



# Genotoxic Consequences in Common Grass Carp (*Ctenopharyngodon idella* Valenciennes, 1844) Exposed to Selected Toxic Metals

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## Abstract

The primary aim of the current study was to recognize the biomarker approach as the finest tool to identify the geno-toxicological effects of copper, chromium, and lead inside the blood of grass carp using micronucleus test and comet assay. The induced micronuclei frequency in response to the administered concentrations of LC50 metals was discovered in the erythrocytes of metal-exposed fish at four-time intervals. The genotoxic effect of these metals with respect to the formation of micronuclei was ranked as chromium > lead > copper. Percentages of other cellular and nuclear abnormalities were also determined in the exposed blood films. Equally, the genotoxic studies in terms of comet assay in fish blood revealed significant deviations  $p < 0.05$  against each of the studied metal at their respective time interval as compared with the healthy fish group. However, induced frequency of micronuclei and the calculated DNA damage were not found to be duration dependent. Consequently, copper, chromium, and lead have been explored as cytotoxic elements that can be responsible for inducing genotoxic effects in fish existing aquatic habitats.

**Keywords** Copper · Chromium · Lead · *Ctenopharyngodon idella* · Comet assay · Micronucleus

## Introduction

Water pollution mainly caused by toxic metals is the growing concern in recent years [1]. Primarily, toxic metals have altered the ecological balance by accumulating in the aquatic environment that further decrease the productivity and fecundity of aquatic life. It also affects the humans that rely on these organisms as a major source of protein [2].

Due to anthropogenic activities, the concentration of metals when exceeds the standard recommended limits of [3] the World Health Organization (WHO) and the United States Environment Protection Agency (USEPA) [4] becomes toxic to organisms by forming complex compounds within the cells of living organisms [5].

The deadliest contaminant accumulated by fish and hazardous to humans in the form of heavy metals are categorized as arsenic,

cadmium, chromium, copper, lead, nickel, and zinc; all of which cause risks to the environment as well as human health [6]. Besides high toxicity level, heavy metals are non-degradable, i.e., they remain in the ecosystem long after their emission is ceased [7], that results in irreversible physiological damage [8].

Metals enter the environment through natural and anthropogenic sources [9]. Natural sources include weathering, eruption of volcanoes [10], soil erosion of metal ions, metal leaching, atmospheric deposition and metal corrosion, sediment re-suspension, and evaporation of metal from different water sources to soil and groundwater [11], whereas anthropogenic sources include mining and smelting processes; domestic and agricultural use of metals and their compounds; insecticides and pesticides used for crops; sewage discharge [10]; industrial uses, for instance, petroleum combustion, nuclear power plants and high tension lines, plastics, textiles, microelectronics, wood preservation, and paper processing plants [12]; and coal combustion which is listed as the most important man-made source [13].

Fish is specifically sensitive against environmental changes and is the most susceptible aquatic organism to toxic substances present in water as compared with mammals. Hence, it can be used as a bioindicator to monitor the toxic stress caused by pollution [14]. Fish biomarkers

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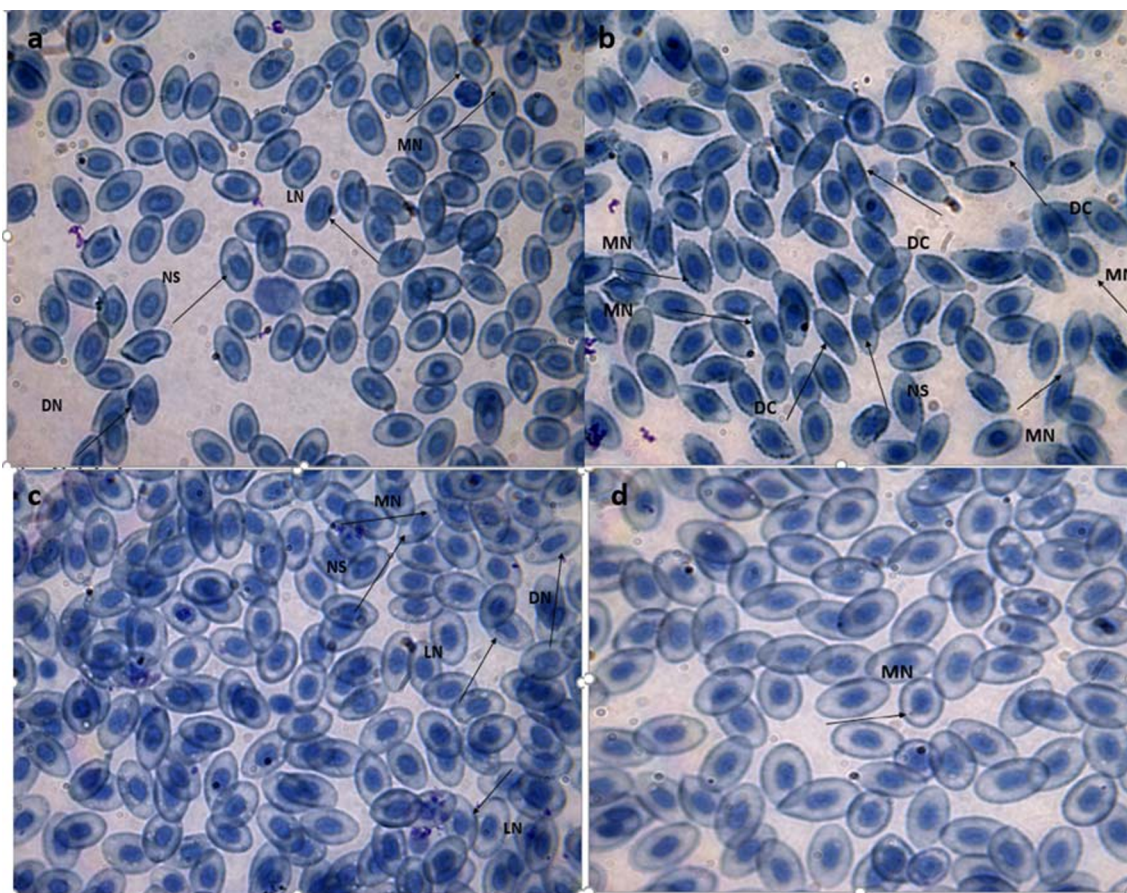
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**Table 1** Representation of cellular and nuclear abnormalities in fish blood exposed to selected metals

