, 2.76±0.00 and 347.21±0.10 µm, respectively. This study indicated that arsenic, in the natural water bodies of Pakistan, can induce DNA damage in peripheral blood erythrocytes of fish.

Introduction

Various geogenic and anthropogenic activities caused the release of toxic metals in aquatic ecosystem (Reimer *et al*., 2002). Fish appear as pragmatic indicator for assessment of pollutants in aquatic environments (Vutukuru *et al*., 2005; Javed, 2012). In the fish body, arsenic exists in different oxidation state: these include methylated arsenic, arseno-sugar and arseno-lipids. All these forms of arsenic vary in their toxicity. Different combinations of these oxidation states are believed to be responsible for the pathophysiology of arsenic (Bears *et al*., 2006). Although much work was done to assess the acute toxicity of arsenic to fish by different workers (Roy *et al*., 2006; Ghosh *et al*., 2006) but its genotoxic effects on different fish systems

are yet to be characterized. Exposure to lethal concentrations of arsenic resulted in pronounced disturbance of physiological processes occurring in fish body (Bears *et al*., 2006) and also induced the production of stress proteins (Roy and Bhattacharya, 2005).

The increased production of stress proteins is responsible for induction of oxidative stress in fish (Bhattacharya and Bhattacharya, 2007). As a result of oxidative damage due to reactive oxygen species, DNA strand breakage occurs which represents a major class of DNA damage under oxidative stress. It is also responsible for various modifications in DNA bases (Cadet *et al*., 1997). Arsenic has long been observed genotoxic both in in-vivo and in-vitro conditions (Dopp *et al*., 2004). It causes DNA damage either through reactive oxygen species production or modulating the levels of antioxidant compounds i.e. glutathione (Bhattacharya and Bhattacharya, 2007), interruption in methylation reactions taking place in cell (Zaho *et al*., 1997) or by blocking DNA repair system (Hartwig, 1998).

Along with DNA strand breakage arsenic also has the ability to cause formation of DNA-protein cross links (Gebel *et al*., 1998). Among various approaches used for the evaluation of DNA strand breakage, Comet Assay has been used as most reliable, responsive and fast technique for assessment of genotoxic potentials of environmental pollutants on fish (Avishai *et al*., 2002). This assay is used for the detection of single/double strand breakage and alkali labile sites, induced in individual eukaryotic cells by physical and chemical pollutants (Kim *et al*., 2002). This assay has also been reported to be applied to the peripheral erythrocytes of various fish species exposed to diverse genetic toxicants (Matsumoto *et al*., 2006). It allows the investigators to assess the DNA damage at initial level by measuring the migration of broken DNA fragments in gel embedded cells (Bombail *et al*., 2001). Thus, present study was conducted to determine the genotoxic effects of arsenic in peripheral blood erythrocytes of four freshwater fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *catla catla* and *Ctenopharyngodon idella*. For this experiment it was hypothesized that different fish species will show variable ability to tolerate adverse effects of metals that would cause genotoxicity in fish blood erythrocytes.

Materials and Methods

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 arsenic trioxide (As_2O_3 : 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. Fish fingerlings (150-day old) belonging to four species with following average wet weights and total lengths were selected for acute toxicity trials:

Table for fish Wet weight and Length

Fish Species	Average Wet Weight (g)	Average Total Length (mm)
Labeo rohita	14.47±0.43	110.33±2.95
Cirrhina mrigala	11.28±0.47	101.53±1.17
Catla catla	19.66±0.24	121.42±2.40
Ctenopharyngodon idella	10.58±0.33	99.78±1.63

Ten fish of each species were placed in aquarium 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. 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 S.M.E.W.W (1989). The analyzed concentrations of arsenic 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.

Arsenic Induced DNA Damage in Fish

The experiments were conducted to determine the extent of DNA damage in the peripheral blood erythrocytes of four fresh water fish species exposed to arsenic. For this purpose, 150-day old fish of each species were acclimatized to laboratory conditions in cemented tanks prior to the experiments. To determine DNA damage, each fish species was exposed to four different sub-lethal concentrations (17%, 25%, 33% and 50% of 96-hr LC_{50}) of arsenic, separately, for a period of 30 days. Each test was conducted with three replications. Temperature, pH and hardness of each test medium were maintained at 30C^{o}, 7.5 and 300mgL^{-1}, respectively. During exposure period, the fish were fed to satiation twice daily on feed (34% Digestible Protein and 3.00k cal/g Digestible Energy).

