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Assessment of genotoxicity through DNA damage analysis in Indian major carps induced by acute exposure to Chakbandi drain water

Muhammad Arshad Rana*, Tayyaba Sultana, Salma Sultana, Bilal Hussain

Department of Zoology, Government College University, Faisalabad, Pakistan

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Key words: Fingerlings, Toxicity, Erythrocytes, Comets, Frequency, Pollution

Abstract

In this study, Chakbandi drain's composite water concentrations were collected from the selected sites in the month of April, May and June, 2016 and applied to fingerling's of three Indian major carps i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria. After determining the LC_{50}, the sub-lethal dilutions i.e. 20%, 25%, 30%, 35% and 40% of drain water were tested for three month's acute toxicity trial. All the water quality parameters i.e. DO, pH, temperature, conductivity, TDS, salinity, TSS, BOD and COD and concentration of selected heavy metals (Cu, Cr, Mn, Cd, Co, Ni, Sn, Hg, Zn and Pb) recorded from drain water were found above the permissible limits as described by FAO/ WHO. During current research, the DNA damage in fish peripheral erythrocytes was quantified by using different categories of damaged cells that were arbitrarily defined according to the tail length (size) of the comets. Maximum frequency of DNA damaged cells was recorded in erythrocytes of *Cirrhinus mrigala* when compared with *Labeo rohita* and *Catla catla*. *Cirrhinus mrigala* showed maximum average tail DNA length and average tail moment than *Labeo rohita* and *Catla catla*. Conclusively, DNA fragmentation as biomarker approach was found to be reliable for the assessment of genotoxicity and environmental pollution. Moreover, findings of this study are helpful as an early warning for environmental monitoring strategies.

***Corresponding Author:** Muhammad Arshad Rana ✉ marshadrana719@gmail.com

Introduction

Industrialization and population explosion has increased the municipal waste water which in return pollute the water bodies. Rapid economic and industrial development has been producing enormous quantity of toxic synthetic chemicals, heavy metals and other pollutants which are constantly introducing into our environment. These pollutants are extremely poisonous and capable of causing serious diseases in living organisms (Atienzar *et al*., 2000; Devaraj *et al*., 2014; Zaqoot *et al*., 2017).

Aquatic environmental pollution is of great concern due to extreme hazardous effects of pollutants that are discharged from domestic waste and industrial chemicals or effluents into riverine water through different drains. Domestic and industrial waste water contains high concentrations of dissolved solids and suspended inorganic and organic toxic compounds. Dissolved organic compounds are found that mostly comprised of detergents, soap, carbohydrates, proteins, fats, lignin and their byproducts. These pollutants directly and indirectly alter the genome of aquatic organisms especially in fishes (Villela *et al*., 2006; Nhapi *et al*., 2011; Perera *et al*., 2015).

Now a days, aquatic pollution has become the burning issue and alarming problem in Pakistan as domestic sewage and industrial contaminants which contain bulk of toxic chemical compounds, including especially the heavy metals that are constantly discharged into aquatic environments. These heavy metals have drastically toxic effects on aquatic organisms carps (Javed, 2005; Hayat *et al*., 2007) causes the reduction of haemoglobin that effect the oxygen binding capacity in these aquatic organisms (Bonga, 1997; Ruane *et al*., 1999; Adeyemo, 2005).

A wide variety of domestic and industrial pollutants directly or indirectly are affecting DNA and causing genotoxicity in aquatic organisms especially in fishes (Shakir *et al*., 2015; Sultana *et al*., 2016). Impacts of these chemical compounds can drastically lead to abnormal physiological activities and cause detrimental effects on growth, development, reproduction and behavior in aquatic organisms (Bistodeau *et al*., 2006; Giesy *et al*., 2000; Lee & Peart, 2000; Ginebreda *et al*., 2014). The detrimental genotoxic effects are considered the endpoints for the assessment of pollution related toxicity (Bolognes & Cirillo, 2014).