	Treated groups	Single micronucleus frequency	Double micronucleus frequency	Deformed nucleus %	Nuclear shift %	Lobed nucleus %	Irregular nucleus %	Deformed cells %	Microcytes %	Vacuolated cells %	Swollen cells %
Cu	Control	0.3	0.0	0.4	0.3	0.0	0.0	4.8	0.1	0.0	0.8
	L-24H	0.6	0	1.0	1.0	0.3	0	1.7	0.7 <sup>a</sup>	0.0	0.0 <sup>b</sup>
	C-48H	1.3	0.0	0.4	0.4	0.1	0.1 <sup>a</sup>	4.6	0.2	0.0	0.0 <sup>b</sup>
	50-72H	0.3	0.0	0.2 <sup>b</sup>	1.0	0.5 <sup>a</sup>	0.1 <sup>a</sup>	1.7	0.6	0.0	0.4
	96H	0.6	0.0	0.8	1.0	0.0 <sup>b</sup>	0.1 <sup>a</sup>	1.8	0.5	0.0	0.4
Cr	24H	1.8	0.0	0.4	0.8	0.0 <sup>b</sup>	0.0	2.0	0.1 <sup>b</sup>	0.0	0.0 <sup>b</sup>
	L-48H	0.1 <sup>b</sup>	0.0	0.4	0.2 <sup>b</sup>	0.0 <sup>b</sup>	0.0	0.4 <sup>b</sup>	0.7 <sup>a</sup>	0.0	1.6 <sup>a</sup>
	C-72H	0.1 <sup>b</sup>	0.0	0.3	1.9	0.1	0.0	2.4	0.4	0.0	0.0 <sup>b</sup>
	50-96H	0.8	0.2 <sup>a</sup>	2.6 <sup>a</sup>	3.1 <sup>a</sup>	0.1	0.0	1.4	0.1 <sup>b</sup>	0.0	0.5
Pb	24H	2.0 <sup>a</sup>	0.0	1.0	1.4	0.4	0.0	3.5	0.3	0.2 <sup>a</sup>	0.4
	L-48H	0.2	0.0	0.4	1.3	0.3	0.0	1.2	0.2	0.0	0.0 <sup>b</sup>
	C-72H	0.1 <sup>b</sup>	0.0	1.0	1.1	0.1	0.0	3.3	0.5	0.0	0.7
	50-96H	0.3	0.0	0.3	0.8	0.3	0.0	7.5 <sup>a</sup>	0.4	0.0	0.5

<sup>a</sup> The maximum obtained percentages of cellular and nuclear anomalies among the administered metals during exposure hours

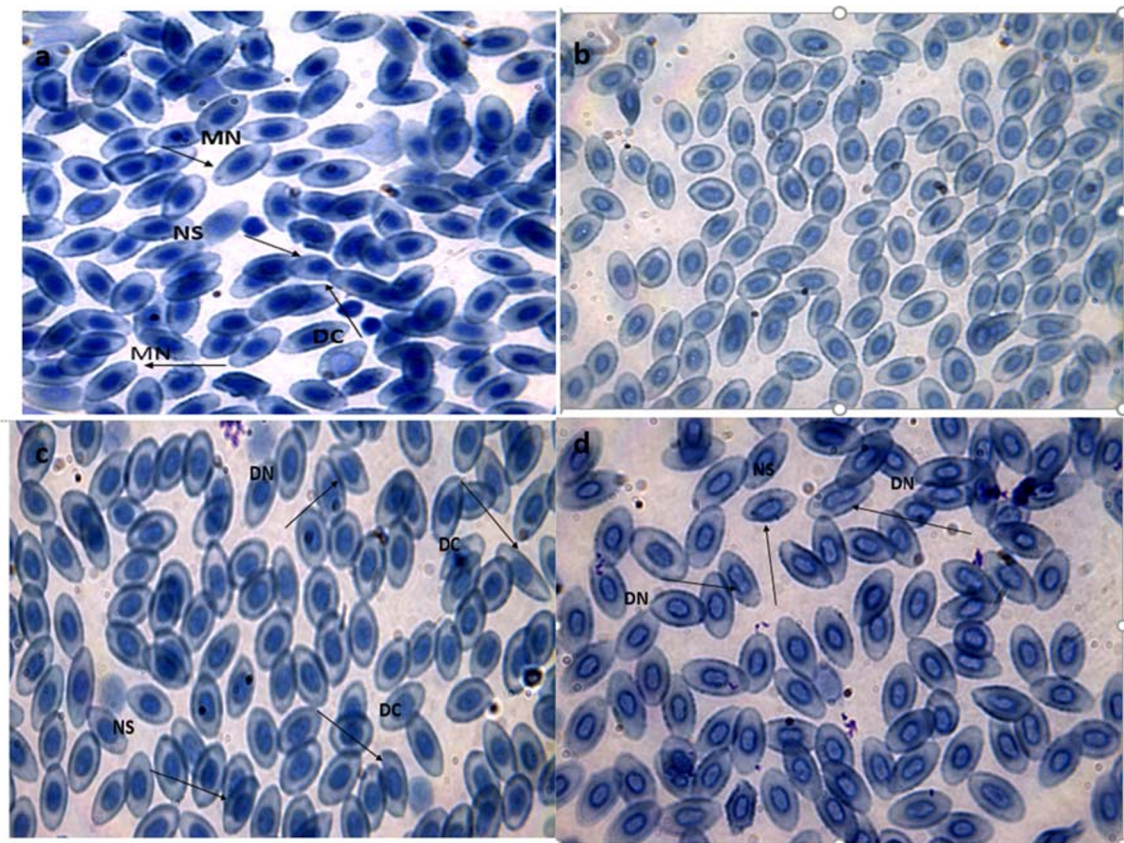
<sup>b</sup> The minimum scored percentages of cellular and nuclear anomalies among the administered metals during exposure hours