The control fish were kept in clean metal free water for comparison as negative control while Cyclophosphamide (Sigma) was used as positive control. Fish blood samples were processed for Comet assay according to Singh *et al*. (1988) with minor modifications. After 30-day metallic ions exposure period, blood samples were collected from the caudal vein of fish through sterilized syringe and processed for Comet assay. One hundred and fifty cells were scored and examined randomly under Epi-Fluorescence microscope (N-400M, American Scope; UK) equipped with light source of mercury short are reflector lamp filters for ethidium bromide at 400 X magnification and low lux (MD-800, American Scope; UK) camera. Cells with no DNA damage possess intact nuclei without a

tail, whereas cells with DNA damage showed comet like appearance. The length of DNA migration in the comet tail is an estimate of DNA damage (Grover *et al*., 2003). The cells with no head or dispersed head were regarded as apoptotic cells and were not included in the analyses. The DNA damage was quantified by visual classification of cells into five categories "comets" corresponding to the tail length, undamaged: Type 0, low-level damage: Type I, medium-level damage: Type II, high-level damage: Type III and complete damage: Type IV. TriTek CometScore^{TM} (Summerduck, USA) software was used to measure the comet tail length of damaged cells (Costa *et al*., 2011; Jose *et al*., 2011) and cumulative tail length of all examined cells (n=50 per replicate). The 96-hr LC_{50} and lethal concentrations of arsenic for four fish species were determined, separately, by using the Probit analysis method (Hamilton, 1977). Mean values of 96-hr LC_{50} and lethal concentrations for each fish species were obtained at 95% confidence intervals. MINITAB computer program, based on Probit method, was used to statistically analyze the fish mortality data. Data were statistically analyzed by using Factorial design (RCBD). Means were compared for statistical differences through Tukey's student Newnan-Keul test (Steel *et al*., 1996). Both parametric and non-parametric tests were used in order to evaluate differences at 0.05 level of significance. Normality of distribution of data was assessed through Analysis of Variance (ANOVA).

Results and Discussion

Acute Toxicity Tests: Tolerance limits of four fish species, in-terms of 96-hr LC_{50} and lethal concentration varied significantly (Table 1). Among four fish species *Labeo rohita* appeared significantly ($p<0.05$) least sensitive to arsenic, followed by that of *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Catla catla*. However, *Catla catla* appeared significantly ($p<0.05$) more sensitive to arsenic with 96-hr LC_{50} and lethal concentration values of 10.16±0.22 and 14.05±0.20 mgL^{-1}, respectively (Table 1).

Arsenic induced DNA Damage in Peripheral Blood Erythrocytes of Fish

a. *Labeo rohita*: Table 2 shows significantly variable proportions of damaged nuclei in the peripheral blood erythrocytes of fish. However, the proportion of damage is changed with the exposure concentrations of arsenic. At 50%, 33% and 25% LC_{50} of arsenic, significantly ($p<0.05$) higher damage was observed in the nuclei of fish erythrocytes as compared positive control. Among the exposure concentrations, 50% LC_{50} caused significantly maximum GDI of 2.15±0.18, followed by that of 33% positive control, 25%, 17% LC_{50} and negative control. However, GDI computed at 33% LC_{50} and positive control treatment did not vary significantly ($p<0.05$). Cumulative tail length of comets, induced due to various concentrations of arsenic, positive and negative controls, ranged between the mean values of 195.40±0.40 and 3.69±40 µm with statistically significant difference at $p<0.05$.

b.*Cirrhina mrigala*: In the fish blood, the frequency of DNA damaged cells increased significantly with concomitant increase in exposure concentration of arsenic. However, mean damage (86.68±1.15%) caused by 50% arsenic LC_{50} was significantly higher than that caused by positive control 51.33±4.16%. The mean GDI values in fish varied significantly ($p<0.05$) due to various concentrations of arsenic, positive and negative controls that followed the order: 50% > 33% > 25% > 17% > positive control

> negative control. Similarly, the incidence of cumulative tail length of comets was higher at 50% LC_{50} (347.21± 1.00 µm), followed by that at 33%, 25%, 17% LC_{50}, positive control and negative control with significant ($p<0.05$) difference among them (Table 3).