Aquatic ecosystems receive a number of toxic substances, including heavy metals (Zaqoot *et al*., 2017) that are discharged from industrial, domestic and other man-made products. These heavy metals are of great importance, due to their toxicity, bioaccumulation potential and ability to induce damage in DNA. Heavy metal contamination in aquatic biota has devastating impacts on aquatic ecosystem, diversity of aquatic organism and ecological balance between ecosystem, organism and environment (Vosyliene & Jankaite, 2006; Kumar *et al*., 2008; Noor & Zutshi, 2016; El-Bassir *et al*., 2017). Among aquatic animal species, fish are the most important bioindicator that cannot protect themselves from devastating effects of the toxicants. Fish are aquatic bioindicators and frequently used as sentinel animal due to the peculiar role in food chain, food webs, bioaccumulation of pollutants and their sensitivity to lower concentration of mutagenic compounds (Lopes *et al*., 2001; Mohammad & Osman, 2014; Yancheva *et al*., 2016).

The quality of water directly effects the quality and quantity of fish species in a directly proportional relationship. The changes in any of the water quality parameter severely affect the aquatic organisms, especially fish (Greig *et al*., 2005; Gupta *et al*., 2017). Even slight fluctuations in water quality parameters directly induce the variety of stresses among fish species because their homeostatic mechanisms are highly dependent on existing conditions in their immediate surrounding parameters (Nussey *et al*., 1995). In the same context such as pH plays a pivotal role in metabolism and maintenance of homeostasis of fresh water animals (Wood *et al*., 1989). Extreme fluctuations in pH value in the aquatic environment are reported to cause disturbance in acid-base, ion regulation, fish growth, reproduction and even mortality (Evans *et al*., 2005 and Zanibomi-Filho *et al*., 2009).

Fish inhabit in close contact with the aquatic environment, and are considered to be more susceptible to different changes in their aquatic environment which may cause haematological alterations (Wilson & Taylor, 1993).

Fish are selected as a model organism in genotoxicological studies due to their sensitivity as bio-indicator for water quality assessment. Hence, fish can highlight the detrimental effects of new chemicals that are discharged into the aquatic environment (Gupta *et al.*, 2017; Sabullah *et al.*, 2015) and can respond to toxic pollutants in the similar way as higher vertebrates (Al-Sabti & Metcalfe, 1995). Fish have greater ability to metabolize xenobiotics and accumulating pollutants (Grisolia & Corderio, 2000). They are capable of inhabiting practically in all the levels of aquatic habitat and hence have the prime importance on commercial and recreational basis (Moustafa & El-Sayed, 2014). They perform different prominent pivotal roles in tropic web such as bioaccumulation of environmental toxicants, bioassimilation and biotransformation of xenobiotics via cytochrome 450-dependent oxidative metabolism like mammals. Moreover, they respond to mutagens even at very minute concentration (Gksoyr *et al.*, 1991).

Aquatic organisms are continuously exposed to a complex mixture of pollutants which include parent chemical compounds and their transformation by products that induce detrimental multiple damages in organ functioning and reduce biological diversity drastically at organism, population and ecosystem levels (Sabullah *et al.*, 2015; Zaqoot *et al.*, 2017). Different levels of water pollution produce DNA fragmentation, infertility and variety of DNA damage in aquatic animals especially in fish which is effected by the exposure of industrial effluents containing heavy metals, environmental toxins, oxidative stress, genetic and other toxicological factors (Kousar & Javed, 2015).

Comet assay is the most reliable tests due to its authenticity and application in genotoxic assessment of pollution levels in aquatic environments (Mallins *et al.*, 2011; Obiakor *et al.*, 2012).

Excessive amount of heavy metals in industrial waste water causes the mutations in the chromosomes of aquatic organism and causing the micronuclei formation that hinder the DNA repair mechanism (Gebel, 2001) particularly lead and mercury that increase the frequency of micronuclei in fish species (Bolognesi *et al.*, 1999).

The current study was planned to determine the effects of municipal and industrial wastewater on fish through DNA damage (genome instability).

Material and methods

Experimental setup

In this study, after the determination of LC_{50} Chakbandi drain's composite water concentration of five selected sites was prepared. The five sub-lethal dilutions (20%, 25%, 30%, 35%, 40%,) of this composite water concentration was applied to fingerlings approximately 11- 13 g of three fish species i.e. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria. All the three fish species were distributed into two groups, one as sub-lethal exposure group (experimental) which was further subdivided into five sublethal concentrations (20-40%) and other as control group. 30 fish of the three fish species were distributed equally into 18 glass aquaria (80L). The experimental glass aquaria were aerated continuously with an air pump through well quipped capillary system. The percentage concentrations of drain water were prepared on the basis of volume to volume (v/v) ratio.

