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Research Article

Comparative genotoxic and histopathological effects of copper nanoparticles and copper chloride in goldfish (*Carassius auratus*)

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Abstract

The purpose of the current study was to ascertain the comparative effects of compounds, copper (II) oxide nanoparticles (nCuO) and copper salt ($CuCl_2$), on the genotoxicity and histopathology of goldfish (*Carassius auratus*) for acute exposures of 24, 48, 72, and 96 h (concentrations: 100, 150, 200, and 1, 1.5, 2 mg/L nCuO and $CuCl_2$, respectively). The peripheral erythrocytes were obtained for that purpose from the caudal vein in each individual. The Comet assay and micronucleus test were used to evaluate genotoxicity, and gill tissues were prepared for histopathology. High levels of nCuO and $CuCl_2$ concentrations and acute exposure (96-hour) increased the percentage of DNA in the comet tails, olive tail moments, and micronucleus counts showed genotoxic effects on exposed fish. Also, the gill tissue alterations were observed and changed as the lifting of the respiratory epithelium, hyperplasia of the lamellar epithelium, incomplete, and complete fusion of several lamellae, and hypertrophy. Genotoxicity for nCuO and $CuCl_2$ was discovered to be a concentration and time-dependent phenomenon. In general, experimental groups showed significant impacts (p=0.05) on concentrations and exposure periods, which may contribute to understanding the mechanism of nCuO and $CuCl_2$-induced genotoxicity in the fish.

Introduction

Water pollution is a global problem caused by anthropogenic activities such as industrial discharge, agricultural runoff, and urbanization, which endanger aquatic ecosystems and especially fish populations. Among numerous pollutants such as heavy metals, herbicides, and chemical compounds, oxidative stress has been identified as an important mechanism (Fuat Gulhan e*t al*., 2012; Selamoglu *et al*., 2015; Caglar *et al*., 2019). This stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses activated by pollutants through direct ROS generation, enzyme stimulation and antioxidant system disruption. Fish respond to oxidative stress by altering enzyme activity, biomarker levels and gene expression, eliciting physiological adaptations and disease (Orun *et al*., 2005; Talas *et al.*, 2008; Selamoglu, 2011; Kakoolaki *et al*., 2013; Talas *et al*., 2014).

To maintain the long-term viability of aquatic ecosystems, mitigation techniques include environmental management, regulatory policy and multidisciplinary research. Aquatic products and animals are indispensable components of the human diet and play a crucial role in the global aquatic product industry, serving consumers worldwide. Therefore, safeguarding our aquatic environments against pollution is imperative due to its diverse environmental and ecological ramifications. The emissions of volatile organic substances and the contamination of water by oil chemicals and various hazardous agents pose significant threats to aquatic ecosystems and the organisms inhabiting them. Among these organisms, fish stand out as essential nutrients in the human diet and as a prominent commodity in the global aquatic product market. Consequently, there is an urgent need to prioritize the enhancement of aquatic products and their health. This necessitates a concerted focus on oxidative stress studies and the adoption of sustainable practices aimed at protecting fish populations and preserving the integrity of aquatic ecosystems (Ates *et al*., 2008; Kakoolaki *et al.*, 2013; Mesut, 2021; Selamoglu, 2021). By proactively addressing these challenges, we can reduce the negative impacts of pollution on aquatic environments while maintaining high quality aquatic products and fish species.

One of the most prevalent transition metals in nature, copper (Cu) serves a variety of functions in organisms and is a necessary nutrient (Burke and Handy, 2005). Cu contamination is pervasive in the aquatic environment. Copper is frequently found in aquatic systems from both natural and man-made sources. Geological deposits, volcanic activity, weathering and erosion of rocks and soils, and geological deposits are all natural sources of copper in surface water. Aquatic system pollution is a serious environmental issue that is becoming more and more global. Aside from cadmium, copper is one of the most often used metals that cause pollution. In several industrial and agricultural operations, people use copper (Carvalho and Fernandes, 2008; Kumar *et al*., 2021). It is a part of several fungicides, algaecides, bactericides, and herbicides, particularly in the forms of sulfate, hydroxide, oxychloride, carbonate, citrate, or gluconate (Ali *et al*., 2019). Therefore,

contamination will unavoidably happen by Cu forms. Due to human and natural inputs, waterborne Cu has been identified in the environment in high amounts (Cu, 0.04-560 g/L) (Agency, 2007; Oliveira *et al*., 2008; Yusni and Ifanda, 2020).