**Fig. 1** Blood smears of *C. idella* fish exposed to **a** 24 h of LC50 Cu showing micronucleus (MN), nucleus shift (NS), lobed nucleus (LN), and deformed nucleus (DN); **b** 48 h of LC50 Cu showing micronucleus (MN), nucleus shift (NS), and deformed cell (DC); **c** 72 h of LC50 Cu

showing micronucleus (MN), nucleus shift (NS), deformed nucleus (DN), and lobed nucleus (LN); and **d** 96 h of LC50 Cu showing micronucleus (MN)





**Fig. 2** Blood smears of *C. idella* fish exposed to **a** 24 h of LC50 Cr showing micronucleus (MN), deformed cell (DC), and nucleus shift (NS); **b** 48 h of LC50 Cr showing red blood cells with the least

abnormalities; **c** 72 h of LC50 Cr showing nuclear shift (NS), deformed nucleus (DN), and deformed cell (DC); **d** 96 h of LC50 Cr showing nuclear shift (NS) and deformed nucleus (DN)

include the evaluation of biomolecular, cellular, and physiological changes against the toxicity caused by metal exposure and have become the tool of interest in the recent years because of several reasons; for instance, it gives caution towards an early solution and precise measurement against the toxic metals showing the different toxicity level of complex mixtures [15].

Genotoxic effects are produced in fish because of potentially toxic metal exposure that causes various anomalies in the form of chromosomal abnormalities, gene frequencies variation, and the generation of micro nucleated blood cells [16]. The micronucleus assay technique is considered as a biomarker tool to diagnose all such variations. Comet assay on the other hand is extensively used reliable technique for finding out the genotoxicity (DNA damage) in aquatic organisms against the heavy metals [17] that can be applied to cells of liver, kidney, gill, and blood.

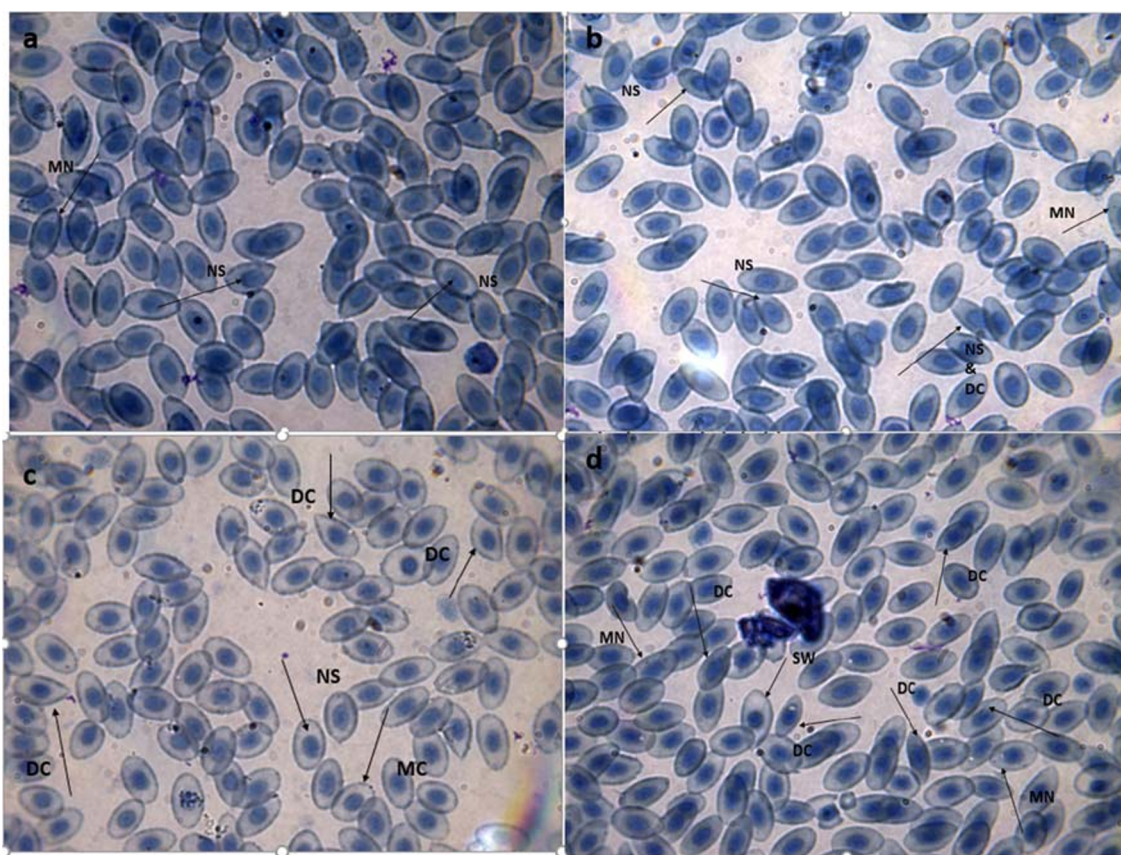
Micronuclei (MN) are formed after the event of DNA damage within a cell additional to the core nucleus as small extranuclear bodies. These bodies are formed during mitosis after cell division when chromosomes, whole chromatids, and acentric fragments are excluded eventually from the daughter nuclei [18, 19]. It detects the

clastogenic as well as aneugenic effects (causing structural and numerical changes in chromosomes respectively) of widespread toxic compounds [20]. Other nuclear alterations like fragmented, budded, blebbed, notched, or bi nuclei are also determined additional with MN assay as high-quality cytotoxic indicators [21].

