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*Original Research*

# Comet Assay Evaluation of Cadmium Chloride-Induced DNA Damage in *Cyprinus carpio* (Common Carp) and the Genoprotective Role of Selenium

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**ABSTRACT**

Heavy metal contamination in aquatic environments represents a significant threat to biodiversity and the sustainability of aquatic resources, with cadmium being among the most toxic pollutants due to its high bioaccumulation potential and genotoxic effects. This study investigates the genotoxicity induced by cadmium chloride (0.78 ppm and 1.56 ppm) in *Cyprinus carpio* (Common carp). It evaluates the ameliorative efficacy of selenomethionine (0.25 ppm and 0.50 ppm) as a genoprotective agent. The comet assay, a widely used technique in genetic toxicology, was employed to quantify DNA damage in fish tissues, providing a sensitive and reliable measure of genotoxic effects. The results revealed a dose-dependent increase in DNA strand breaks following cadmium chloride exposure, indicating a significant genotoxicity. Conversely, co-treatment with selenomethionine demonstrated a notable reduction in DNA damage, highlighting its potential to mitigate cadmium-induced genotoxicity. These findings enhance our understanding of heavy metal toxicity and instill hope for the potential of selenomethionine as a sustainable intervention to protect aquatic life. From a societal perspective, protecting fish health is crucial for global food security and the well-being of millions who rely on fisheries for their livelihoods. The protective capacity of selenium underscores the promise of sustainable, environmentally safe interventions to combat pollution, foster ecological resilience, and contribute to preserving our natural resources for future generations. This study identifies the knowledge gaps and provides a comprehensive understanding of DNA damage

assessment through the prism of the comet assay, while highlighting the protective role of selenium in alleviating cadmium-induced genotoxicity.

## INTRODUCTION

Freshwater resources are essential for sustaining life and maintaining ecological balance. However, rapid industrialization, urbanization, population growth, and unsustainable exploitation of natural resources have significantly degraded water quality (Carolin et al. 2017, Joseph et al. 2019, Qiao et al. 2020, Vardhan et al. 2019, Sharma et al. 2023, Saravanan et al. 2024). Heavy metals are particularly concerning among the major pollutants due to their persistence, toxicity, and bioaccumulative nature (Niu et al. 2020, Singh et al. 2011, Tchounwou, 2012, Wu et al. 2016). Cadmium (Cd), widely used in industrial processes, is one of the most toxic heavy metals and frequently enters aquatic ecosystems through specific activities such as mining, smelting, electroplating, battery production, and phosphate fertilizers. For instance, cadmium can be released into the environment by extracting and processing ores in mining. Similarly, in battery production, cadmium is a common component of rechargeable batteries, and its disposal can lead to environmental contamination (Khan et al. 2022, Das et al. 2023, Rahoui et al. 2014, Irfan et al. 2021, Hayat et al. 2019, Kubier et al. 2019).

Due to its high solubility and mobility, cadmium easily accumulates in aquatic organisms, especially fish, posing severe and potentially catastrophic health risks to aquatic life and humans through the food chain (Mielcarek et al. 2022). It disrupts vital cellular processes such as DNA replication, repair, and apoptosis, causing oxidative stress and elevated reactive oxygen species (ROS) levels that result in DNA strand breaks and mutations (Liao et al. 2021, Liu et al. 2022, Qu & Zheng 2024, Hakem 2008, Wang 2015). Chronic exposure leads to cancer and systemic toxicity in multiple organs (Taysı, 2024, Obaiah et al. 2020, Ferro et al. 2021, Noor et al. 2020, Zheng et al. 2021). Even at low concentrations, cadmium exhibits genotoxicity and is classified as a Group 1 human carcinogen (IARC 1993), underlining the gravity of the situation.

The common carp (*Cyprinus carpio*), a widely consumed and economically important freshwater fish, is an ideal bioindicator due to its physiological adaptability (Mancera-Rodríguez et al. 2024). Selenium (Se), a trace element with antioxidant properties, plays a critical role in cellular defence against oxidative damage (Kora 2018, Li et al. 2023). In its organic form, selenomethionine (SeMet), Se enhances antioxidant enzyme activity and mitigates oxidative stress-induced DNA damage (Wenfi Jia 2023, Marieke Swinkels 2020).

This study advances current understanding in aquatic toxicology by providing detailed evidence of cadmium-induced genotoxicity in *C. carpio*, and by evaluating the protective efficacy of SeMet across multiple tissues using the comet assay. While earlier studies have established cadmium's bioaccumulation and genotoxic potential in fish (Cuypers et al., 2010; Pandey et al., 2011), limited research has comprehensively assessed tissue-specific DNA damage patterns alongside the mitigating effects of selenium-based antioxidants. By demonstrating that SeMet significantly reduces genotoxicity in a dose-dependent manner, particularly in gill, kidney, and blood tissues, this study builds on prior work (Elia et al., 2011; Kieliszek & Błażejak, 2013) and addresses knowledge gaps in selenium's role in oxidative stress regulation and DNA repair. Furthermore, the validation of the Genetic Damage Index (GDI) as a sensitive metric enhances the methodological framework for genotoxicity screening. These findings might contribute significantly to the existing literature advocating for the integration of antioxidant-based interventions in pollution mitigation and aquaculture health management.

## 2. MATERIALS AND METHODS

### 2.1. Experimental setup and Chemicals

Juvenile *C. carpio* (single breed) with an average length of 12.52 ± 2.00 cm and a mean weight of 24.00 ± 2.00 g were obtained from the Tamil Nadu Fisheries Development Corporation Limited (TNFDC), Aliyar, located in Coimbatore district, a renowned source of high-quality fish for research. The fish were transported to the laboratory in oxygenated water tanks to ensure safe handling. Upon arrival, they underwent prophylactic treatment by immersion in a 0.05% potassium permanganate ($KMnO_4$) solution for 2 minutes, repeated twice, to prevent dermal infections. Fish were acclimated for one month under controlled laboratory conditions, following the protocol described by Palaniappan and Karthikeyan (2022). Waste materials, including faecal matter, were siphoned out daily to minimize ammonia content in the tanks (Company et al. 2010). The fish were fed once daily with boiled eggs and minced goat liver ad libitum at 3% of their body weight. This diet was uniformly provided to both control and exposed groups, and was rapidly consumed, minimizing the possibility of food soaking in contaminated water.