Due to their remarkable physicochemical characteristics, metal oxide nanoparticles (NPs) are often employed in a wide range of consumer goods and industrial technologies. Nanomaterials are employed in a variety of home furnishings and appliances, the production of textiles and electronics, the creation of medical devices, and bioremediation technology (Montes *et al*., 2012). The items incorporating nanomaterials will be released into aquatic ecosystems and agricultural areas as a result of large-scale manufacture and use (Chatterjee, 2008; Fabrega *et al*., 2011; Scott-Fordsmand *et al*., 2017). Metal oxide NPs are among the most popular nanomaterials, and their possible ecological consequences have attracted a lot of interest. Due to their increased potential for potential uses in the future and the fact that NPs are an addition to personal care products, copper (II) oxide nanoparticles (nCuO) are produced in huge quantities for both industrial and domestic usage (Hochella *et al*., 2019; Ming *et al*., 2020). Additionally, CuO NPs may find use in a variety of industrial settings (Fig. 1). CuO NPs are thus among the most likely environmental pollutants (Nel *et al*., 2006; Klaine *et al*., 2008; Poynton *et al*., 2011; Hou *et al*., 2017).

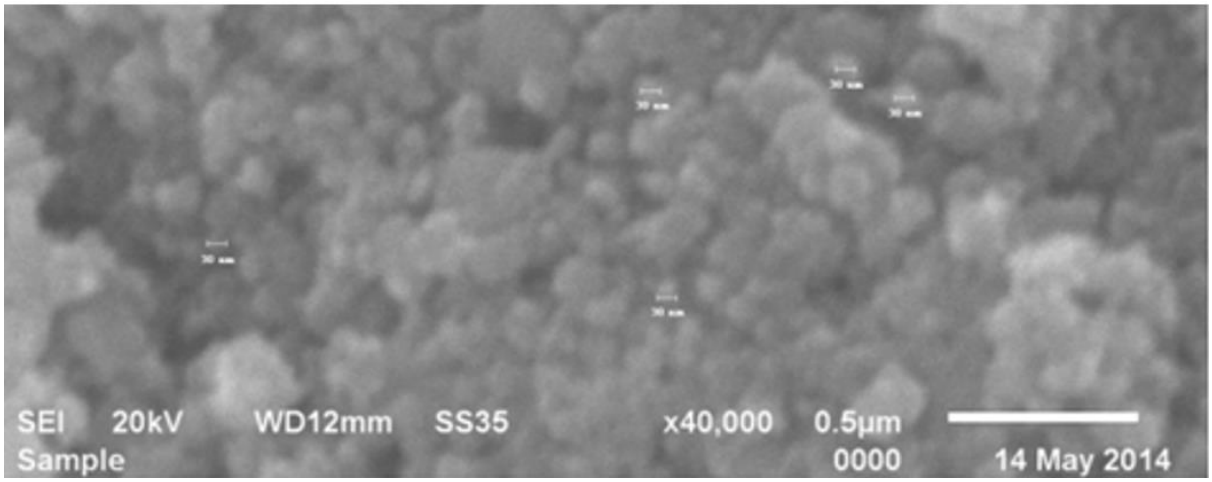

Figure 1: A scanning electron microscope (SEM) image of nCuO.

The significance of determining the toxicological interactions and effects of nCuO and copper salt ($CuCl_2$) combinations on aquatic life is highlighted by the widespread copper pollution. In this study, goldfish are used as a model to evaluate the toxic effects of the combined exposure of nCuO and $CuCl_2$ in freshwater ecosystems on fish. To investigate the potential genotoxic and cytotoxic effects in both structures of copper we used Comet and micronucleus assays as a test for DNA damage and gill tissue for histopathology. The significance of determining the

toxicological interactions and effects of nCuO and $CuCl_2$ combinations on aquatic life is highlighted by the widespread copper pollution.

Materials and methods

Following the commencement of the experiment, the total copper (Cu) contents of all experimental groups were assessed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (at the 1 hour). The goldfish (*Carassius auratus*) were purchased from a commercial supplier (Dogasan Aquarium, Istanbul, Turkey) and were transferred to the laboratory. They were given the commercial base diet of *C. auratus* (38.7% crude protein; 13% crude fat; 14.8% crude fiber ash) and acclimated for a month at 26±1°C under natural photoperiod. At the start and the conclusion of each exposure session, the standard water quality parameters were tested according to established procedures (APHA, AWWA, WPCF 2005). The pH ranged from 7.6 to 8.1±0.1, dissolved oxygen was at 8.3±0.3 mg/L, nitrite was at 0.3 mg/L, and ammonium was at 0.05 mg/l. In this study, 180 goldfish from both sexes (the mean weight and length of 0.72±0.1 g and 4.7±0.1 cm, respectively) were prepared. The experiments were randomly performed on six groups and at four different times, together with a positive (5 mg/L ethyl methanesulfonate) and negative control in three duplicate tanks. There were 10 fish in each tank (40×30×50 cm) with 25 L of dechlorinated tap water. Fish were not fed during the experiments in order not to cause physiological changes.

nCuO (Alfa Aesar, K02S018, Germany) and $CuCl_2$ (Alfa Aesar, 10154919, Germany) were produced in diluted water and supplied to the aquariums. Specific particle size distribution of nCuO nanometallic particles with scanning determined by electron micrographs (SEM). Subnanometer-sized nanoparticles with solubility, as determined from SEM images, ensure no aggregation is observed. It has been found to be about 30nm in the regions. This result is commercially obtained nano copper (II) is compatible with the property given in the material data sheet of the oxide (Fig. 1). For the acute studies, four distinct nCuo and $CuCl_2$ concentrations (nominal concentrations: 100, 150, 200, and 1, 1.5, 2 mg/L for nCuO and $CuCl_2$) and four time periods (24, 48, 72, and 96 h). The acute values of Copper compounds in goldfish, presented as 96-h median lethal doses, were used to choose the test concentrations (Kahru and Dubourguier, 2010).