Single-gel electrophoresis or comet assay is another sensitive and simple method for assessing the DNA damage in fish exposed to various toxic metals [22]. Since the DNA repair in fish is slower than the cells of mammals [23], the impact of toxic metals is diagnosed by the induced free radicals formed through breakages of phosphodiester linkages inside the DNA molecules [24].

Considering the substantial role of blood parameters in piscine health, the present study was therefore designed to find the genotoxic consequences of the selected toxic metals—copper (Cu), chromium (Cr), and lead (Pb)—in *Ctenopharyngodon idella*. Fish had been selected as a model organism since they are capable to inhabit almost the entire habitats of different aquatic zones and occupy countless commercial value. The genotoxic assessment was consequently done by using sensitive and reliable techniques on fish blood in terms of micronuclei and comet assay.





**Fig. 3** Blood smears of *C. idella* fish exposed to **a** 24 h of LC50 Pb showing nuclear shift (NS) and micronucleus (MN); **b** 48 h of LC50 Pb showing nuclear shift (NS) and micronucleus (MN) and deformed cell

(DC); **c** 72 h of LC50 Cr showing nuclear shift (NS), deformed cell (DC), and microcyte (MC); and **d** 96 h of LC50 Pb showing deformed cell (DC), micronucleus (MN), and swollen cell (SW)

## Materials and Methods

### Sample Collection and Maintenance

Freshwater grass carps (*Ctenopharyngodon idella*;  $8.5 \pm 5.5$  cm,  $9.5 \pm 6.5$  g) of same age group irrespective of sexes were procured from government carp hatcheries located in Mardan and Peshawar cities of Khyber Pakhtunkhwa province of Pakistan. Fish were acclimatized to the laboratory conditions in large plastic water tubs of 750-l capacity, approximately for 2 weeks prior to the experimentation.

The experimentation was conducted in glass aquaria ( $36 \times 11.5 \times 12$ ) of 80-l volume approximately, aerated with the help of air pumps and air stone diffusers. Glass aquaria were filled with dechlorinated tap water and enclosed with wire nettings for preventing fish escape. The glass tanks were washed before and after each experiment with distilled water for the removal of impurities and other dirt particles. Fish specimens after adjustment were then transferred to the glass tanks for the commencement of the experiment. Fish were not fed during the exposure period of the experiments. Animal maintenance and experimentation were processed in accordance with the

guidelines for use and care of laboratory animals by the Laboratory Animals Science Association [25].

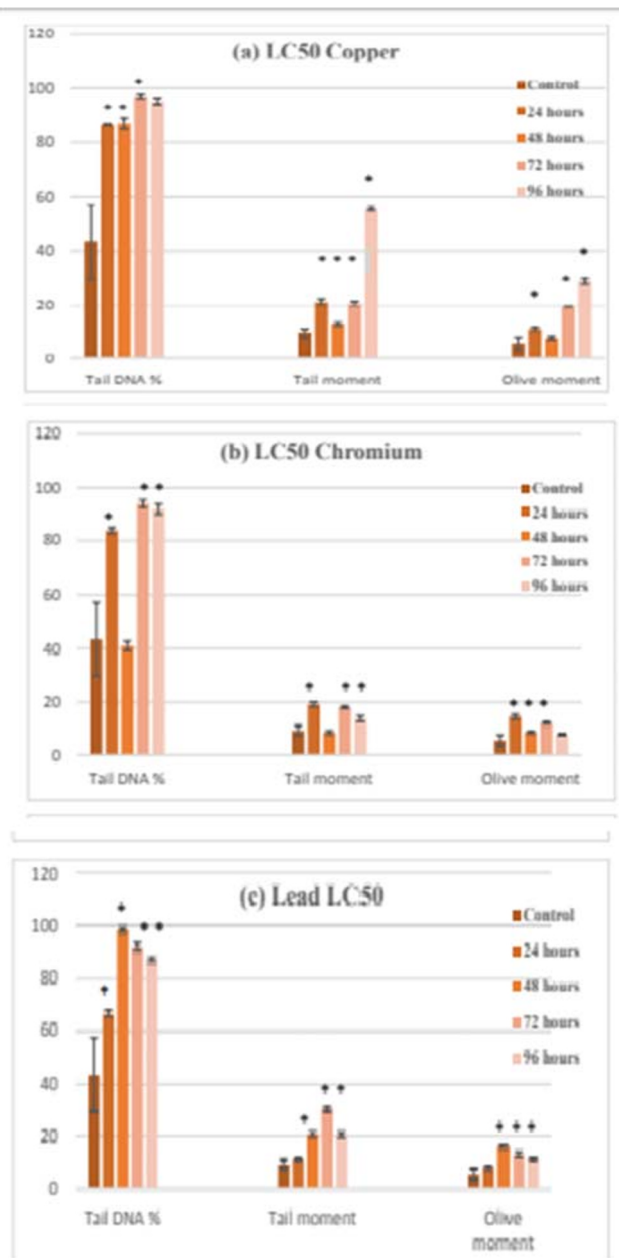
Water quality parameters in both tanks were measured and kept within the acceptable limits throughout the study course in accordance with the standard methods [26]. The mean  $\pm$  standard deviation values obtained for the water quality parameters were as follows: pH  $7.12 \pm 0.12$ , temperature  $25 \pm 0.81$  °C, electrical conductivity concentration (ECC)  $220.53 \pm 11.60$   $\mu\text{S}/\text{cm}$ , total dissolved solids concentration (TDS)  $144.66 \pm 3.39$   $\text{mg L}^{-1}$ , total suspended solids concentration (TSS)  $8.66 \pm 3.29$   $\text{mg L}^{-1}$ , total hardness concentration (CaCo3)  $86.29 \pm 1.59$   $\text{mg L}^{-1}$ , total alkalinity concentration  $102.73 \pm 2.38$   $\text{mg L}^{-1}$ , chloride concentration  $21.64 \pm 0.92$   $\text{mg L}^{-1}$ , and dissolved oxygen (DO)  $7.93 \pm 0.41$   $\text{mg L}^{-1}$ .