Water quality parameters were meticulously monitored and consistently maintained within optimal ranges throughout the experimental period: temperature at 26.7 ± 1.6 °C, dissolved oxygen between 6.5–8.5 mg/L, pH from 6.5 to 7.5, nitrite concentrations of 0.06–0.1 mg/L, nitrate between 1–3.5 mg/L, total hardness at 154 ± 1.7 mg/L (as $CaCO_3$), and total ammonia levels between 0.1–0.3 mg/L. All exposures were conducted under natural photoperiod conditions. All chemicals utilized were of analytical grade from Himedia, India.

### 2.2. Experimental Design for Lethal Toxicity Tests

After a month of acclimatization, healthy adult common carp weighing 45-50 grams were randomly assigned to acclimatization groups, with 10 fish per group. The study comprised nine experimental groups randomly assigned to treatment groups, with 10 fish per group, including a control group with no exposure (1). The other groups are a cadmium chloride ($CdCl_2$) group exposed to sub-lethal concentrations of 0.78 ppm (2) and 1.56 ppm (3); a SeMet-only group exposed to either 0.25 ppm (4) and 0.50 ppm SeMet (5); co-exposure groups treated with 0.78 ppm $CdCl_2$ plus 0.25 SeMet (6) and 0.50 ppm SeMet (7), and another set of co-exposure groups treated with 1.56 ppm $CdCl_2$ plus 0.25 SeMet (7) and 0.50 ppm SeMet (8) based on prior studies (Ta et al. 2018, Mechlaoui et al. 2019), in a semi-static system with the change of test water every day to maintain the concentration of the chemical. The selection of concentration 0.25 and 0.50 ppm for SeMet is based on the earlier work done by Elia et al., 2011. This resulted in a total of 9 distinct experimental conditions. Taking into account the five time intervals, each treatment condition involved 50 fish (9 × 10 fish × 5 time intervals). In the present study, water is used as a vehicle control as water is a neutral substance and does not contain any selenium. All treatments were conducted in triplicate and analyzed at five time intervals: 24, 48, 72, 96, and 120 hours. At each time, the animals were euthanized, and samples from the gills, liver, kidney, and peripheral blood were collected.

### 2.3. Cell Isolation and Preparation

Cell isolation and preparation were carried out with utmost care and precision. *C. carpio* specimens were anesthetized using clove oil (AQUI-S®, Aquatic Anaesthetic, Aqua World LTD, India) at a concentration of 0.05 ml/L for 2–3 minutes, following the protocol described by Husen & Sharma (2015). Blood samples were collected aseptically from the caudal vein using heparinized syringes and immediately transferred into labeled microtubes. After anesthesia, the gill arches, liver, and kidney were carefully excised using sterile scalpels. The excised tissues were rinsed thoroughly three times with sterile phosphate-buffered saline (PBS) to eliminate blood, debris, and surface contaminants. Clean tissues were placed into labeled Petri dishes and finely minced with sterile scalpels to create a homogeneous tissue suspension, from which any solid fragments were carefully removed.

The homogenized tissue suspensions were transferred into labeled microtubes using sterile tips and incubated with 10 ml of 0.25% trypsin-EDTA solution (Merck, Merck Specialities Pvt. Ltd., Mumbai, India) to facilitate enzymatic dissociation. These tubes were placed on a rotating platform at ambient temperature for 10 minutes. Enzymatic activity was halted by adding 5 ml of fetal calf serum (FCS) to each microtube. Subsequently, the cell suspensions were transferred to fresh, labeled microtubes and centrifuged at 800 rpm for 10 minutes in a pre-cooled bench-top centrifuge. After centrifugation, the supernatant was discarded, and the resulting cell pellets were resuspended in 10 ml of FCS. As worth noting, all procedures were carried out on ice to preserve cell viability and prevent thermal degradation, as recommended by Klobučar et al. (2012).

### 2.4. Comet Assay

The alkaline comet assay was meticulously carried out following established protocols. The mixed cell suspension (100 µL) was mixed with 200 µL of 2% low-melting point agarose (maintained at 37°C) and evenly layered onto microscope slides previously coated with a thin film of 0.5% normal-melting agarose. A coverslip was gently placed on top to ensure uniform spreading, and the slides were subsequently cooled at 4°C for 5 minutes to allow gel solidification, as described by Rojas et al. (1999). Slides were further cooled in a steel tray placed on ice to reinforce solidification for at least 3 minutes. After the initial coverslip was removed, a final overlay of 100 µL of 0.5% low-melting-point agarose was added, followed by another coverslip, and allowed to set fully (Tice et al. 2000).

Subsequently, the coverslip was carefully removed, and the slides were immersed in a freshly prepared lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, pH 10) for 1 hour to facilitate cellular lysis. The slides were then gently rinsed with redistilled water and placed in a horizontal electrophoresis chamber containing cold electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13). Slides were incubated in the buffer for 40 minutes at 4°C before electrophoresis, which was carried out at 1 V/cm and 300 mA for 25 minutes to allow DNA unwinding. After electrophoresis, the slides were rinsed three times with a neutralization buffer containing 0.4 M Tris (pH 7.5). All procedures were performed under dim yellow light to prevent light-induced DNA damage. Slides were stained with 80 µl of ethidium bromide (20 µg/ml) for 5 minutes, rinsed with cold distilled water to remove excess stain, air-dried, and mounted with coverslips. DNA migration patterns were visualized using a fluorescence microscope (NIKON Eclipse 400). DNA damage was evaluated by a subjective visual scoring system (scoring 100 nuclei per fish). Undamaged cells appeared with intact nuclei, while DNA-damaged cells exhibited a characteristic 'comet' appearance, where the tail represented fragmented DNA. Cells exhibiting no heads or fully dispersed heads, indicative of apoptosis, were excluded from the analysis.