To reduce changes brought on by fish metabolism, the volatilization of less stable chemicals, and organism catabolites, the test water was replaced every 24 hours (80%) by providing semi-static test conditions. After exposure times, blood samples were taken from the caudal vena of anesthetized fish using heparinized syringes (100 mg/L MS-222, Sigma Aldrich). The fish were put back in their tank after a five-minute recovery time in well water.

Genotoxicity

Trypan blue dye was used to examine the cells' viability before the experiment began. Goldfish were used as test subjects for the comet assay, which measures DNA strand breaks (single-strand breaks and alkali-

labile sites). Two fish were removed from each aquarium at each sample period after 24, 48, 72, and 96 hours of exposure and given buffered MS-222 anesthesia. To avoid causing more DNA damage, all of the experimental procedures were carried out in the dark beneath a yellow bulb. The alkaline comet test was carried out using a modified version of the Tice *et al*. Technique (Tice *et al*., 2000). Briefly, the erythrocytes (10µL) were resuspended in cooled PBS (pH 7.4) buffer after the peripheral blood samples were taken from the caudal vasculature using a 1 mL heparinized syringe. The suspension (65 µL) was then combined with 100 µL of 0.65% (w/v) agarose with a normal melting point before being applied on a frosted slide that had already been precoated with 0.65% (w/v) agarose with a high melting point. The microscope slide was then submerged in a cold (4°C) lysate solution that contained recently added 1% Triton X100 and 10% DMSO and contained 2.5 M NaCl, 10 mM Na_2EDTA, 10 mM Tris-HCl, and 1% SDS. To facilitate DNA unwinding, the slide was incubated in a newly made alkaline buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 13) for 30 min after 2 h. In the same buffer, electrophoresis was performed at 4 °C for 30 min at 15 V and 300 mA. After being neutralized with a 0,4 M Tris solution (pH 7.59), the slides were immediately stained with 75 L EtBr (10 g/mL) for viewing under a fluorescence microscope (BX51TF, Olympus, Japan) with a 530 nm excitation filter, a 590 nm emission filter, a digital camera (Kameram A640 FL). A computer-based image processing system was used to make the genotoxic observations and analysis (Kameram Komet Module, Micro System Ltd. Turkey). For data analysis of DNA damage percentage in the tail and olive tail moment, 100 cells from each replication slide were randomly chosen. The fraction of DNA-damaged cells with more than 5% damaged DNA in the tail was used to establish the DNA damage percentage. The amount of DNA in the tail distribution and the distance between the head and the tail were calculated to create the Olive tail moment (Olive *et al*., 2012). To reduce score fluctuation, each slide was examined by a single observer while remaining completely blind. All chemicals used in these processes were bought from Sigma-Aldrich.

Micronucleus

The slides that would be stained with fish blood were maintained in a solution of 99% ethanol and hydrochloric acid for 10 minutes before being rinsed with distilled water (dH_2O). The slides' edges were covered with fish blood samples. After soaking in 99% ethyl alcohol for ten minutes, the examples were fixed. The slides were run through dH_2O after the ready-made 5% Giemsa (Sigma-Aldrich) stain was applied to the smear preparations. Canadian balsam (Entellan) was used to seal the stained preparations so they could be examined under a light microscope (BX51TF, Olympus, Japan). Four distinct zones were chosen and 250 cells were counted with the 100 x lens. The images were captured using the Image Pro Express 6.0 software. To quantify the frequency of notched nuclei, lobed nuclei, budding, fragmenting, and micronucleated (MN) cells per 1000 cells (‰), 1000 erythrocytes

were scored from each slide at a magnification of 1000 x. %MN is determined by dividing the total number of cells by the number of cells that contain MN.

Histopathology

After 96 hours, fish from the negative control group (negative) and nCuO and $CuCl_2$ exposure groups were removed and dissected gill tissues were processed for histological analysis. Fixation was made in 10% neutral formalin with 0.03% eosin (F5304, Sigma) 24. After fixation for 24 h, gill tissues were dehydrated through a graded series of ethanol for 30 minutes each, cleared in xylene, and infiltrated in paraffin (56-58^{o}C). Sections of 7-10 μm were prepared from paraffin blocks by using a rotary microtome. Staining with Hematoxylin dye (Hematoxylin Solution, Mayer's) for 15 minutes and in Eosin solution (EosinYY-Solution 0.5% Aqueous for Microscopy, Merck) for 5 minutes. Histological preparations were randomly examined three times, and the results from each observation were combined for the final results. Histopathological images were captured using an Olympus BH2 microscope with an image analyzer system (Media Cybernetics, Silver Spring, MD).