## Experimental Procedure

### Exposure Concentration

Analytical grade heavy metals in the form of salts were used in the present study, i.e., copper as  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , chromium III as  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ , and lead as  $\text{Pb}(\text{NO}_3)_2$  were purchased





**Fig. 4** Error bar graphs a, b, and c represents DNA damage caused by selected metals to different exposure periods. Values expressed as mean  $\pm$  SD.  $p < 0.05$ ;  $n = 3$ . The superscript shows a statistically significant difference from control values

from (Merck, Germany) through a local distributor. The whole experimentation was ethically approved with regular health checks of fish throughout the research work.

A total of four groups of fish were placed in separate aquaria. One control group with non-exposed healthy fish was used for the entire experimentation, whereas groups I, II, and III were exposed to dosages of LC50 Cu, Cr, and Pb each for 24, 48, 72, and 96 h respectively. Each metal-exposed group contained ten fish per exposure period. LC50 Cu concentrations for the exposure duration were 1.5, 1.4, 1.2, and

1 mg L<sup>-1</sup>, LC50 Cr was 25.5, 22.5, 20, and 18 mg L<sup>-1</sup>, and LC50 Pb was 250, 235, 225, and 216 mg L<sup>-1</sup> respectively. Fish samples ( $n = 3$ ) of each metal-exposed group were removed after each time interval and anesthetized with MS222 (tricaine methanesulfonate, Sigma, Germany, CAS No.886-86-2), at a concentration of 0.1 g L<sup>-1</sup> prior to dissection and morphometric measurements [27]. Afterwards, the blood samples ( $n = 3$ ) were collected against the administered metals through heparinized syringes from the caudal vein of the exposed fish at the end of the respective time interval.

## Blood Analysis

### 1. Micronuclei (MN) Test

Micronuclei test serves as an index for cytogenetic damage caused by the toxic metals. For finding out the genotoxic effects of these metals in grass carp, micronuclei (MN) test was performed by the devised method of Fenech [28] with slight modification.

**Procedure** Thin blood smears were prepared by placing a drop of blood on a clean slide and spreading it with another slide placed at an angle of 45°. The dried slides were fixed in chilled methanol for 20 min, air-dried, and stained with Giemsa stain by suggested protocol of Khan [29]. Three slides per fish were prepared.

### Identification of Cellular Alterations Under a Microscope

The stained slides after getting dried were evaluated for micronuclei at  $\times 1000$  magnification using Leica Microscope (New York Microscope Company) equipped with a digital camera (Canon, Japan). Images were captured for each field of view, analyzed by using standard procedures implemented in Digimizer [30] an image analysis software (version 4.1.1.0), and manually scored for the micronuclei and other abnormalities of the cells (1000/slide). The MN were identified by adopting the proposed criteria of [31].

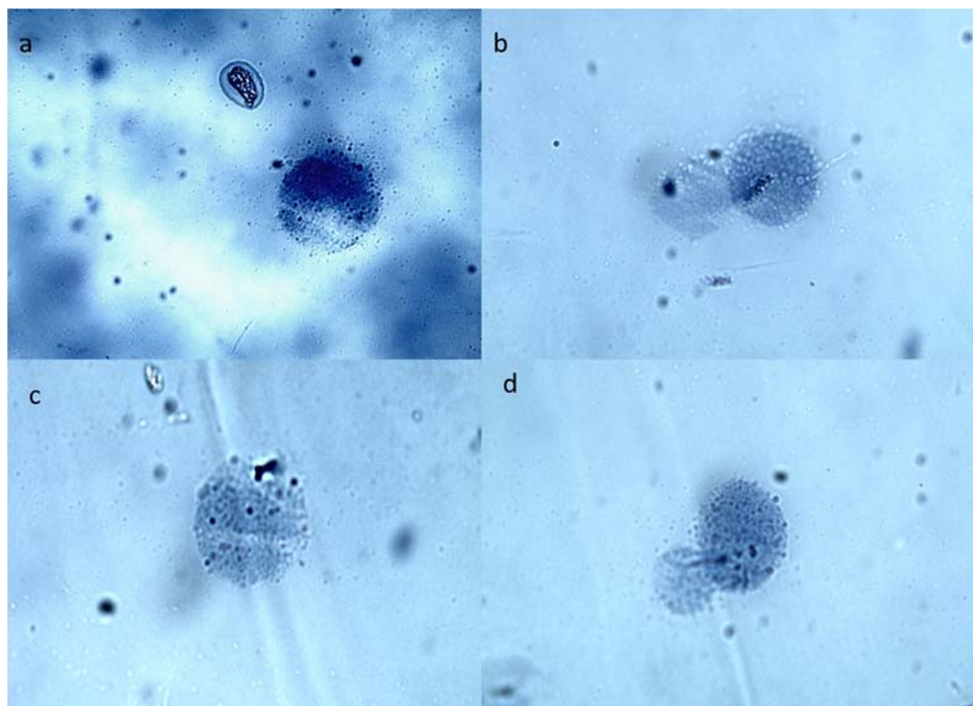
The frequency of MN for each treatment was calculated by the following formula:

$$\text{MN frequency (\%)} = \frac{\text{Number of cells with MN}}{\text{Total number of counted cells}} \times 100$$