DNA damage studies

Venous blood was collected in heparin coated tubes from caudal vein of the respective fish species of Indian Major Carps after domestic waste water and industrial waste water exposure experiment for three month. Fresh blood of fish was used for Comet assay test to identify the DNA damage with slight modifications of Dhawan *et al.*, 2009 and Singh *et al.*, 1988 methods.

First of all single-cell suspensions were prepared and these suspensions were embedded in agarose gel of

low melting point on the frosted microscope slide and these slides were placed into the lysing buffer in order to get DNA unwinding. The subsequent electrophoresis, neutralization and staining with ethidium bromide visualized the individual cell and the DNA on florescent microscope. After the staining process, slides were covered with cover slips and observed under the florescent microscope at 40x magnification. Total 100 cells were scored from each slide for each sample. All the slides were scored with great care. The cells were evaluated for the quantitative and qualitative potential of DNA damage by measuring the length and percentage of migrated DNA by software Tri Tek Comet Score™ Freeware. All the necessary components of comet assay were applied to express DNA damage as head DNA length, tail DNA length, % DNA in head, % DNA in tail and tail moment.

Results

During current research, the DNA damage in fish peripheral erythrocytes was quantified by using different categories of damaged cells that were arbitrarily defined according to the tail length (size) of the comets and percentage of damaged cells was calculated by using the following formula:

Percentage of damaged cells (PDC %) =

$$\frac{\text{Type I + Type II + Type III + Type IV}}{\text{S/ 100}}$$

Where,

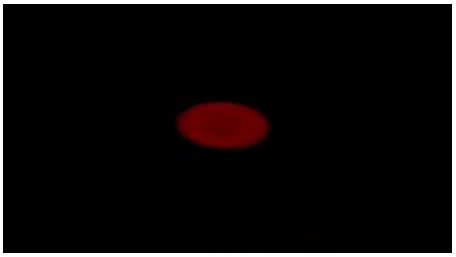
S = Total no. of cells scored

Peripheral blood erythrocytes of fish exposed to various concentrations of drain water were divided into five categories according to the size of comet tail. Form these categories Genetic Damage Indices (GDI) of peripheral erythrocytes were calculated by using the following formula:

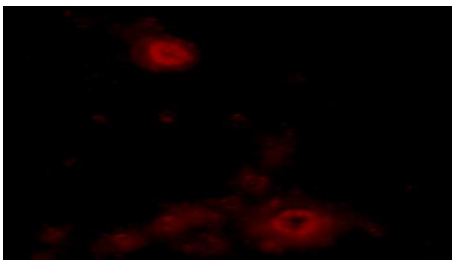
$$\text{GDI} = \frac{\text{(Type I) +2(Type II) + 3(Type III) + 4(Type IV)}}{\text{Type 0 + Type I + Type II + Type III + Type IV}}$$

Tail length of comets is more frequently used parameter for the determination of DNA damage. Image analyzing systems have become more powerful that allow the definition of DNA damage parameters such as comet tail length. During this study, the tail length of comets was measured by using TriTek Comet Score™ (Summerduck, USA) software while cumulative tail length (µm) was obtained by adding the tail lengths of all the examined cells (n = 100 per replicate).

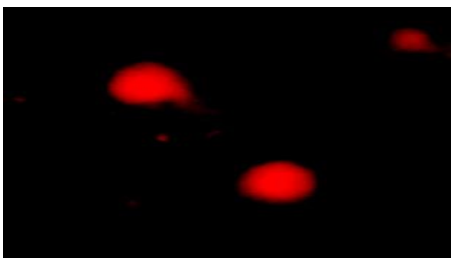
Fingerlings of three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* were exposed to different drain water concentrations containing heavy metals for 90 days. Five different drain water concentrations viz. DW-1 (20%), DW-2 (25%), DW3 (30%), DW-4 (35%) and DW-5 (40%) of LC_{50} were tested on the fish species in glass aquaria for their genotoxic effects on fish peripheral erythrocytes by using Comet assay. The damaged DNA in the peripheral erythrocytes of each fish species was divided arbitrarily into five categories.