Statistical Analysis

The averages and standard deviations (SD) of each independent experiment that was carried out in triplicate were given as experimental data. SPSS for Windows version 11.0 was used to conduct all statistical analyses (SPSS Inc., Chicago, IL, USA). The difference between groups was examined using a three-independent-samples test, which was followed by a non-parametric Mann- Whitney U test. *p*-value 0.05, significance was determined.

Results

In the exposure groups (nCuO: 100, 150, and 200 mg/L and $CuCl_2$: 1, 1.5, and 2 mg/L) of goldfish at 24, 48, 72, and 96 h with positive, negative controls, comet formations reflecting the DNA profile migrated out of the nucleus was shown by fluorescence staining. When the comet images obtained from the positive control and application groups were compared with the negative control, it was seen that the tail formation increased significantly (p=0.05). The Comet images were analyzed using a computer program, and DNA damage levels were presented as % DNA tail and olive tail moment. We discovered that the specimens that were exposed to all concentrations of nCuO and $CuCl_2$ had significantly more DNA damage than the negative and positive control after performing the comet assay on erythrocytes (Fig. 2). We discovered high comet scores when we compared the positive control (66 ±5.43% Tail DNA and 46±3.21 Olive tail moment) and experimental groups. The highest comet score compared to the positive control was seen in fish exposed to nCuO. It was observed that the experimental groups exposed to both chemicals had higher DNA damage than the positive control (Table 1, Figs. 2 to 4)

After staining and monitoring under the light microscope, the frequencies of MNs generated outside the main nucleus as a result of genetic damage were assessed. When the MN pictures from the positive, negative, and experimental groups were

compared to the negative and positive controls, MN formation was determined to be significantly increased (p=0.05). The quantity of MN increased rapidly, especially as the exposure period increased (Fig. 5).

Histological images of the gills obtained from the positive, negative, and experimental groups were compared with the negative and positive control.

Table 1: nCuO and $CuCl_2$ at various concentrations have an impact on comet properties in the erythrocytes of *Carassius auratus*.

	Concentration	Time	Tail % DNA	Olive tail moment
	Negative		28 ± 1.3	10 ± 0.7
	Positive	24h	66 ± 5.43	46 ± 3.21
nCuO	100 mg/L	24h	65 ± 3.78	52 ± 1.36
		48h	74 ± 3.9	59 ± 2.11
		72h	78 ± 3.45	66 ± 2.21
		96h	81 ± 4.45	73 ± 3.3
	150 mg/L	24h	71 ± 3.65	59 ± 1.7
		48h	79 ± 2.7	80 ± 4.87
		72h	77 ± 3.9	66 ± 2.4
		96h	85 ± 3.15	76 ± 2.6
	200 mg/L	24h	69 ± 2.21	58 ± 1.91
		48h	73 ± 4.6	65 ± 4.21
		72h	80 ± 3.57	68 ± 2.13
		96h	86 ± 3.15	79 ± 3.78
$CuCl_2$	1 mg/L	24h	76 ± 2.78	52 ± 2.9
		48h	61 ± 2.96	59 ± 2.87
		72h	81 ± 5.12	66 ± 2.45
		96h	80 ± 3.38	73 ± 2.24
	1.5 mg/L	24h	78 ± 2.7	59 ± 1.36
		48h	62 ± 2.6	80 ± 3.91
		72h	74 ± 2.13	66 ± 2.74
		96h	82 ± 4.78	76 ± 2.87
	2 mg/L	24h	76 ± 2.9	58 ± 1.5
		48h	65 ± 2.78	65 ± 2.04
		72h	82 ± 3.46	68 ± 2.19
		96h	80 ± 5.24	79 ± 3.21