The morphologically altered red blood cells along with nuclear anomalies were placed in other described categories such as deformed cells (wrinkled, elongated, small, sickled shaped), microcytes, swollen/enlarged cells, vacuolated cells, deformed nucleus, nucleus shifted in position, lobed nucleus, and irregular nucleus [32–34].

### 2. Single-Cell Gel Electrophoresis/Comet Assay

**Fig. 5** Giemsa-stained comets of *C. idella* fish exposed to **a** 24 h of LC50 Cu, **b** 48 h of LC50 Cu, **c** 72 h of LC50 Cu, and **d** 96 h of LC50 Cu



The DNA damage or genotoxicity was premeditated through the comet assay technique [35] with slight modifications in the Molecular Biology Laboratory of Department of Zoology, University of Peshawar, followed by Giemsa stain microscopy for visualization of DNA comets.

#### Giemsa Staining for Comet Assay

In the present study, instead of using the expensive SYBR Green fluorescence method, DNA comets were stained by the most widely used Giemsa stain (azur-eosin-methylene blue solution). This protocol of visualization of comet DNA and its compatibility with Giemsa stain was established in the laboratory recommended for the first time by Osipov [36].

#### Visualization of DNA Comets

For scoring, each slide was divided into six equal parts and random images were captured by using a common bright-field optical microscope (Leica Microscope, New York Microscope Company) integrated with a digital camera (Canon, Japan). About 50 cells were automatically measured by computerized image analysis system using OpenComet-imagej-v1.3.1 software per metal per exposure period [37] giving different comet tail parameters. Among the various available parameters, three were selected for the evaluation of DNA damage, i.e., olive moment, tail moment, and amount of DNA in comet tail (tail DNA percentage).

#### Statistical Analysis

The obtained data was recorded as mean  $\pm$  standard deviation. One-way analysis of variance (IBM SPSS statistics 20) determined significant differences between untreated and treated variables. All tests used a significance level of  $p < 0.05$ . The obtained data was plotted on error bar graphs to see the values conveniently.

## Results

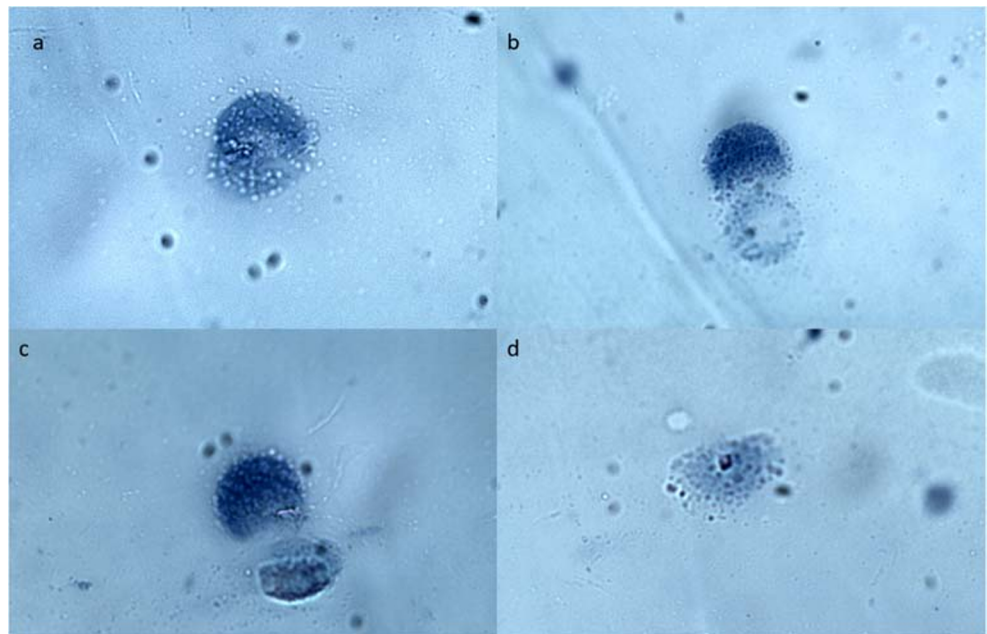
### Geno Toxicological Studies

#### Micronucleus Test Assay

The red blood cell alterations were observed in *C. idella* for the LC50 metal-treated groups of the three metals against the selected periods. In the metal-exposed groups, greater percentage of DNA damage, i.e., 1.8% and 2.0% of micronucleus induction was scored in Cr- and Pb-exposed blood samples during 24 h of treatment period as shown in Table 1. Double micronucleus frequency (0.2%), maximum nuclear deformity (2.6%), and nuclear shift (3.1%) were observed in Cr-exposed blood sample during 96 h of the exposure period. Noteworthy, for the cellular anomalies like cell distortion, minimum deformity of 0.4% was revealed in Cr-exposed blood samples as compared with that of Cu and Pb, whereas maximum cellular distortion of 7.5% was recorded for Pb-exposed blood samples at 96 h of the exposure period. Cr-exposed blood cells at 48 h were nearly normal in