The degree of DNA damage was determined with great care by classifying cells into five categories based on the extent of tail migration: Type 0 (no damage), Type I (low damage), Type II (moderate damage), Type III (high damage), and Type IV (extensive damage). This visual scoring method allowed for a semi-quantitative genotoxicity assessment (Grover et al. 2003).

Due to the requirement of a large number of fish across multiple time points and replicates, additional positive and negative control groups were not included in this study to maintain ethical and logistical feasibility.

### 2.5. Statistical analysis

DNA damage was assessed using the alkaline comet assay, scoring 100 cells per fish and classifying them into five categories based on tail length (Type 0 to Type IV). The percentage of DNA-damaged cells (Types II–IV) and genetic damage index (GDI) were calculated for each tissue (Collins et al., 2023). A thorough statistical analysis was conducted using one-way ANOVA followed by Tukey's HSD post hoc test, with significance set at $p < 0.05$, to ensure the robustness of our conclusions.

$$GDI = (Type\ 1 + Type\ II + Type\ III + Type\ IV)/(Type\ 0 + Type\ I + Type\ II + Type\ III + Type\ IV) \quad \ldots(1)$$

$$\%\ of\ DNA\ damage = Type\ II + Type\ III + Type\ IV \quad \ldots(2)$$

## 3. RESULTS AND DISCUSSION

This study explored the genotoxic effects of $CdCl_2$ and the protective role of SeMet in *C. carpio*. Alkaline comet assay was employed to evaluate DNA damage in gill, liver, kidney, and blood tissues. The results revealed a significant, dose- and time-dependent increase in DNA damage in all tissues following cadmium exposure, while SeMet demonstrated an apparent protective, antioxidant effect.

In the fields of molecular biology and environmental science, the isolation of DNA constitutes a fundamental aspect of genetic research. Genotoxicity assays play a critical role in the evaluation of the effects of environmental stressors on aquatic organisms. Among the wide variety of aquatic life, fish are particularly significant as bioindicators; they provide valuable insights into the ecological health of their habitats and the consequences of pollution. This underscores the importance of acquiring high-quality DNA from fish tissues and assessing DNA damage through methodologies such as the comet assay, which is vital for the conservation of aquatic resources.

Fish, widely acknowledged as effective bioindicators for evaluating metal pollution in aquatic ecosystems played a pivotal role in this study. *C. carpio*, chosen as the model species, is prominent in aquaculture, frequently used in toxicological research, and serves as a sentinel organism due to its broad geographical distribution and sensitivity to environmental stressors (Forouhar Vajargah et al. 2018, Farhangi & Jafaryan, 2019, García-Medina et al. 2022, Yancheva et al. 2022).

The alkaline comet assay, a crucial tool in this study, was initially developed by Singh et al. (1988) and adapted for fish erythrocytes. It is a sensitive method for detecting DNA strand breaks (Jiang et al. 2023). This assay has been extensively used across various tissues, including gills, liver, and blood, for both *in vivo* and *in vitro* assessments following exposure to xenobiotics (Bajpayee et al. 2016). Blood, due to its accessibility and the predominance of red blood cells (RBCS), is commonly used for genotoxicity testing. Solid tissues like liver and kidney require careful dissociation to preserve DNA integrity and avoid artifact formation (Collins et al. 2023, Jha 2023).

The gill, liver, kidney, and blood tissues of the control group showed the maximum number of only Type 0 and Type I nucleoids (Fig. 5A). The tissues of the exposed group showed the presence of Type II, Type III, and Type IV nucleoids (Fig. 5B) along with Type 0 and Type 1 nucleoids.

### 3.1. DNA Damage in Gill Nucleoids

The results of this study demonstrate that $CdCl_2$ exerts statistically significant genotoxic effects on the gill tissue of *C. carpio*, as reflected by the elevated percentage of DNA-damaged cells and the GDI. The observed increase in DNA damage was both dose- and time-dependent, with the highest levels recorded at 1.56 ppm after 120 hours of exposure (GDI = 0.387, $p < 0.05$, Fig. 1). These findings are consistent with earlier research showing that cadmium exposure compromises DNA integrity in fish gills, primarily due to oxidative stress-induced strand breaks and impaired DNA repair mechanisms (Pandey et al. 2011, Ghosh & Indra, 2018). Javed et al. (2016) found that thermal power plant effluent leads to concomitant damage to DNA in the gill and liver of the fish *C. punctatus*. A significantly higher mean tail length was observed in the exposed group compared to the fish in the control group.

As the primary site of metal uptake in aquatic organisms, gills are particularly vulnerable to waterborne toxicants such as cadmium. Their large surface area, rich vascularization, and direct contact with the external environment facilitate rapid metal accumulation. Cadmium disrupts cellular homeostasis, interferes with calcium signaling, and induces ROS, which lead to oxidative DNA damage (Genchi et al. 2020). The significant increase in GDI values in cadmium-exposed groups confirms the extent of nuclear damage and supports its utility as a sensitive quantitative index for genotoxicity assessment. GDI values, representing the percentage of

DNA-damaged cells, are crucial in understanding the genotoxic effects of cadmium. Importantly, the non-genotoxic nature of SeMet was validated by the low GDI values in SeMet-alone treatments, which remained statistically indistinguishable from controls ($p > 0.05$).

Co-exposure groups demonstrated the protective role of SeMet against cadmium-induced genotoxicity. Notably, 0.50 ppm SeMet significantly reduced ($p < 0.05$) DNA damage in gill tissues across all time points, with the most marked reduction observed at 96 h, where the GDI dropped from 0.385 (Cd-alone group) to 0.146 (SeMet co-treated group). This effect is attributed to the ability of SeMet to enhance antioxidant defences by activating glutathione peroxidase and other selenoproteins that neutralize ROS and prevent oxidative DNA damage (McKelvey et al. 2015, Tchounwou et al. 2012). The greater reduction at the higher SeMet dose suggests a dose-dependent protective effect. The antioxidant properties of SeMet, such as suppressing lipid peroxidation and stabilizing cellular membranes, further contribute to its genoprotective function (Hashtjin et al. 2025).