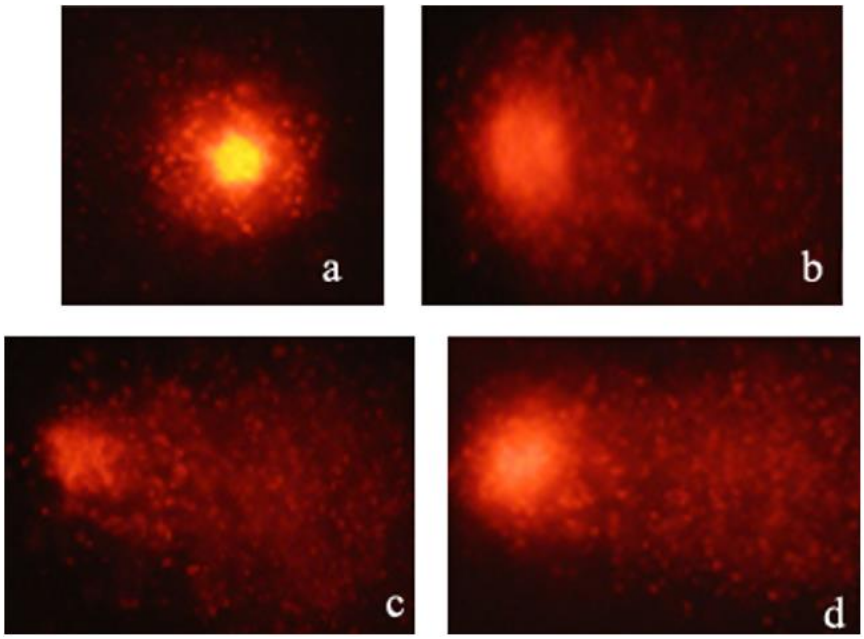

Figure 2: Erythrocytes of *C. auratus* showing: (a) negative control DNA, (b) positive control DNA, (c) DNA damaged after exposure to nCuO and (d) DNA damaged after exposure to $CuCl_2$.

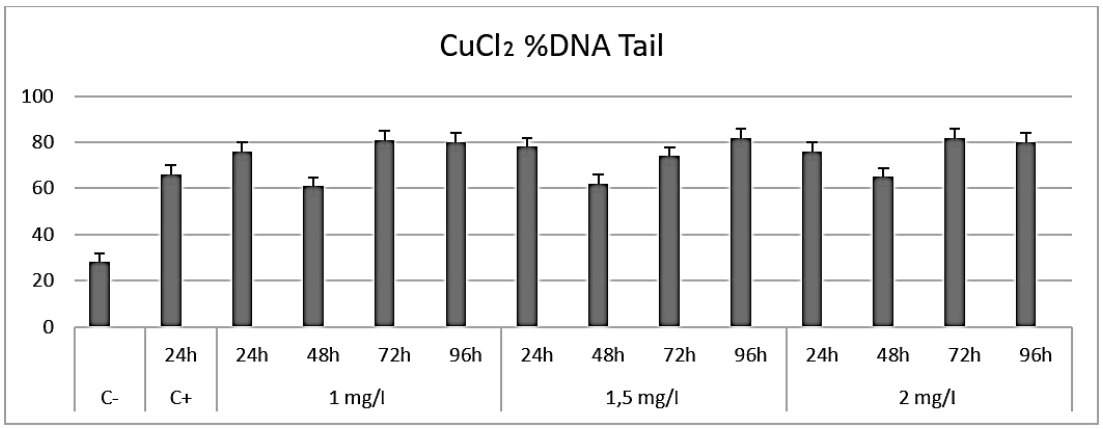

Figure 3: Showing tail % DNA and standard error bars as a result of exposure to nCuO at different concentrations and durations with positive and negative control in goldfish (p<0.05).

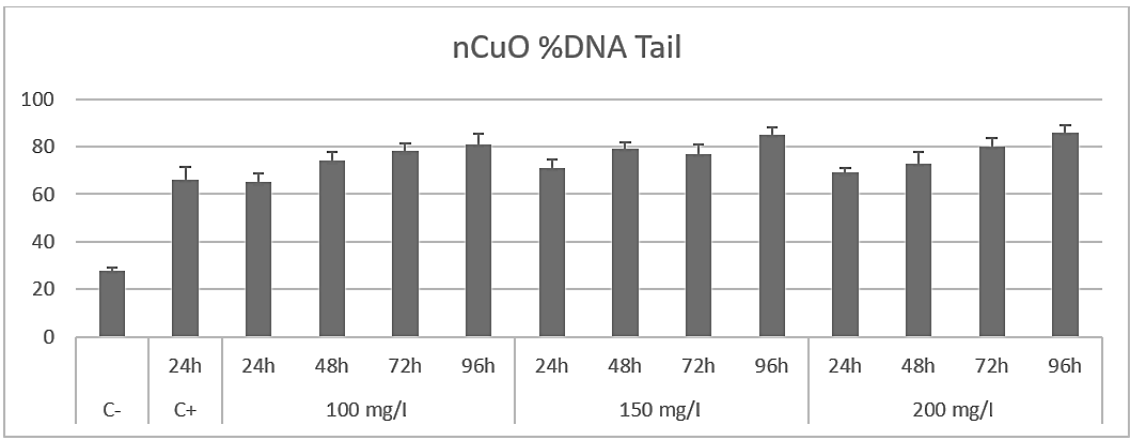

Figure 4: Showing tail % DNA and standard error bars as a result of exposure to $CuCl_2$ at different concentrations and durations with positive and negative control in goldfish (p<0.05).