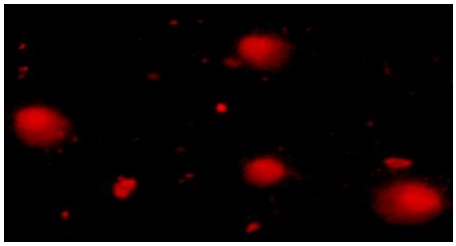
a. Type 0 (Undamaged)



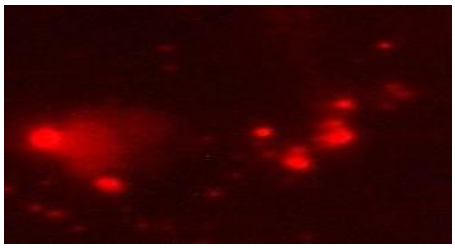
b. Type I (Low level damage)



c. Type II (Medium level damage)



d. Type III (High level damage)



e. Type IV (Complete damage)

Fig. 1. Overall DNA damage level categories as shown in a, b, c, d, and e.

The fish data was collected, on the basis of above mentioned DNA damage classification, at each drain water concentration. From the above mentioned types, the proportions of damaged nuclei, percentage of damaged cells and genetic damage index (GDI) were obtained. Tail lengths of comets were also measured by using computer software.

Analysis of variance for DNA damage identified in all the three fish species

Data presented in Table 1, showed the analysis of variance comparing the fish species and the drain water concentrations (treatments) with respect to DNA damage for average head length, average tail length, average head DNA length percentage, average tail DNA length percentage and tail moment. The mean average tail length, average head DNA length percentage, average tail DNA length percentage and tail moment showed the highly significant ($p<0.01$) differences than the average head length which shows the non-significant ($p>0.05$) differences in different fish species whereas highly significant ($p<0.01$) differences in drain water concentrations (treatment). The species x treatment wise, mean average head length, average head DNA percentage showed the non-significant ($p>0.05$) differences whereas average tail length, average tail DNA length percentage and tail moment showed the highly significant ($p<0.01$) differences.

In *Catla catla*, the maximum mean average tail length (7.11±0.17), average tail DNA percentage (7.70±0.24) and tail moment (0.61±0.02) were recorded from the drain water concentration DW-5 and minimum mean average tail length (5.72±0.06), average tail DNA percentage (4.20±0.06) and tail moment (0.38±0.01) were recorded from the DW-1. In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different mean average tail length (2.21±0.05), average tail DNA percentage (3.30±0.13), average head DNA% (96.70±2.45), and tail moment (0.04±0.00) as compared to experimental fish. In *Labeo rohita*, the mean average tail length (7.85±0.12), average tail DNA percentage (10.50±0.20) and tail moment (0.73±0.02) were recorded from the drain water concentration DW-5 and minimum mean average tail length (5.25±0.05), average tail DNA percentage (5.50±0.06) and tail moment (0.41±0.01) were recorded from the DW-1.

The controlled *Labeo rohita* showed significantly ($p<0.05$) different average tail length (2.35±0.07), average tail DNA percentage (2.50±0.03), average head DNA% (97.5±0.03) and tail moment (0.06±0.00) as compared to all drain water concentration exposed fish. In *Cirrhinus mrigala,* the maximum mean average tail length (8.89±0.21), average tail DNA percentage (13.30±0.42) and tail moment (0.83±0.02) were recorded from the drain water concentration DW-5 and minimum mean average tail length (6.27±0.09), average tail DNA percentage (6.00±0.10) and tail moment (0.37±0.01) were recorded from the DW-1. The controlled *Cirrhinus mrigala* showed significantly ($p<0.05$) different average tail length (2.47±0.05), average tail DNA percentage (5.10±0.12) and tail moment (0.09±0.00) as compared to all drain water concentration exposed fish. Overall maximum mean average head length (22.91±0.49) and average head DNA percentage (94.70±0.63) was recorded in the erythrocytes of *Catla catla* and minimum mean average head length (22.52±0.23) and average head DNA percentage (91.20±1.25) was recorded in the erythrocytes of *Cirrhinus mirgala*. Maximum mean average tail length (6.95±0.97) was recorded in the erythrocytes of *Cirrhinus mrigala* and minimum mean average tail length (5.67±0.72) was recorded in the erythrocytes of *Catla catla* (Table 2).