c.*Catla catla*: The DNA damage in peripheral blood erythrocyte of fish, caused by different concentrations of arsenic, varied significantly with the highest mean damage of 70.67±3.06% at 50% arsenic LC_{50}, followed by that of 33%, positive control, 25%, 17% of LC_{50} and negative control. Exposure of arsenic at 50% LC_{50} caused significantly higher GDI with the mean value of 2.18±0.07 while significantly lower GDI was observed due to negative control treatment. The cumulative tail length of comets, induced due to various arsenic concentrations showed significant differences with the mean higher cumulative tail length of 190.45±0.45 µm. observed at 50% arsenic LC_{50} that was significantly higher than that induced due to positive control (Table 4).

d.*Ctenopharyngodon idella*: In *Ctenopharyngodon idella*, the percentage of damaged cells (%), genetic damage index (GDI) and cumulative tail length of comets (µm), observed at 50% arsenic LC_{50} exposure, were significantly higher than that of positive control treatment. However, the GDI values showed non-significant difference at 25% and 33% arsenic LC_{50} exposures (Table5).

Figure 1 shows DNA damage in peripheral blood erythrocytes of fish in-terms of percentage of damaged cells, GDI and cumulative tail length of comets. Among four fish species, *Cirrhina mrigala* exhibited higher DNA damage in-terms of percentage of damaged cells, GDI and cumulative tail length of comets, followed by that of *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla*.

Industrial advancement has great contribution in the release of a variety of toxic chemicals, including metals, into the aquatic environments of Pakistan (Jabeen *et al*., 2012). These pollutants not only disturb the physico-chemical properties of the water bodies but also influence the aquatic food chain causing physiological and cytogenetic alterations in the aquatic animals (Barbosa *et al*., 2009). It is observed during present investigation that the toxicity of arsenic to four fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* varied significantly ($P<0.05$). The variation in tolerance limits of four fish species for arsenic is attributed to their physiological differences and species-specificity to interact against heavy metals (Svecevicius, 2010) or /and due to differences in the synthesis of metallothionein (Hollis *et al*., 2001). Metallothionein works as metal-chelating agent that plays an important role in the regulation, detoxification and depuration of metals in aquatic organisms, through oxygen free radical scavenging actions and metal binding (Kalpaxis *et al*., 2003). Javid *et al*. (2007), Azmat *et al*. (2012) and Yaqub and Javed (2012) also reported variability in the sensitivity of Indian major carps towards toxicity of metals.

Contamination of heavy metals appeared seriously alarming to the aquatic organisms especially fish due to their ability to induce oxidative stress through production of reactive oxygen species that leads to oxidation of various proteins, lipids and DNA (Sevcikova *et al*., 2011). In this context, arsenic exhibited higher ability to generate reactive oxygen species (ROS) that would cause oxidative DNA damage.

Table.1 Acute toxicity of arsenic 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	30.00±0.02 **a**	27.78 - 31.78	40.16±0.04 **a**	37.13 - 46.88
Cirrhina mrigala	24.50±0.05 **b**	23.17 - 25.53	32.06±0.02 **b**	30.34 - 35.06
Catla catla	10.16±0.22 **d**	9.42 - 10.70	14.05±0.20 **d**	13.16 - 15.69
Ctenopharyngodon idella	22.17±0.02 **c**	20.82 - 23.20	29.67±0.02 **c**	28.03 - 32.49

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

Table.2 DNA damage measured in peripheral blood erythrocytes of *Labeo rohita* exposed to arsenic

Treatments	Exposure Concentrations ($mg\ L^{-1}$)	Un-damaged Nuclei (%)	Proportions of Damaged Nuclei (%)				% age of Damaged Cells (II+III+IV)	Genetic Damage Index *(GDI)	Cumulative Tail Length (µm)
		Type 0	Type I	Type II	Type III	Type IV			
Labeo rohita									
Negative control	0.00	92.67±3.06 **a**	5.33±2.31 **f**	2.00±2.00 **f**	0.00±0.00 **e**	0.00±0.00 **f**	2.00±2.00 **f**	0.09±0.05 **e**	3.69±0.40 **f**
Positive control	CP (20 μgg^{-1})	33.33±1.15 **c**	22.67±2.31 **c**	10.00±2.00 **e**	9.33±1.15 **b**	24.67±1.15 **a**	44.00±3.46 **d**	1.69±0.08 **b**	118.66±0.18 **c**
17% of LC_{50}	5.10	48.00±2.00 **b**	19.33±1.15 **d**	21.33±2.31 **d**	9.33±1.15 **b**	2.00±2.00 **e**	32.67±5.46 **e**	0.98±2.00 **d**	78.00±0.44 **e**
25% of LC_{50}	7.50	9.33±1.15 **d**	39.33±1.15 **a**	44.67±1.15 **c**	3.33±1.15 **d**	3.33±3.06 **d**	51.33±1.15 **c**	1.52±0.07 **c**	112.87±0.65 **d**
33% of LC_{50}	9.90	6.00±2.00 **e**	33.33±4.16 **b**	49.33±3.06 **a**	6.00±3.46 **c**	5.33±3.06 **c**	60.67±4.16 **b**	1.71±0.13 **b**	167.22±0.22 **b**
50% of LC_{50}	15.00	8.67±1.15 **d**	15.33±2.31 **e**	46.00±3.46 **b**	12.67±1.15**a**	17.33±6.43 **b**	76.00±3.46 **a**	2.15±0.18 **a**	195.40±0.40 **a**