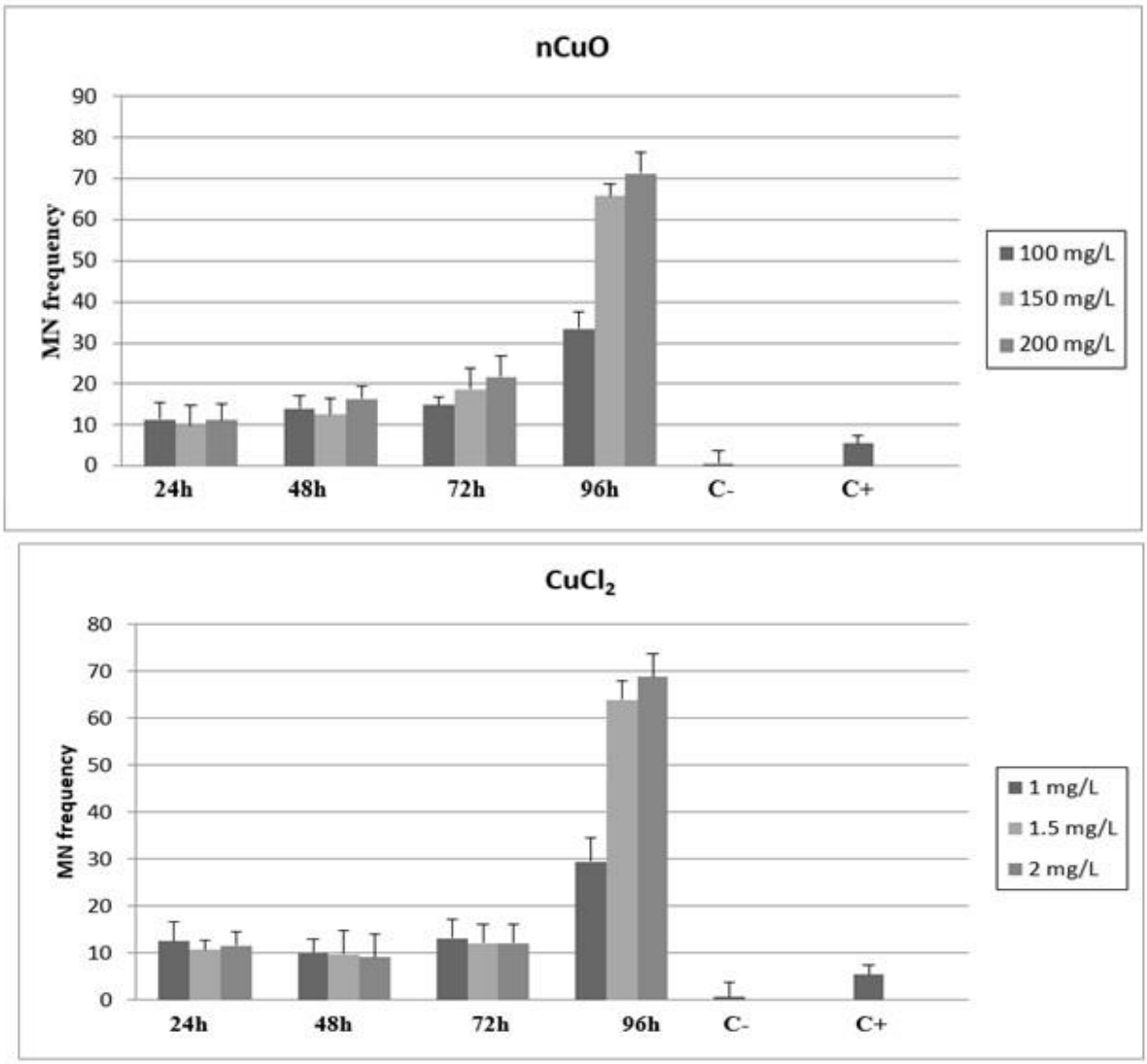

Figure 5: MN counts are shown on a graph of comparative frequency in the erythrocytes of goldfish after exposure to nCuO and $CuCl_2$ ($p<0.05$).

Significant deteriorations in the gill epithelium such as uplift, edema, fusion of secondary lamellae, hyperplasia, and hypertrophy were observed that would prevent even the function of the gills (Table 2). The images obtained as a result of histopathological staining revealed the differentiation in the gill structure (Fig. 6).

Table 2: Histopathological evaluation of the findings of nCuO and $CuCl_2$ administration groups.

Concentration	nCuO			$CuCl_2$		
	100 mg/L	150 mg/L	200 mg/L	1 mg/L	1.5 mg/L	2mg/L
Degeneration of secondary lamellae	+	+	++	+	+	+
Epithelial lift	+	++	+++	+	++	++
Fusion	+	++	+++	+	++	++
Hyperplasia	+	++	++	+	+	++
Hypertrophy	+	++	+++	++	+	++
Edema	-	+	++	-	+	+