Table 1. Analysis of variance showing comparison between species and treatment for Avg. cells identified other nuclear abnormalities.

Source of variation	Degrees of freedom	Mean squares				
		Avg. head length	Avg. tail length	Avg. head DNA (%)	Avg. tail DNA (%)	Tail moment
Species	2	0.692^{NS}	8.638**	55.33**	55.327**	0.08007**
Treatment	5	5.302**	36.221**	55.52**	55.519**	0.49959**
S x T	10	1.249^{NS}	0.990**	3.16^{NS}	3.163**	0.00737**
Error	36	1.108	0.064	10.99	0.115	0.00049
Total	53					

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01).

Table 2. DMR-test showing the comparison of mean ± SE for avg. cells identified other nuclear abnormalities.

Species	Avg. head length	Avg. tail length	Avg. head DNA (%)	Avg. tail DNA (%)	Tail moment
C. catla	22.91±0.49A	5.67±0.72B	94.70±0.63A	5.30±0.63C	0.41±0.082B
L. rohita	22.73±0.37A	5.86±0.81B	93.13±1.21B	6.87±1.21B	0.48±0.096B
C. mrigala	22.52±0.23A	6.95±0.97A	91.20±1.25C	8.80±1.25A	0.55±0.112A

Means similar letters are statistically non-significant (P>0.05).

Data presented in Table 3, showed the analysis of variance comparing the fish species and the drain water concentrations (treatments) with respect to DNA damaged cells and genetic damage index (GDI). The mean DNA damaged cells and genetic damage index (GDI) showed the highly significant (p<0.01) differences in different fish species, drain water concentrations (treatment) and species x treatment wise interaction. In *Catla catla*, the maximum mean DNA damaged cells (12.00±0.17) and genetic damage index (0.22±0.004) were recorded from the drain water concentration DW-5 and minimum DNA damaged cells (6.00±0.12) genetic damage index (0.13±0.002) were recorded from the drain water concentration DW-1. In *Catla catla*, the controlled fish showed significantly (p<0.05) different mean DNA damaged cells (3.00±0.06) and genetic damage index (0.03±0.00) recorded from the drain water concentration as compared to experimental fish. In *Labeo rohita*, the maximum mean DNA damaged cells (14.00±0.35) and genetic damage index (0.26±0.010) were recorded from the drain water concentration DW-5 and minimum mean DNA damaged cells (7.00±0.12) and genetic damage index (0.11±0.001) were recorded from the drain water concentration DW-1.

The controlled *Labeo rohita* showed significantly (p<0.05) different DNA damaged cells (4.00±0.12) and genetic damage index (0.04±0.00) as compared to all drain water concentration exposed fish.

Table 3. Analysis of variance showing comparison between species and treatment for damaged cells (%) and genetic damage index.

Source of variation	Degrees of freedom	Mean squares	
		DNA damaged cells (%)	Genetic damage index (GDI)
Species	2	78.167**	0.027267**
Treatment	5	129.367**	0.063827**
S x T	10	2.367**	0.002247**
Error	36	0.128	0.000046
Total	53		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01).

In *Cirrhinus mrigala,* the mean DNA damaged cells (17.00±0.23) and genetic damage index (0.34±0.006) were recorded from the drain water concentration DW-5 and minimum mean DNA damaged cells (10.00±0.17) and genetic damage index (0.16±0.001) were recorded from the drain water concentration DW-1. The controlled *Cirrhinus mrigala* showed significantly (p<0.05) different DNA damaged cells (4.00±0.12) and genetic damage index (0.04±0.001) as compared to all drain water concentration exposed fish.

Overall maximum mean DNA damaged cells (11.833±1.851) and genetic damage index (0.217±0.043) was recorded in the erythrocytes of *Cirrhinus mrigala* and minimum mean DNA damaged cells (7.667±1.282) and genetic damage index (0.140±0.06) was recorded in the erythrocytes of *Catla catla* (Table 4).

Table 4. DMR-test showing the comparison of mean ± SE for damaged cells (%) and genetic damage index.

Species	DNA damaged cells (%)	Genetic damage index(GDI)
C. catla	7.667±1.282C	0.140±0.026B
L. rohita	9.667±1.542B	0.167±0.036B
C. mrigala	11.833±1.851A	0.217±0.043A

Means similar letters are statistically non-significant (P>0.05).