The means with similar letters in a single column for each variable are statistically non-significant at p<0.05.

***GDI = {Type I + 2(Type II) + 3(Type III) + 4(Type IV) / Type 0 + Type I + Type II + Type III + Type IV}; CP = Cyclophosphamide**

Table.3 DNA damage measured in peripheral blood erythrocytes of *Cirrhina mrigala* exposed to arsenic

Treatments	Exposure Concentrations (mg L^{-1})	Un-damaged Nuclei (%)	Proportions of Damaged Nuclei (%)				% age of Damaged Cells (II+III+IV)	Genetic Damage Index *(GDI)	Cumulative Tail Length (µm)
		Type 0	Type I	Type II	Type III	Type IV			
Cirrhina mrigala									
Negative control	0.00	95.33±1.15 **a**	3.33±1.15 **f**	1.33±1.15 **d**	0.00±0.00 **f**	0.00±0.00 **f**	1.33±1.15 **f**	0.06±0.02 **d**	3.78±0.23 **f**
Positive control	CP (20 $\mu g g^{-1}$)	24.00±3.46 **b**	24.67±1.15 **b**	26.00±2.00 **b**	14.67±3.06 **e**	10.67±1.15 **b**	51.33±4.16 **d**	1.63±0.13 **c**	121.20±0.13 **e**
17% of LC_{50}	4.17	11.33±1.15 **c**	40.67±3.06 **a**	16.00±2.00 **c**	30.00±2.00 **d**	2.00±0.00 **e**	48.00±4.00 **e**	1.71±1.59 **c**	139.72±0.18 **d**
25% of LC_{50}	6.13	5.33±1.15 **e**	18.67±1.15 **c**	17.33±1.15 **c**	54.67±1.15 **a**	4.00±0.00 **d**	76.00±2.00 **c**	2.33±0.04 **b**	240.24±0.21 **c**
33% of LC_{50}	8.09	7.33±1.15 **d**	10.00±2.00 **e**	32.00±2.00 **a**	42.00±2.00 **c**	8.67±1.15 **c**	82.67±2.31 **b**	2.35±0.04 **b**	307.75±0.20 **b**
50% of LC_{50}	12.25	1.33±1.15 **f**	12.00±2.00 **d**	17.33±2.31 **c**	48.00±2.00 **b**	21.33±1.15 **a**	86.67±1.15 **a**	2.76±0.00 **a**	347.21±0.10 **a**

The means with similar letters in a single column for each variable are statistically non-significant at p<0.05.

***GDI = {Type I + 2(Type II) + 3(Type III) + 4(Type IV) / Type 0 + Type I + Type II + Type III + Type IV}; CP = Cyclophosphamide**

Table.4 DNA damage measured in peripheral blood erythrocytes of *Catla catla* exposed to arsenic