+:less, ++:moderate, +++:severe

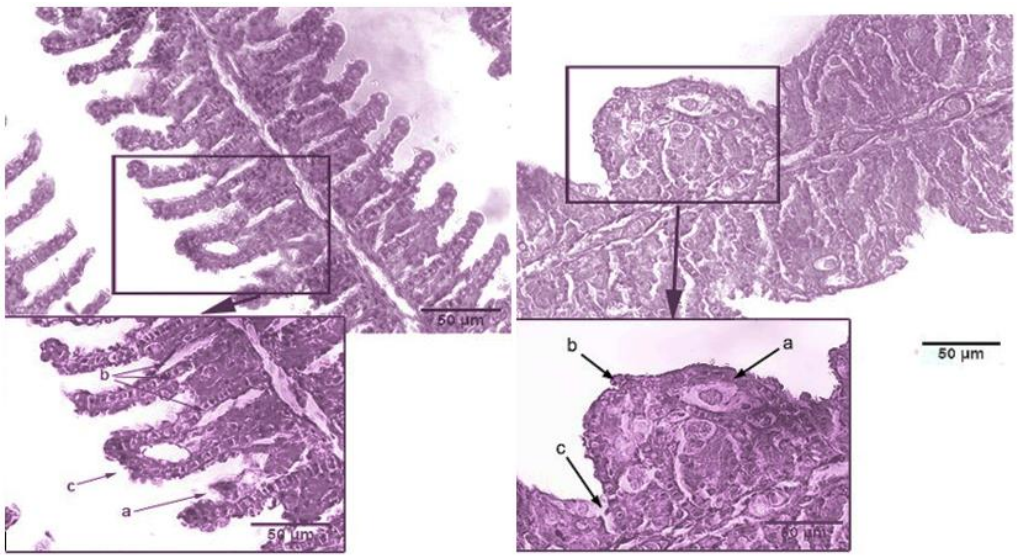

Figure 6: Micrograph showing the gill histopathology of goldfish treated with nCuO and $CuCl_2$ respectively a) mucous hypertrophy b) lamella epithelial removal c) fusion of four secondary lamellae.

Discussion

The primary objective of this study was to systematically collect and analyze data to elucidate the genotoxic and histopathological impacts of nCuO and $CuCl_2$ on *Carassius auratus*, commonly known as goldfish. This experiment aimed to determine the differential effects of these copper-based compounds at various concentrations, exploring their potential to induce DNA damage and cellular alterations within the tissues of the exposed organisms. By employing a range of biomarkers for genotoxicity and histopathological assessment, the study sought to provide a comprehensive overview of the cellular and molecular disruptions occurring in response to these specific contaminants. The results of this research are intended to contribute valuable insights into the toxicological profiles of nCuO and $CuCl_2$, thereby informing risk assessment and management strategies in aquatic environments where these compounds are prevalent contaminants (Kahru and Dubourguier, 2010).

Behavioral changes in fish were monitored throughout chemical administration (Sharma *et al*., 2019). In this study, it was found that since there was an excessively dark environment in all concentrations of nCuO, the fish swam close to the surface of the water and there was a decrease in their movement. In $CuCl_2$ concentrations, active swimming rates decreased and they moved more towards the bottom. In a similar study, Tilton *et al*. reported that active swimming rates of zebrafish, in a mixture of copper chloride and chlorpyrifos, decreased as in their study (Tilton *et al*., 2011).

Results showed that the comet test was more sensitive in fish studies than the micronucleus test, and they suggested that the two tests, which revealed DNA damage from distinct perspectives, may be combined (Ternjej *et al*., 2010; Pavlica *et al*., 2011). The integrated biomarker response index, which integrates the numerous biomarkers in a multivariate data set, can offer a comprehensive harmful effect of pollution on organisms. Many

field and laboratory environmental risk assessment studies have used this strategy. However, till recently, no investigations on the harmful effects of TiO_2 NPs at projected ecologically relevant concentrations on marine scallop have been published (Kim *et al*., 2010; Kim *et al*., 2013; Kim and Jung, 2016). Blood cells are susceptible to potentially harmful compounds, as indicated by research involving $CuSO_4$ molecules (Beninca *et al*., 2012). Researchers who performed the comet test to demonstrate genotoxicity discovered time- and concentration-dependent increases in the exposed groups as compared to the control groups by examining the proportion of DNA tail damage (Ghisi Nde *et al*., 2011; Pavlica *et al*., 2011; Beninca *et al*., 2012). Many researchers have shown that the comet test is an inexpensive, rapid and reliable test for detecting genotoxicity (Mohanty *et al.*, 2011; Sponchiado *et al*., 2011; Kousar and Javed, 2015; Chelomin *et al*., 2017; Kaygisiz and Ciğerci, 2017; Sehirli *et al*., 2017; Jafar *et al*., 2019; Atila *et al.*, 2020; Moller *et al*., 2020; Boyadzhiev *et al*., 2022). In our study, as a result of the examinations made with the comet test, DNA damages were more clearly seen with the increase in concentration and exposure time, in accordance with the literature. Our results showed that there was more damage in the exposed groups compared to the positive control. Studies have shown that copper nanomaterials cause more DNA damage compared to other nanomaterials. It has also been found that the comet test is sensitive and accurate in revealing potential nanomaterial DNA damage (Boyadzhiev *et al*., 2022). Studies have shown that copper nanomaterials cause more DNA damage compared to other nanomaterials They have also found that the comet test is sensitive and accurate in revealing potential nanomaterial DNA damage (Boyadzhiev *et al*., 2022).