Graphical representation of DNA damage in all the three fish species

Fig. 1, showed the avg. tail DNA length percentage in all the three fish species exposed to drain water concentrations. Maximum average tail DNA length percentage was recorded in erythrocytes of *Cirrhinus mrigala* from DW-5 (13.3%) and DW-2 (11.1%) as compared to *Labeo rohita* from DW-5 (10.5%) and DW-2 (9.8%) and *Catla catla* from DW-5 (7.7%) and DW-2 (6.1%) respectively. Minimum average tail DNA length percentage was recorded in erythrocytes of all the control fish species.

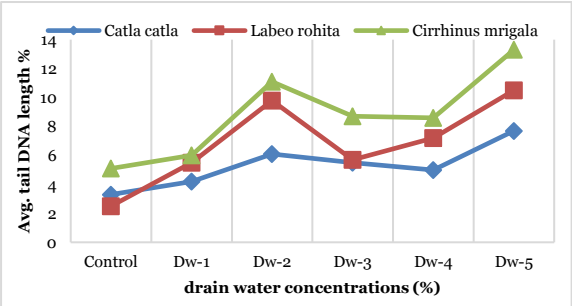


Fig. 2. Line graph representing the average tail DNA length % in all the three fish species.

Fig. 2, showed the frequency of DNA damaged cells in all the three fish species exposed to drain water concentrations.

Maximum frequency of DNA damaged cells was recorded in erythrocytes of *Cirrhinus mrigala* from DW-5 (17%) and DW-2 (15%) as compared to *Labeo rohita* DW-5 (14%) and DW-2 (13%) and *Catla catla* from DW-5 (12%) and DW-2 (10%) respectively.

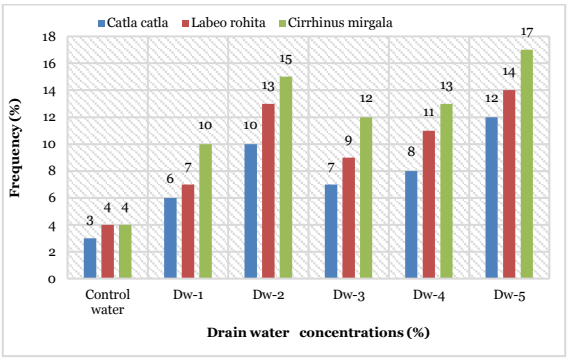


Fig.3. Bargraph representing the frequency of DNA damaged cells in all the three fish species exposed to different drain water concentrations.

Discussion

In the present investigation, the DNA damage in fish peripheral erythrocytes was quantified by using different categories of damaged cells that were arbitrarily defined according to the tail length (size) of the comets. The proportions of damaged nuclei, percentage of damaged cells and genetic damage index (GDI) were calculated. The average tail length, average head DNA length percentage average tail DNA length percentage and tail movement shows highly significant ($p<0.01$) differences than the average head length which showed the almost non-significant ($p>0.05$) results in different fish species and significant ($p>0.01$) results in drain water concentrations. Maximum mean average tail length, average tail DNA length percentage and tail moment was identified in the erythrocytes of all the drain water treated fish species as compared to control fish. Maximum mean average tail length (6.95±0.97), average tail DNA length percentage (8.80±1.25) and tail moment (0.55±0.112) were recorded in *Cirrhinus mrigala* and minimum mean average tail length (5.67±0.72) average tail DNA length percentage (5.30 ± 0.63) and tail moment (0.41±0.082) were recorded in *Catla catla*.

All the drain water treated fish species showed significantly ($p<0.05$) maximum DNA damage in the erythrocytes of blood as compared to the control group. Maximum mean average head DNA length percentage (94.70 ± 0.63) were recorded in *Catla catla* as compared to *Labeo rohita* (93.13 ± 1.21) and *Cirrhinus mirgala* (91.20 ± 1.25) respectively.

The DNA damaged cells percentage and genetic damage index shows highly significant ($p<0.01$) differences in different fish species and drain water concentrations (treatment). Maximum mean DNA damaged cells and GDI was identified in the erythrocytes of all the drain water treated fish species as compared to control fish. Maximum mean DNA damage cells (11.833 ± 1.851) and GDI (0.217 ± 0.043) was recorded in *Cirrhinus mrigala* and minimum mean DNA damage cells (7.667 ± 1.282) and GDI (0.140 ± 0.026) were recorded in *Catla catla*.