Treatments	Exposure Concentrations (mg L^{-1})	Un-damaged Nuclei (%)	Proportions of Damaged Nuclei (%)				% age of Damaged Cells (II+III+IV)	Genetic Damage Index *(GDI)	Cumulative Tail Length (µm)
		Type 0	Type I	Type II	Type III	Type IV			
Catla catla									
Negative control	0.00	91.33±1.15 **a**	8.00±0.00 **f**	0.67±1.15 **f**	0.00±0.00 **e**	0.00±0.00 **e**	0.67±1.15 **f**	0.09±0.02 **e**	3.24±0.29 **f**
Positive control	CP (20 $\mu g g^{-1}$)	29.33±1.15 **d**	26.00±2.00 **c**	10.00±0.00 **d**	17.33±2.31**a**	18.00±0.00 **b**	45.33±2.31 **c**	1.69±0.04 **b**	120.00±0.80 **c**
17% of LC_{50}	1.73	66.00±4.00 **b**	21.33±2.31 **d**	8.00±0.00 **e**	2.67±1.15 **d**	2.00±3.46 **d**	12.67±4.62 **e**	0.53±0.16 **d**	52.12±0.10 **e**
25% of LC_{50}	2.54	48.00±2.00 **c**	28.00±3.46 **b**	11.33±1.15 **c**	9.33±1.15 **c**	3.33±3.06 **c**	24.00±5.29 **d**	0.92±0.14 **c**	107.36±0.06 **d**
33% of LC_{50}	3.35	17.33±3.06 **e**	34.67±2.31 **a**	18.67±3.06 **b**	12.00±2.00 **b**	17.33±1.15 **b**	48.00±5.29 **b**	1.77±0.11 **b**	148.70±0.29 **b**
50% of LC_{50}	5.08	18.00±2.00 **e**	11.33±1.15 **e**	29.33±3.06 **a**	17.33±1.15 **a**	24.00±2.00 **a**	70.67±3.06 **a**	2.18±0.07 **a**	190.45±0.45 **a**

The means with similar letters in a single column for each variable are statistically non-significant at p<0.05.

***GDI = {Type I + 2(Type II) + 3(Type III) + 4(Type IV) / Type 0 + Type I + Type II + Type III + Type IV}; CP = Cyclophosphamide**

Table.5 DNA damage measured in peripheral blood erythrocytes of *Ctenopharyngodon idella* exposed to arsenic

Treatments	Exposure Concentrations (mg L^{-1})	Un-damaged Nuclei (%)	Proportions of Damaged Nuclei (%)				% age of Damaged Cells (II+III+IV)	Genetic Damage Index *(GDI)	Cumulative Tail Length (µm)
		Type 0	Type I	Type II	Type III	Type IV			
Ctenopharyngodon idella									
Negative control	0.00	92.00±2.00 **a**	6.00±2.00 **e**	2.00±2.00 **e**	0.00±0.00 **f**	0.00±0.00 **e**	2.00±2.00 **f**	0.10±0.03 **e**	3.81±0.25 **e**
Positive control	CP (20 µgg^{-1})	19.33±2.31 **c**	25.33±1.15 **c**	31.33±1.15 **a**	15.33±1.15 **d**	8.67±2.31 **b**	55.33±1.15 **b**	1.69±0.08 **b**	109.14±0.22 **c**
17% of LC_{50}	3.77	23.33±1.15 **b**	45.33±1.15 **a**	17.33±1.15 **d**	11.33±1.15 **e**	2.67±2.31 **d**	31.33±1.15 **e**	1.25±0.06 **d**	90.12±0.16 **d**
25% of LC_{50}	5.54	22.67±2.31 **b**	24.67±2.31 **c**	28.00±2.00 **b**	22.00±0.00 **b**	2.67±3.06 **d**	52.67±4.62 **c**	1.57±0.13 **c**	121.00±0.21 **c**
33% of LC_{50}	7.32	22.00±0.00 **b**	31.33±1.15 **b**	20.00±0.00 **c**	20.00±0.00 **c**	6.67±1.15 **c**	46.67±1.15 **d**	1.58±0.03 **c**	128.11±0.22 **b**
50% of LC_{50}	11.09	4.67±2.31 **d**	20.67±1.15 **d**	26.67±1.15 **b**	38.67±2.31 **a**	9.33±2.31 **a**	74.67±3.06 **a**	2.27±0.11 **a**	184.53±0.21 **a**

The means with similar letters in a single column for each variable are statistically non-significant at p<0.05.

***GDI = {Type I + 2(Type II) + 3(Type III) + 4(Type IV) / Type 0 + Type I + Type II + Type III + Type IV}; CP = Cyclophosphamide**