The dose-dependent efficacy of SeMet in mitigating DNA damage underscores its therapeutic potential. The 0.50 ppm dose consistently outperformed the 0.25 ppm dose, leading to a marked reduction in GDI values (Fig. 1). This emphasizes the role of SeMet as a powerful antioxidant and cytoprotective agent. These findings support the hormetic nature of selenium, which offers protective effects at low doses but may become toxic at higher concentrations (Angelone et al. 2024). Importantly, the significant reduction ($p < 0.05$) in genotoxicity with SeMet co-treatment instills confidence in its applicability in pollution mitigation strategies, reinforcing its potential in sustainable aquaculture health management.

While SeMet co-treatment showed a reduction in genotoxic damage compared to cadmium-only groups, in many instances, the damage levels remained significantly elevated relative to controls. For example, in liver tissues at 120 hours, the GDI values remained high despite SeMet exposure, indicating only partial, rather than substantial, protection. Thus, the genoprotective effect of SeMet could be interpreted as moderate attenuation genomic integrity.

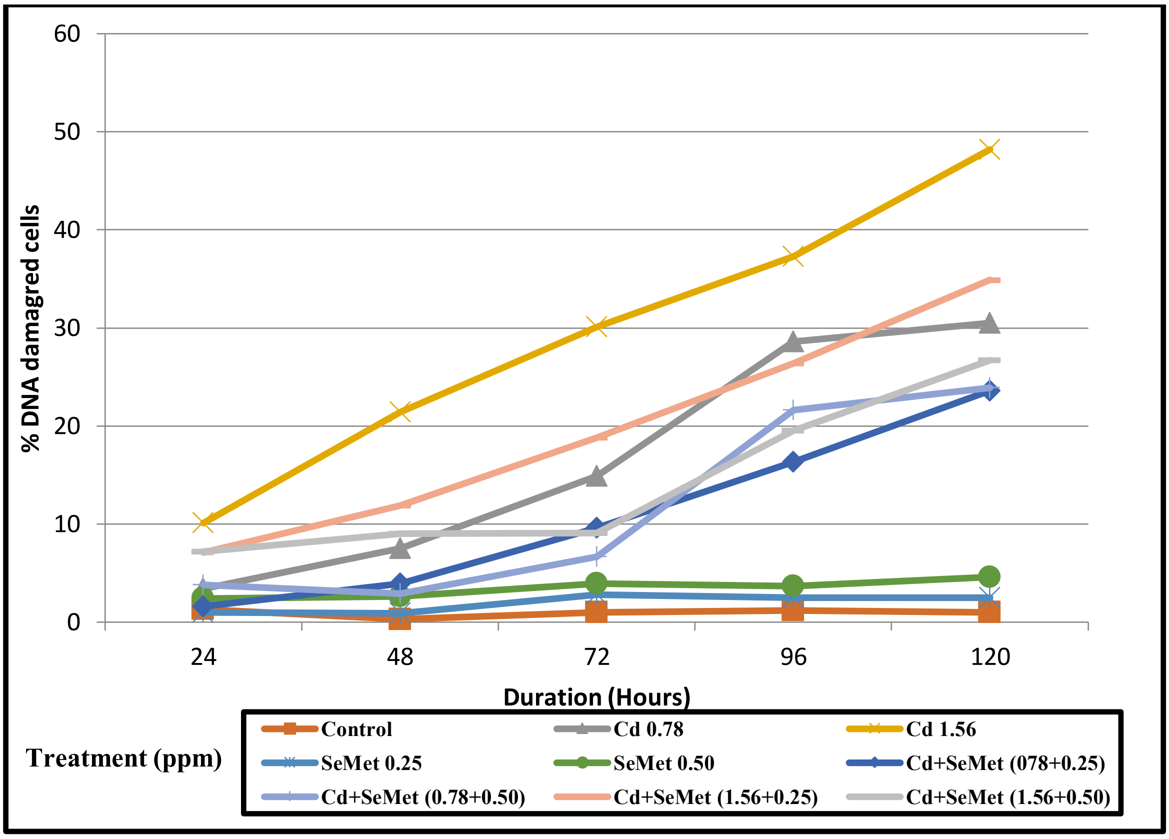


**Fig. 1:** Percentage of DNA damage in the gills of *C. carpio*

### 3.2. DNA Damage in Liver Cells

The liver, a central organ for detoxification and metabolic processing, is alarmingly vulnerable to pronounced DNA damage following $CdCl_2$ exposure. The percentage of DNA-damaged cells and the GDI increase in a precise dose- and time-dependent manner, with the most severe genotoxic effects recorded in the 1.56 ppm group at 120 hours. This vulnerability, underscored by the liver's pivotal role in metal accumulation and oxidative stress regulation (Minarik et al. 2014, Ardeshir et al. 2017), highlights the urgent need for research in this area. Cadmium exerts its genotoxic effects primarily through generating ROS, depletion of intracellular antioxidants such as glutathione, and disruption of DNA repair pathways (Cuypers et al. 2010).

Co-administration of SeMet offers a promising solution to address cadmium-induced hepatic DNA damage. At 72 hours, the higher SeMet dose (0.50 ppm) significantly reduces DNA damage from 27.0% to 9.8% ($p < 0.05$), demonstrating its potential as an effective antioxidant *via* activation of selenoenzymes such as glutathione peroxidase (Kieliszek & Błażejak 2013). However, this protective effect is not a permanent solution; elevated DNA damage persists later. The unexpectedly high GDI value (0.590) (Fig. 2) observed in the Cd+SeMet (1.56+0.25 ppm) group at 120 hours suggests that lower SeMet doses may be insufficient to counteract cadmium toxicity or may alter metal retention and distribution within hepatic tissue (Wang et al. 2024).