The present findings are in line with findings of Ramesh & Agarajan (2013) who studied the DNA damage through comet assay in the blood samples of fish species *Clarias batrachus* and found comet tail in the individuals exposed to the effluents while control showed normal structures. They also observed increasing pattern in the tail with increasing concentration of the effluents. The present findings are also in line with findings Kumar *et al.* (2011) who investigated the extent of genotoxicity of the fresh water fish species *Channa punctatus* under sub lethal concentrations by using the comet assay and micronucleus test. Micro nuclear frequency determined was significantly higher in treated specimen as compared to the control group. All the tissues of fish showed that increase in DNA damage is concentration dependent.

The present findings are in line with findings of Kousar and Javed (2015) who evaluated the genotoxic impacts on four different freshwater fish species viz. *Catla catla, Labeo rohita*, *Cirrhinus mrigala* and *Ctenopharyngodon idella* through Comet assay. All the fish species reflected prominently different extant of DNA damage in terms of damaged cells % age, cumulative tail length of comets, genetic damage index against the metals exposure. Fish species *Cirrhinus mrigala* reflected the higher damaged cells % age, genetic damage index and cumulative tail length of comets as compared to other three fish species. However, *Catla catla* indicated the lower damaged cells % age and GDI respectively as in the present study.

In the present study, among drain water treated fish species, maximum mean DNA damage was recorded in the fish species exposed to DW-5 concentrations. Maximum mean DNA damage cells (14.333 ± 1.453) and GDI (0.273 ± 0.035) was recorded from DW-5 and minimum mean DNA damage cells (7.667 ± 1.202) and GDI (0.133 ± 0.015) was recorded from DW-1 concentrations. All the drain water treated fish species reflected significantly ($p<0.05$) maximum DNA damage in the erythrocytes of blood as compared to control group. Maximum frequency of DNA damaged cells was recorded in erythrocytes of *Cirrhinus mirgala* from DW-5(17%) and DW-4 (15%) as compared to *Labeo rohita* DW-5 (14%) and DW-4 (13%) and *Catla catla* from DW-5 (12%) and DW-4 (10%) respectively. Maximum GDI was recorded in erythrocytes of *Cirrhinus mirgala* from DW-5 (0.34) and DW-4 (0.29) as compared to *Labeo rohita* from DW-5 (0.26) and DW-4 (0.26) and *Catla catla* from DW-5 (0.22) and DW-4 (0.18), respectively.

The present study for the use of comet assay is also supported by Dikilitas *et al.* (2009) who reported that Comet assay is most advanced and recent technique to be used for identification of DNA damage and repair mechanism. Cavas *et al.* (2007) also reported that comet assay is reliable and most widely used for the proper characterization of DNA damage parameters like; tail moment (TM), tail intensity (tail % DNA) and tail length (tail DNA). Andrade *et al.* (2004) also described the comet assay and micronucleus test to identify the effects of genotoxicity through municipal waste water, agriculture runoff and industrial pollutants.

Results of present study reflected that maximum percentage of DNA damaged cells was identified in *Cirrhinus mrigala* (11.833 ± 1.851) as compared to *Labeo rohita* (9.667± 1.542) and *Catla catla* (7.667± 1.282) all from DW-5. Similarly, *Cirrhinus mrigala* shows maximum mean tail DNA length (µm) followed by *Labeo rohita* and *Catla catla,* respectively. Tail moment used to access genotoxic level, and maximum mean tail moment (um) was identified in *Cirrhinus mrigala* compared to mean tail DNA moment in *Labeo rohita* and *Catla catla,* respectively. Similarly, genetic damaged index (GDI) recorded was maximum in *Cirrhinus mrigala* as compared to *Labeo rohita* and *Catla catla,* respectively. Results of the present study confirmed that all the three fish species exposed to different drain water concentrations showed the significant ($p<0.05$) variations with respect to DNA damaged cells (%), mean DNA tail length, mean DNA tail moment and genetic damaged index respectively. These findings are in corroborate with the findings of several workers (Shirani *et al.*, 2012; Kousar & Javed, 2015; Santamaria *et al.*, 2016; Zizy *et al.*, 2016).

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