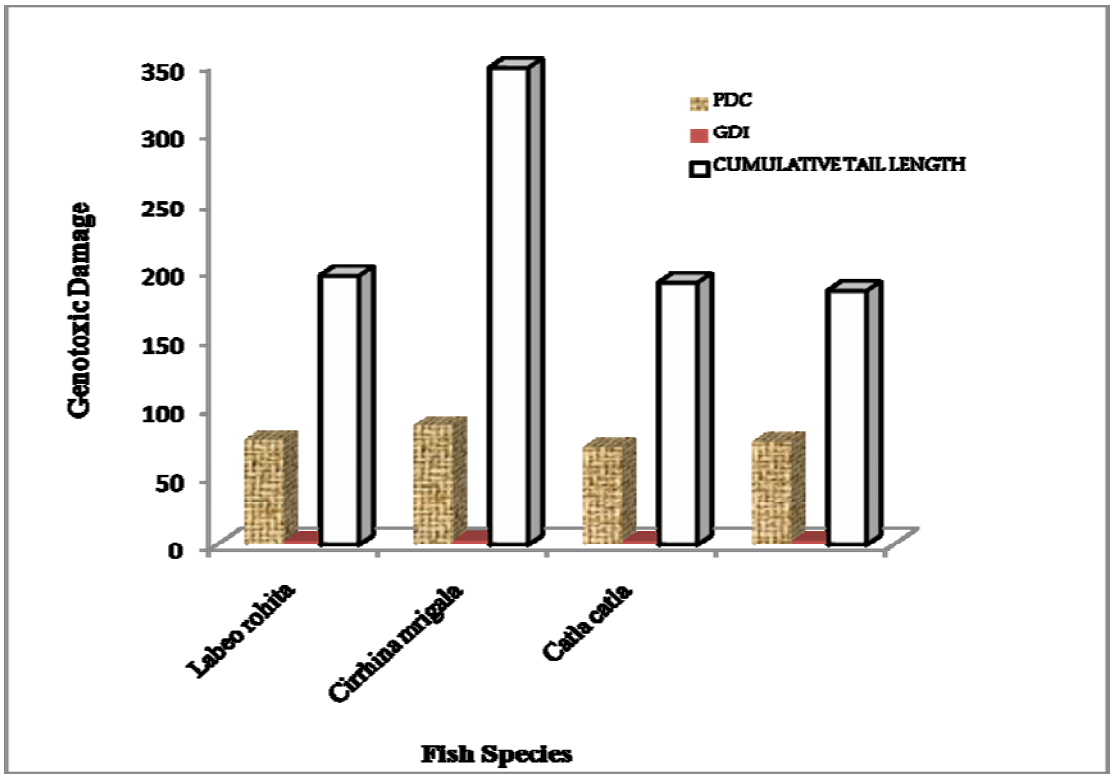

Figure.I Genotoxic damage in peripheral erythrocytes of fish (*Ctenopharyngodon idela*) due to arsenic exposure

Thus, generation of ROS and inhibition of DNA repair would lead to oxidative stress induced genomic instability in fish (Rossman, 2003). During present investigation, in the fish erythrocytes, the extent of DNA damage, in-terms of percentage of damaged cells, GDI and cumulative tail length of comets, increased significantly with concomitant increase in concentration of arsenic. Kumar *et al*. (2013) also reported the genotoxic potential of arsenic at different exposure concentrations in *Channa punctatus* and *Carassius auratus*.

The fish, *Oreochromis mossambicus* exposed to different concentrations of arsenic exhibited concentration dependent increase in DNA damage in the blood cells that was significantly higher than control fish (Ahmed *et al*., 2011). Kopjar *et al*. (2008) reported that DNA damage in-terms of comet tail length, induced in peripheral erythrocytes of loaches (*Cobitis elongate*), increased significantly due to toxicity of industrial effluents containing arsenic, copper, mercury, chromium, managanese and strontium. Pereira *et al*. (2013) observed the genotoxic effects of cadmium and aluminum on the embryonic cells of zebra fish by using Comet assay that showed significantly higher double strand breakage due to aluminum than cadmium exposure. During present experiment, when four fish species were compared for their sensitivity towards arsenic, *Cirrhina mrigala* exhibited higher DNA damage in terms of percentage of damaged cells, GDI and cumulative tail length of comets, followed by that of *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla*. Nagpure *et al*. (2008)

observed species-specific variations in comet tail lengths of *Labeo rohita* and *P. puntius*. Significantly higher percentages of tail DNA in *Cirrhina mrigala* indicate its higher susceptibility to metals, under study, than the other three fish species. All the four experimental fish species, showed variable responses towards arsenic toxicity due to their physiological differences and species-specificity to interact against various metals. Moreover, the genotoxic potentials of arsenic suggested a serious concern towards its potential danger to the survival and growth of fish, under study, in the natural aquatic habitats.

The present was concluded that arsenic caused concentration dependent increase in DNA damage in peripheral blood erythrocytes of all the four fish species. Hence, by using comet essay, *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* can suitably be used as bio indicators of metallic ion pollution in the natural aquatic habitat.

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