As a selenium-containing amino acid, SeMet serves as a precursor for key selenoproteins such as GPx and thioredoxin reductase, which are essential for neutralizing ROS and maintaining cellular redox homeostasis (Zuo et al. 2019, Wande et al. 2020). These exciting findings reveal the liver's sensitivity to heavy metal damage, emphasizing the importance of ongoing research into better antioxidant treatments. While SeMet shows great potential in protecting our genes, its success depends on finding the correct dose and timing. This compelling dose-dependent response illustrates the hormetic nature of selenium, demonstrating its protective benefits at low concentrations while emphasizing the necessity for optimal levels to ensure safety and efficacy.

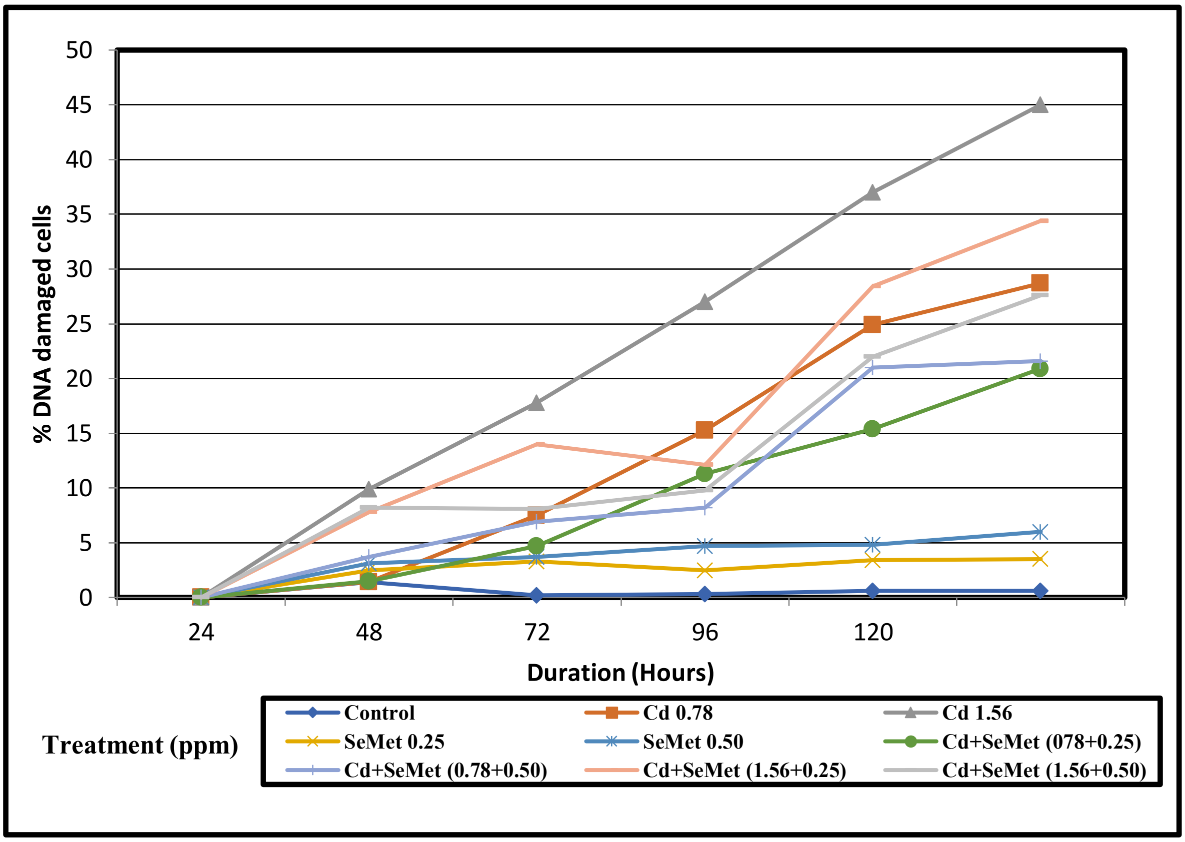


**Fig. 2:** Percentage of DNA damage in the liver of *C. carpio*

### 3.3. DNA Damage in Kidney Nucleoids

The kidney, an essential organ responsible for osmoregulation and excretion, exhibited significant genotoxic responses following exposure to $CdCl_2$. DNA damage, assessed by the percentage of damaged cells and the GDI, increased in a precise dose- and time-dependent manner. The most severe DNA damage was observed in the 1.56 ppm Cd group at 120 hours, where 48.2% of kidney cells exhibited DNA damage. The GDI peaked at 0.568 (Fig. 3). These values were significantly elevated compared to the control group, which showed only 1.0% damage and a GDI of 0.044 ($p < 0.05$).

At earlier exposure durations, $CdCl_2$ still elicited pronounced genotoxic effects. Specifically, at 72 hours, fish exposed to 1.56 ppm of cadmium exhibited 30.1% DNA damage and a GDI of 0.372, while those treated with 0.78 ppm showed 14.9% damage and a GDI of 0.286 (Fig. 3). Both values were significantly elevated compared to the control group ($p < 0.05$), underscoring the potent genotoxicity of cadmium. These findings are consistent with earlier studies indicating that cadmium induces DNA strand breaks and oxidative base lesions, primarily through ROS and disruption of DNA repair mechanisms (Cuypers et al., 2010; Thévenod, 2009). The pronounced accumulation of cadmium in renal tissues is attributed to its high affinity for metallothioneins, which prolongs its retention and enhances its nephrotoxic impact.