**Fig. 6** Giemsa-stained comets of *C. idella* fish exposed to **a** 24 h of LC50 Cr, **b** 48 h of LC50 Cr, **c** 72 h of LC50 Cr, and **d** 96 h of LC50 Cr



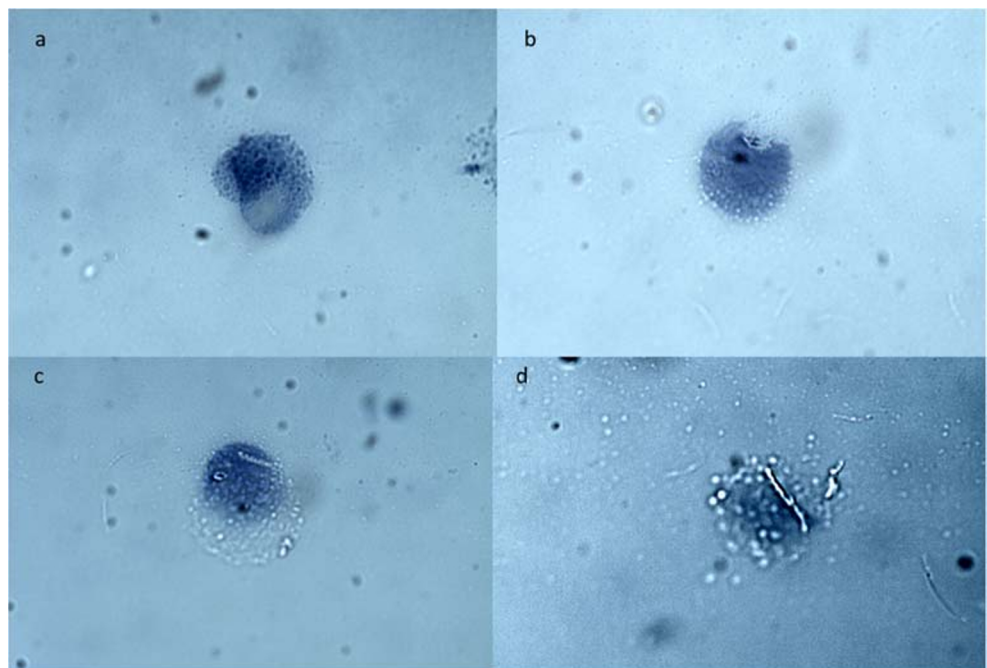
shape. Table 1 and Figs. 1, 2, and 3 represent detail observations of micronucleus induction and nuclear and cellular abnormalities against the studied metals at their respective exposure period.

#### Comet Assay

Giemsa-stained images as shown in Figs. 5, 6, and 7 were analyzed subsequently for LC50 metal exposures. The comet tail parameters were recorded for the three metals at different exposure periods. The comparison of comet image of control and the metal-exposed groups showed significant

genotoxicity ( $p < 0.05$ ) at the exposure intervals as shown in Fig. 4. Images were analyzed subsequently for the metal-treated group. The comet tail parameters were recorded for the three metals at different exposure periods. For LC50 copper, the mean tail DNA % of the control group was  $43.39 \pm 13.82$ . The mean tail DNA % trended significantly in succeeding order as compared with the control group with the highest value at 72 h ( $97.06 \pm 0.80$ ) followed by 48 ( $87.13 \pm 1.79$ ) and 24 h ( $86.79 \pm 0.26$ ) respectively. Non-significant increase was seen for 96 h of exposure. The tail moment ( $\mu\text{m}$ ) parameter depicted a significant increase from

**Fig. 7** Giemsa-stained comets of *C. idella* fish exposed to **a** 24 h of LC50 Pb, **b** 48 h of LC50 Pb, **c** 72 h of LC50 Pb, and **d** 96 h of LC50 Pb



the mean values of  $9.3067 \pm 1.85371$  of the control group to the mean values of  $21.18 \pm 1.03$  (24 h),  $12.98 \pm 0.68$  (48 h),  $20.63 \pm 0.76$  (72 h), and  $55.41 \pm 0.69$  (96 h) of the treated groups respectively. The mean values of olive moment ( $\mu\text{m}$ ) parameter for the reference group were  $5.57 \pm 2.10$  while a significant ( $p < 0.05$ , Fig. 4) increase in the mean values of the treated group was recorded at 24 ( $11.17 \pm 0.57$ ), 72 ( $19.25 \pm 0.28$ ), and 96 ( $28.73 \pm 1.18$ ) hours respectively.

Afterwards, comet tail parameters were patterned for LC50 chromium for the four exposure intervals. In the case of the mean % of tail DNA, greater significant value was recorded for 72 h ( $94.02 \pm 1.38$ ) followed by 96 h ( $92.04 \pm 1.78$ ) and 24 h ( $83.92 \pm 1.19$ ) respectively. The 48-h exposure period had a non-significant decrease when compared with the control group. Likewise, the tail and olive moment ( $\mu\text{m}$ ) of the chromium-treated groups had increased mean values with a statistically significant difference in contrast to the mean values of  $9.30 \pm 1.85$  and  $5.57 \pm 2.10$  for the reference groups respectively. The significant recorded values for the mean tail moment ( $\mu\text{m}$ ) are outlined as ( $19.25 \pm 0.96$ ) 24 h > ( $18.14 \pm 0.54$ ) 72 h > ( $13.70 \pm 1.09$ ) 96 h respectively whereas for the olive tail moment ( $\mu\text{m}$ ), the values in terms of significant DNA damage are sequenced as ( $14.45 \pm 0.91$ ) 24 h > ( $12.08 \pm 0.47$ ) 72 h > ( $8.68 \pm 0.57$ ) 48 h of exposure duration respectively. Insignificant mean values were obtained for tail and olive moment ( $\mu\text{m}$ ) at 48 and 96 h of exposure time intervals.