The studies demonstrated how a variety of heavy metals increased MN counts and can be used on blood and other tissue cells (Bolognesi and Hayashi, 2011; Obiakor *et al*., 2012; Di Bucchianico *et al*., 2013). Depending on the concentration and duration used during the application of the nCuO and $CuCl_2$ linear increase in MN counts was found (Fig. 5). This increase was observed to be greater in fish exposed to nCuO. Semisch *et al*. explained that nCuO particles accumulate more in the nucleus than other copper forms, resulting in an increased level of DNA strand breaks (Semisch *et al*., 2014). A Genotoxic study of *Labeo rohita* revealed the development of micronuclei, supporting the MN test as a biomarker tool for water contamination (Hussain *et al*., 2018). It is worth mentioning that the formation of these nuclear anomalies caused by toxicant exposures may further require explainable studies (Cavas and Ergene-Gozukara, 2003).

Gill tissues are significant target tissues for histopathological alterations because they play a fundamental role in respiration and are in direct contact with water (Wood and Eom, 2021). Copper exposure is known to cause alterations in the gill epithelium such as hypertrophy, hyperplasia, necrosis, and secondary lamellae fusion (Karayakar *et al*., 2010; Ostaszewska *et al*., 2016). Furthermore, copper nanoparticles build up more quickly in the liver and other organs

of living things than in bone tissues (Wang *et al*., 2014). Not only copper forms do not damage tissues and organs, but studies have also reported that pesticides used in agriculture also damage different tissues such as the brain. Continuous use of pesticides enters the aquatic ecosystem and threatens the living organisms in the water. According to current understanding, living organisms may experience oxidative stress as a consequence of the accumulation of hazardous chemicals (Ostaszewska *et al*., 2016). In this case, the air supply will decrease, it will disrupt organ functions, shorten the lifespan of the living thing significantly, and even cause death. (Luzio *et al*., 2013). Zebrafish gill alterations revealed that epithelial cell proliferation as well as edema of main and secondary gill filaments following exposure after 48 hours of exposure to 0.25 mg/L ionic copper and 1.5 mg/L Cu NPs in the literature (Griffitt *et al*., 2007; Griffitt *et al.*, 2008; Griffitt *et al*., 2009). In our study, we observed degeneration of secondary lamellae, epithelial lift, fusions, hyperplasia, hypertrophy, and edema . These alterations contribute to thickening and damaging the surface of the gills which inhibates gas exchange, particularly oxygen uptake. (Al-Bairuty *et al.*, 2013; Hao *et al.*, 2013). As a result of our study, tissue alterations and damages seen in fish exposed to nCuO may have a greater impact on the overall health of aquatic organisms than $CuCl_2$.

Given the critical role of essential metals (Fe, Zn, and Cu) as cofactors in numerous enzymatic processes, their levels in fish are significant due to strict physiological regulation, emphasizing the importance of understanding these concentrations for both fish management and human consumption (Kamaruzzaman *et al*., 2011). They have been reported that the accumulation of these metals at certain levels is not fatal for living organisms or when they are transferred through the food chain. However, the damage that will occur in the accumulation of metals in living organisms can reach levels that can no longer be tolerated by living organisms. These accumulations are seen in different tissues (Caglar *et al*., 2019). Metal accumulation in fish differs between tissues and is affected by the environmental conditions of the environment where the fish live (Unlu *et al.*, 2009). Heavy metals have been found to be more dangerous for aquatic ecosystems, especially for fish feeding in deep waters (Popek *et al*., 2003). After heavy metal accumulation in fish, particularly through the food chain, toxic effects ensue in other organisms. Nonetheless, studies have clearly indicated that the detrimental effects of heavy metal accumulation in fish can be prevented by certain antioxidative substances. One of the most frequently reported antioxidative substances is propolis. (Fuat Gulhan *et al*., 2012; Kakoolaki *et al*., 2013; Talas *et al*., 2014; Selamoglu *et al.*, 2015) and selenium (Ates *et al*., 2008; Talas *et al*., 2008; Selamoglu, 2011).

Exposure of goldfish to nCuO and $CuCl_2$ at relevant concentrations resulted in DNA damages as determined by comet assay of genotoxicity. Histopathological examination revealed that the gill is a target organ for copper toxicity. nCuO caused more gill morphological changes upon exposure, than $CuCl_2$. Both types of copper forms can produce increased oxidative

stress, leading to DNA damage in the case of exposure to copper. MN test is as sensitive as the comet test in detecting genotoxicity. However, more techniques should be used to quantify genotoxic capacity. As a result, copper oxide nanoparticles (nCuO) and copper salt ($CuCl_2$) can cause harmful effects in fish at these concentrations. The release of excess copper concentration into the aquatic environment poses a potential risk to the aquatic environment. As a result of copper accumulation in aquatic organisms, DNA and tissue damage caused by excess copper accumulation, which they transferred to humans through the food chain, can be reduced by propolis, selenium, and different protective compounds reported in the literature.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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