Selenomethionine significantly ameliorated cadmium-induced DNA damage across most treatment groups. At 72 hours, co-exposure to Cd at 0.78 ppm and SeMet at 0.25 ppm resulted in a notable reduction in DNA damage from 14.9% to 9.6%, along with a decline in the GDI from 0.286 to 0.180 ($p < 0.05$). Similarly, at 120 hours, the combination of Cd at 1.56 ppm with SeMet at 0.50 ppm led to a marked decrease in DNA damage from 48.2% to 26.7%, with a corresponding reduction in the GDI from 0.568 to 0.313, underscoring an apparent dose-dependent protective effect ($p < 0.05$). Nonetheless, SeMet did not afford complete genoprotective efficacy in all scenarios. At 96 hours, the group receiving 1.56 ppm Cd with 0.25 ppm SeMet still manifested appreciable DNA damage (26.4%) and a GDI of 0.299. Although these values were significantly diminished compared to the Cd-only group (37.3% and 0.430, respectively), they remained markedly elevated relative to controls ($p < 0.05$). These observations emphasize that SeMet imparts notable antioxidant and cytoprotective benefits, particularly at higher concentrations, and abrogates cadmium-induced genotoxic effects, especially under suboptimal dosing or prolonged exposure.

The findings of this study are of utmost importance, as they underscore the superior sensitivity of the GDI as an indicator of DNA fragmentation severity compared to the percentage of damaged cells alone. While the percentage of damage provides insight into the incidence of genotoxic events, GDI offers a more refined assessment by quantifying the degree and extent of DNA migration, thereby delivering a deeper understanding of the damage magnitude (Kumar et al., 2010). The current findings are consistent with existing literature that emphasizes the capacity of selenium for detoxification in the context of heavy metal toxicity. This protective effect is attributed mainly to the upregulation of antioxidant defences facilitated by selenoproteins such as glutathione peroxidase and thioredoxin reductase (Zhang et al., 2013; Liu et al., 2017). Nevertheless, the inability of even higher SeMet doses to fully restore DNA integrity implies that cadmium-induced nephrotoxicity may involve multifaceted pathological pathways extending beyond oxidative stress. These may include compromised DNA repair mechanisms, mitochondrial dysfunction, and pro-inflammatory responses (Haberland, 2023).

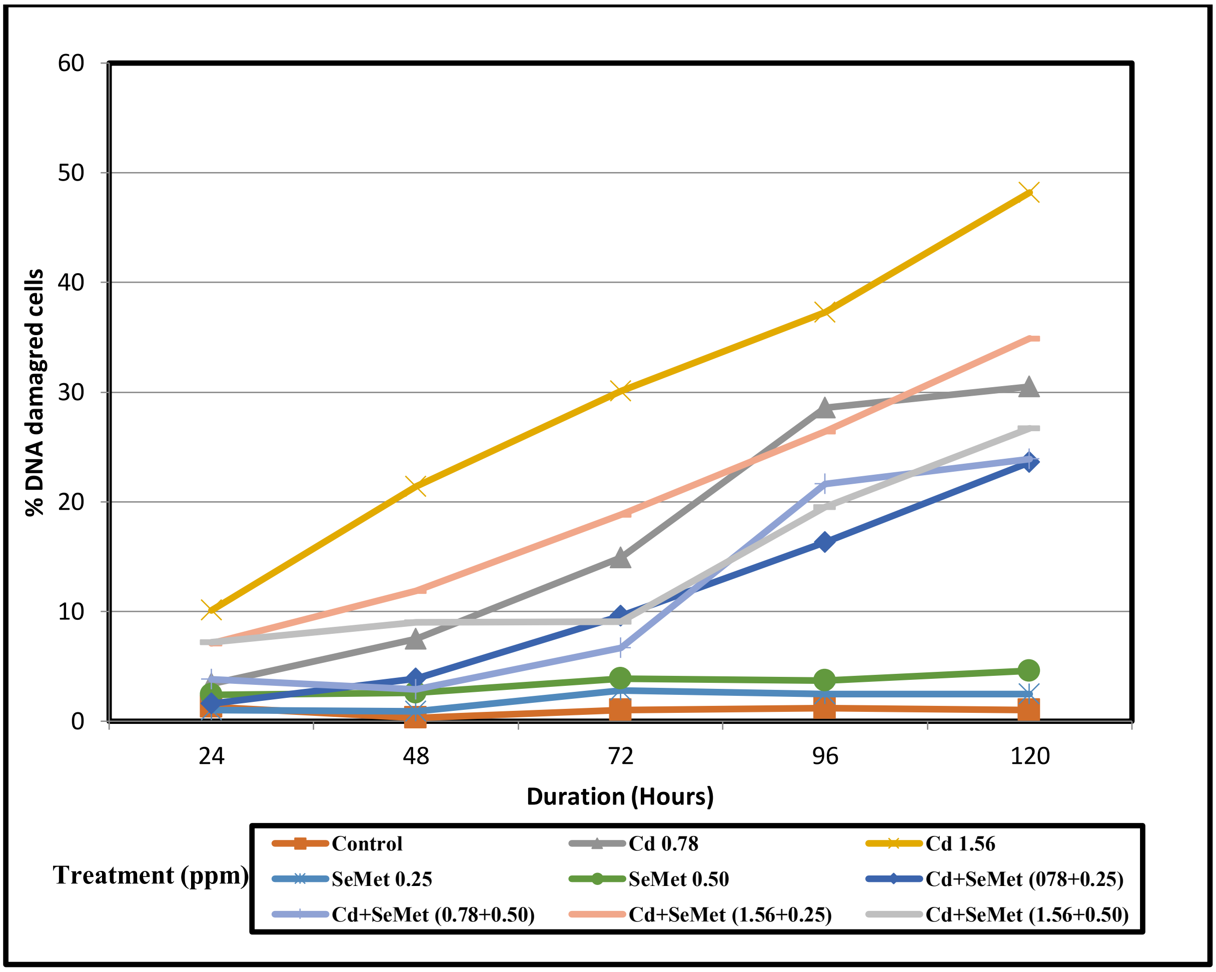


**Fig. 3:** Percentage of DNA damage in the kidney of *C. carpio*

### 3.4. Peripheral Erythrocyte (Blood) DNA Damage

Peripheral erythrocytes of *C. carpio* exhibited a significant genotoxic response to $CdCl_2$ exposure, with both the percentage of DNA-damaged cells and the GDI increasing progressively with time and higher cadmium concentrations ($p < 0.001$ for both parameters across time and dose levels). Similarly, exposure to varying concentrations of arsenic resulted in a significant rise in DNA damage in the blood cells of *Oreochromis mossambicus* compared to the control group (Ahmed et al. 2011). These findings correspond with earlier reports highlighting the high sensitivity of fish erythrocytes to heavy metal-induced genotoxicity due to their nucleated nature and continuous exposure to circulating toxicants (Witeska et al. 2023).