Likewise, DNA damage was also evaluated for the LC50 lead-treated group. Regarding tail DNA %, significant increases ( $p < 0.05$ ) in the mean values of the exposed groups were perceived at all the four exposure intervals as compared with the mean values of  $43.39 \pm 13.82$  for the reference group. Thus, significantly high percentage of DNA damage was seen after 48 h ( $98.54 \pm 0.93$ ) of exposure period followed by 72 ( $91.9 \pm 1.46$ ), 96 ( $87.12 \pm 0.93$ ), and 24 h ( $66.70 \pm 1.07$ ) of exposure period respectively. Similarly, mean values of the tail moment ( $\mu\text{m}$ ) and olive moment ( $\mu\text{m}$ ) had statistically significant difference ( $p < 0.05$ , Fig. 4) at 48, 72, and 96 h of metal administered periods when compared with the mean values of the reference groups.

## Discussion

In the present investigation, positive responses in contrast to the control had been observed in fish blood to the administered metals. Amongst the LC50 metals, Cr surpassed the three metals by producing prominent levels of genotoxic damage whereas Pb ranked as the second chief metal for higher frequencies of MN induction followed subsequently by Cu. The induced micronuclei frequency by LC50 metals was in the following order: Cr > Pb > Cu. Since Cr is mostly used in industries, it

has therefore awakened the maximum interest regarding its genotoxic activity [38, 39]; for instance, through numerous studies, the cytotoxic and genotoxic nature of aquatic Cr in fish cells has been confirmed [20]. Parallel study revealed significant MN induction in fish red blood cells with the increase in treated doses of Cr and the trend slowing down after the 21 days of exposure [21]. Similar substantial genotoxic effects of LC50 Pb and LC50 Cu were depicted in erythrocytes of the three freshwater fish species at 96 h of exposure [40]. Studies have shown the genotoxic effects of metals on fish and other aquatic animals, but the mechanism of genotoxicity is not fully stated with very little knowledge about Cu genotoxicity [18]. The possible path for Cu genotoxicity has been explained as the production of reactive oxygen species causing damage to DNA [19]. The least frequencies of double micronuclei were also induced in the present study as compared with the single micronucleus. These double micronuclei were formed in fish erythrocytes only at the exposition of LC50 Cr metal whereas Cu and Pb depicted no double micronuclei. Double micronuclei formation had been reported in a genotoxic study of *Labeo rohita* confirming the MN assay as a biomarker tool of aquatic pollution [41].

Cellular and nuclear anomalies were documented in comparison with the blood cells of the healthy fish group. The mechanisms of these morphological anomalies have not yet been fully explained though considerable attention has been attained besides the studies of MN induction [42, 43]. Cellular deformity for the metal administered groups was seen in high percentages for Pb followed by Cu and Cr that concur with the study of three freshwater fish species confirming cellular and nuclear anomalies such as lobed, notched, and blebbed nucleus at the 48 h of Pb, Cu, and Cr exposure respectively [40]. Deformed nucleus and nuclear shift had been the next uppermost calculated anomaly amongst the three administered metals, whereas lobed and irregular nuclei were the third frequently seen nuclear aberrations with lowered percentages noted for the reference group. It is worth mentioning that the formation of these nuclear anomalies caused by toxicant exposures may further require explainable studies [44].

Likewise, metal-induced significant alterations were determined in the levels of DNA damage through comet assay. This technique can effectively screen diverse genetic contaminants in the peripheral blood of different fish species [45]. During present research work, concomitant DNA damage has happened in the blood of *C. idella* at the considered concentration of the three metals (Figs. 4, 5, 6, and 7). Similar findings of genetic damage were reported in *Hyphssobrycon luetkenii* caused by metals such as Cu, Cr, Pb, zinc, nickel, and aluminum [46]. In terms of the comet tail parameters, significantly increased percentage of tail DNA had been calculated for the three studied



metals in the exposed fish in contrast to the reference mean values of  $77.83 \pm 1.82$ . Similar higher percentages for tail DNA was determined in *Cirrhina mrigala* followed by *Ctenopharyngodon idella* against copper, zinc, and arsenic [47]. Analogous to current experimental results, significant differences were reported in respect to tail moment and olive moment in fish collected from polluted water [41] and fish exposed to inorganic chromium with maximum genotoxic effects revealed in respect to greater tail DNA % of  $56.57 \pm 0.839$  and olive moment of  $3.83 \pm 0.144$  as compared with the non-administered fish group. Analysis thus has approved the genotoxic effects of Cu, Cr, and Pb in *Ctenopharyngodon idella* with the lowest effects in the healthy fish group.

## Conclusion

Current cyto-genotoxic investigation confirmed the structural genomic alterations along with MN induction. It is one of the newly studied fish analysis in Pakistan that detected the metal toxicological response using comet assay and micronucleus assay. Since the increase in commercial, industrial, and agricultural chemicals has remarkably increased the degree of genetic disorders along with the mortality of the exposed organisms, the effects of such toxicants thus need to be evaluated in aquatic habitats of Pakistan to maintain the function and integrity of cellular DNA in living organisms. Present results also demonstrated the use of low-budget Giemsa-stained comets instead of silver, fluorescent SYBR Green or other costly staining methods that would open opportunities for the application of a low-priced version of the comet assay in genotoxic laboratories.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** Ethical approval for the study was taken from the Ethical Committee, University of Peshawar.

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