Across all exposure durations, the 1.56 ppm Cd group consistently exhibited the highest DNA damage and GDI values, peaking at 36.3% DNA damage and 0.422 the GDI at 120 hours ($p < 0.001$ compared to control and other treatment groups) (Fig. 4). This persistent elevation underscores cadmium's cumulative and enduring genotoxic effects on erythrocytes, likely driven by its inhibition of DNA repair enzymes and its capacity to generate reactive oxygen species (ROS), which cause oxidative DNA damage (Kumar et al. 2024). These results reinforce the effectiveness of comet assay-based parameters as reliable biomarkers of genotoxic stress in aquatic organisms.

The protective effect of SeMet was evident, particularly in co-exposure groups. Co-treatment with SeMet significantly reduced both DNA damage percentage and GDI at all-time points compared to cadmium-only groups ($p < 0.01$), suggesting that SeMet, known for its antioxidant properties, mitigates cadmium-induced oxidative stress by scavenging ROS and enhancing antioxidant defence mechanisms such as glutathione peroxidase activity (Ibrahim et al. 2024). For instance, at 120 hours, co-treatment with SeMet (1.56+0.50 ppm) reduced the GDI from 0.422 to 0.248 (Fig. 4), indicating substantial mitigation of genotoxic effects.

Interestingly, SeMet at 0.50 ppm alone showed a slightly elevated GDI at 24 hours (0.171), possibly reflecting a dose-dependent biphasic or hormetic response, where excess selenium may exert mild pro-oxidant effects under certain conditions ($p < 0.05$ when compared to control). Such behaviour has been reported in fish and other organisms, underscoring the importance of careful dose optimization when using selenium as a protective agent. Peripheral erythrocytes of *C. carpio* thus serve as reliable and early indicators of systemic genotoxic stress following cadmium exposure. Despite the observed protective effects, SeMet co-treatments did not fully restore DNA integrity to control levels ($p < 0.001$), indicating that selenium supplementation, while beneficial, cannot entirely counteract cadmium's multifactorial genotoxicity. This partial protection may be attributed to cadmium's diverse mechanisms of toxicity, including interference with DNA synthesis, induction of apoptosis, and disruption of metal ion homeostasis.

The results suggest that selenium, particularly at higher concentrations, has a clear protective role in mitigating the genotoxic effects of cadmium exposure across various tissues. The antioxidant properties of selenium are likely key to its protective effect, reducing the oxidative stress caused by cadmium and thereby lowering DNA damage. The tissue-specific effects of SeMet indicate that while it offers substantial protection in some tissues (e.g., kidney and gills), it may not fully restore DNA integrity to baseline levels in highly vulnerable tissues such as the liver. However, its role in reducing genotoxicity across all tissues highlights its potential as a protective agent against heavy metal-induced toxicity. It offers a promising avenue for future research and potential solutions to cadmium exposure.

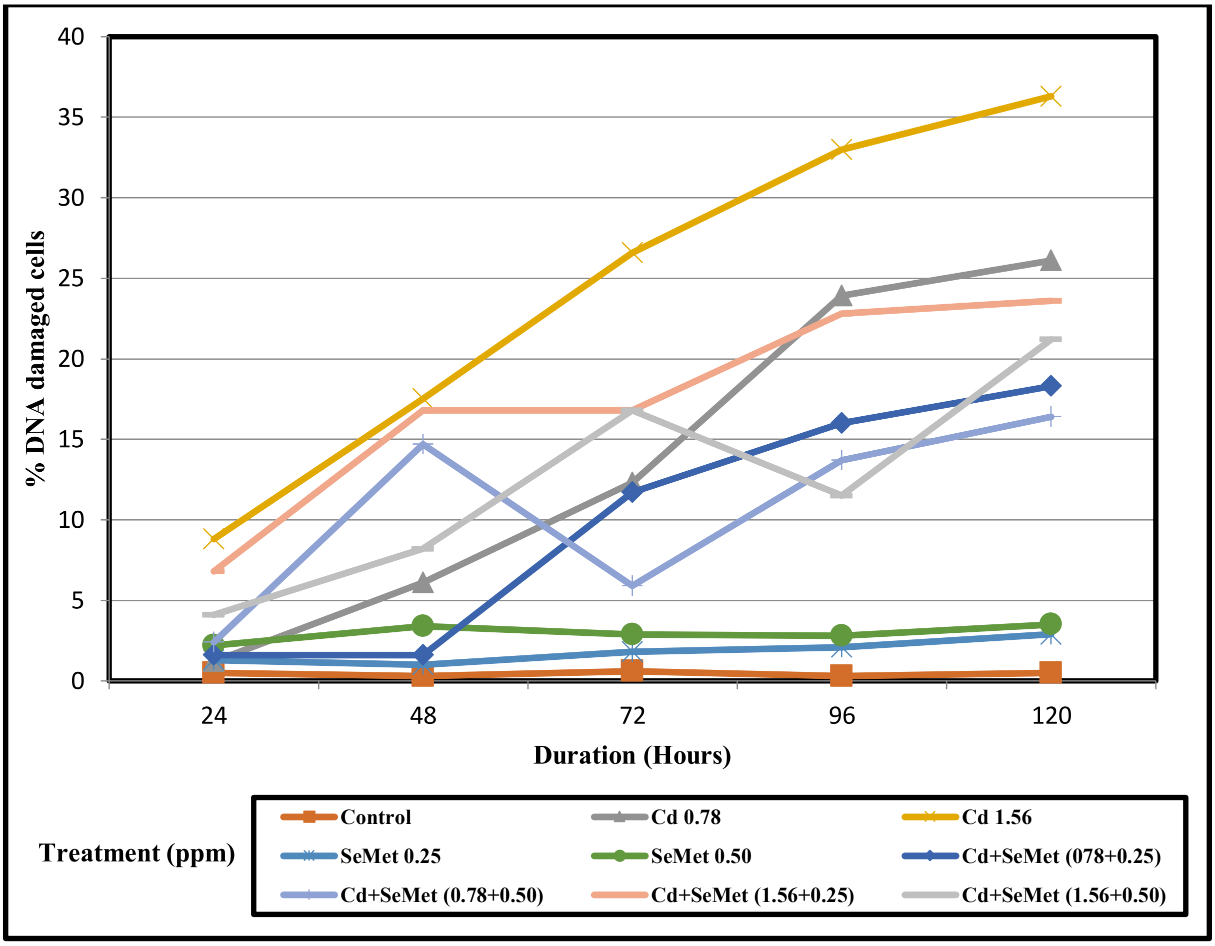


**Fig. 4:** Percentage of DNA damage in the blood of *C. carpio*

The order of tissue sensitivity based on genotoxic damage is: liver > kidney > gill > erythrocytes. Based on the metal pollution index, livers and kidneys, followed by gills, showed maximum overall metal load. The degree of DNA damage (assessed by comet and diphenylamine assays) was relative to the accumulated metals in tissues with species and site specification. Ahmed et al. (2011) reported that exposure to lead chloride resulted in the highest level of DNA damage in the liver tissue of the freshwater fish *Anabas testudineus*, followed by the kidney and gill tissues. Overall, the results suggest that selenium, particularly at higher concentrations, plays a crucial protective role in mitigating the genotoxic effects of cadmium exposure across various tissues. Its antioxidant properties are likely key to its protective effect, reducing the oxidative stress caused by cadmium and thereby lowering DNA damage. The tissue-specific effects of SeMet indicate that while it offers substantial protection in some tissues (e.g., kidney and gills), it may not fully restore DNA integrity to baseline levels in highly vulnerable tissues such as the liver. However, its role in reducing genotoxicity across all tissues highlights its potential as a protective agent against heavy metal-induced toxicity.

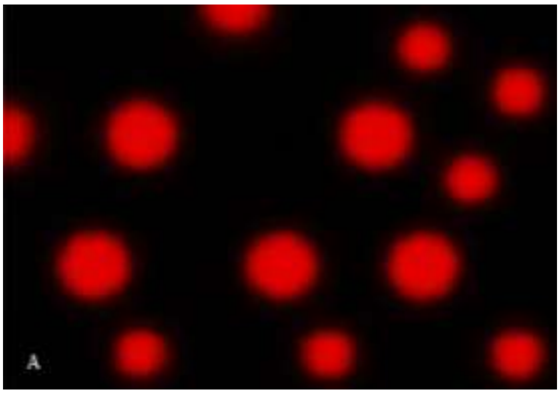


**Fig. 5A:** Control group of *C. carpio* showing no DNA damage nucleoids

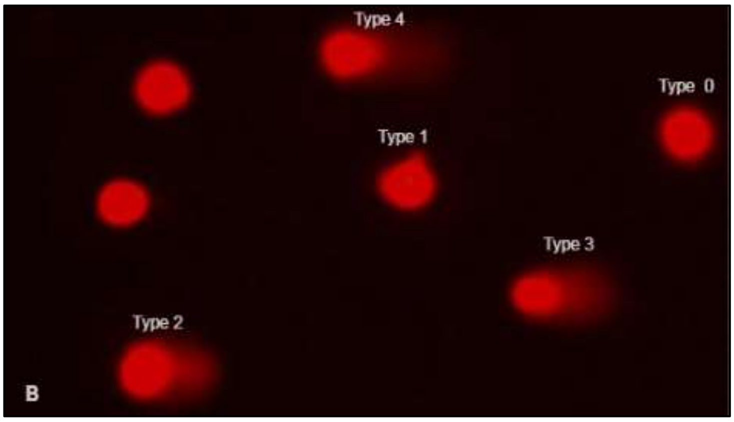


**Fig. 5B:** *C. carpio* exposed to cadmium chloride showing all five types of DNA damage nucleoids

The differential sensitivity of various tissues to cadmium, coupled with the protective efficacy of SeMet, holds significant implications for environmental biomonitoring. The kidneys and liver serve as sensitive biomarkers for assessing heavy metal toxicity, while blood provides a practical and non-lethal option for routine genotoxic screening. In this regard, the comet assay is a robust and reliable early-warning tool for detecting sub-lethal exposure to pollutants in aquatic organisms. This study emphasizes the potential of SeMet as an impactful chemoprotective agent within aquatic toxicology. By substantially mitigating cadmium-induced genotoxicity without inflicting harm, SeMet represents a promising strategy for implementation in aquaculture and environmental remediation, aimed at reducing the ecological consequences of heavy metal pollution in freshwater ecosystems.

## 4. CONCLUSIONS

This study demonstrates that $CdCl_2$ induces significant, dose- and time-dependent DNA damage in *C. carpio*. The kidney and liver show the highest sensitivity due to their detoxification and metal accumulation roles. However, the effective mitigation of cadmium-induced genotoxicity by SeMet underscores its potential as a chemoprotective agent in aquatic toxicology and brings hope for the future of environmental management.

Given *C. carpio*'s importance as a food species, cadmium contamination poses ecological and public health risks. Therefore, future research should investigate the combined effects of pollutants, life-stage variability, and gender-specific responses. These areas of study will provide a more comprehensive understanding of cadmium's full biological impact. Furthermore, these findings strongly advocate applying SeMet in aquaculture and environmental management to reduce heavy metal toxicity and strengthen ecosystem resilience. Incorporation of oxidative stress biomarkers or antioxidant enzyme activity in future would substantiate the antioxidant-based protective role of SeMet .

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