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Comprehension of the zinc chloride's ameliorative apoptotic and genotoxic effects on mice with cadmium-induced hepatotoxicity

Enas Nihad Bayram[a] | **Hanady S. Al-Shmgani**[a*] | **Nahla A. Al-Bakri**[a]

[a]Deprtment of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

[*]*Corresponding author:* hanadi.s.as@ihcoedu.uobaghdad.edu.iq

Abstract Cadmium is a typical heavy metal quite dangerous to humans and animals. Zinc supplementation protects the biological system from Cd toxicity and alleviates Cd-induced toxicity. The present study was assessed to evaluate the preventive effects of zinc chloride ($ZnCl_2$) on male mice with liver damage induced by cadmium chloride ($CdCl_2$). Metals accumulation was quantified in the liver. Body weight, liver weight ratio, lipid peroxidation, caspase 3, and DNA damage were determined in the liver of male mice after receiving an intraperitoneal (IP) a single dose of $CdCl_2$ at 1.5 and 3 mg/kg or/and $ZnCl_2$ 10 mg/kg during 21 days. The LD50 was 6.023 mg/kg for $CdCl_2$ and 89.05 mg/kg for $ZnCl_2$. The results indicate that mice in control and Zn groups gained body weight at the end of the experiment, while other treated groups significantly decreased. The relative weight of the liver revealed a significant increase in experimental groups. In addition, an increase in malondialdehyde level, Metallothionein concentration, and caspase-3 level was detected in Cd and Zn groups alone or in combination. Strand breaks of DNA of hepatocytes showed a significant increase in tail length of groups treated with cadmium. Co-treatment with zinc reduced these parameters compared to those measured in cells treated with cadmium. The outcome of this study implied that cadmium chloride causes oxidative stress, DNA damage, and elevated apoptosis markers in mice livers at low and medium doses. By pinpointing the target organ involved, the study results have also added some understanding of the impacts of zinc chloride injection to ameliorate cadmium toxicity in a low dose at 10 mg/kg.

Keywords: cadmium toxicity, comet assay, oxidative liver markers, zinc protection

1. Introduction

Metals are a natural part of the ecosystem and are difficult to remove due to their long half-life in the body. Due to the increased utilization of a wide range of metals in commerce and daily life, toxic metal pollution has generated significant issues. Non-essential metal cadmium (Cd) can pollute through occupational and non-occupational exposure. Manufacturing paint, plastic, glass, metal alloys, and mining activities are all associated with occupational exposure (Gao et al 2015). Ingestion of metal-contaminated food and water, as well as bioaccumulation in plants and aquatic species, can all result in non-occupational exposure (Waalkes 2003). The ability of cadmium to accumulate in tissue is comparable to that of other heavy metals; reports of cadmium accumulation in the liver, reproductive organs, kidneys, and nervous system have been documented (Rahimzadeh et al 2017). According to research studies, the toxicity of cadmium increases the generation of reactive oxygen species (ROS) and the onset of oxidative stress in tissues (Liu 2009). It has been well established that endogenous antioxidants play a pivotal role in antioxidant defense mechanisms against oxidative impairment, reflecting the protective role of particular biological functions. Cd induces oxidative stress through impairment of the antioxidant enzyme system due to changes in gene expression mechanisms (Rahimzadeh et al 2017). The primary direct biological mechanism reliant on zinc action is what causes Cd's molecular toxicity. It is possible that the Zn in the active site of the mammalian enzyme -aminolevulinate dehydratase, which is dependent on Zn, could be a target of Cd. Zn can, in general, reduce the effects of Cd. The liver significantly influences the oxidative stress caused by Cd Zn homeostasis, which controls how Zn ions are incorporated into a wide range of enzymes essential to the body's metabolism (Rogalska 2011). Zn is crucial for producing metallothionein (MT) biosynthesis in the liver and kidneys (Banni 2010).

Along with the sequestration and detoxification of Cd and other heavy metals, MTs are crucial for the homeostasis of critical metal ions. Additionally, MTs are effective free radical scavengers produced during oxidative stress (Nordberg 2009). Free Cd^{2+} concentrations may increase as a result of either excessive Cd^{2+} exposure or MT deficiency, which may have a range of cytotoxic effects because the mechanism underlying the activity of cadmium is thought to be strongly prooxidative (Klaassen et al 2009; Cuypers et al 2010; Vasak and Meloni 2011; Jomova and Valko 2011). It can directly disrupt the electron transport chain, forming reactive oxygen species (ROS). It can also do so indirectly by

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weakening the enzymatic and nonenzymatic antioxidative barrier (Jomova and Valko 2011; Brzoska et al 2011). Damage to tissues and organs and functional impairment may result from disturbances of the cellular oxidation-reduction equilibrium (Brzoska et al 2011; Thevenod and Lee 2013).

Aspartate-specific cysteine proteases known as caspases cause proteolytic cascades and increase intracellular apoptotic signals. The replacement of Zn^{2+} by Cd^{2+} within the tumor suppressor protein (P53) compromises P53-mediated DNA damage repair or cell cycle arrest, leading to caspase-independent death. Apoptosis brought on by Cd2+ is accompanied by an increase in ROS, which can damage mitochondria, DNA, and proteins (Kowaltowski et al 2009). Calpain, a Cd2+-dependent protease crucial for Cd2+-induced caspase-independent apoptosis at early time points in rat kidney proximal tubule cells, can also be activated by this metal ion (Smith and Schnellmann 2012).

The exposure, dose, and length of exposure all affect how much cadmium enters the biological system. This study was carried out to determine if zinc supplementation decreased cadmium absorption and/or cytotoxicity and the toxicity of cadmium in mice livers.

2. Materials and Methods

2.1. Chemicals

All chemicals and reagents were of analytical grade; zinc chloride ($ZnCl_2$) and Cadmium chloride ($CdCl_2$) were purchased from Sigma-Aldrich (St Louis, MO, USA). Mice Caspase-3 kit was obtained from MyBioSource, Inc. (San Diego, CA, the USA; REF: MBS733100). HNO_3 acid from Suprapure, Merck.

2.2. Animals

Thirty-six adult male mice *(Mus Musculus*) weighing 26 ±5 g were purchased from National Center for Control and Pharmaceutical Research in Baghdad, Iraq. Mice were maintained under controlled illumination (12-12 hr light/dark) and ambient temperature (25 ± 5 °C). Water and feed, a standard rodent diet, were offered ad libitum. The Ethics Committee on Animal Use of the University of Baghdad approved the experimental procedures (N° D.A.487 on Jun 29, 2020).

2.3. Eco-toxicity assessments

To evaluated lethal dose50 (LD50), different doses (1,2,4,8,16) mg/kg BW of $CdCl_2$ and $ZnCl_2$ (12.50, 25, 50, 100, 200) were injected intraperitoneal (IP), one dose for each group (6 mice/group) for 24, 48, 72 hr and percentage of survival mice was calculated according to Ali (2012).

2.4. Experimental Design

After adaptation, the mice were randomized into six experimental groups (n=6 animal/group); all animals were injected intraperitoneal (IP) for 21 consecutive days as the following:

G1: The control group received distal water (DW) only;
G2: received 0.1 ml of $ZnCl_2$ solution at concentration 10 mg/kg;
G3: received 0.1 ml of $CdCl_2$ solution at concentration 1.5 mg/kg;
G4: received 0.1 ml of $ZnCl_2$ solution at concentration 10 mg/kg, and after 30 mints, received 0.1 ml of $CdCl_2$ at concentration 1.5 mg/kg;
G5: received $CdCl_2$ solution at concentration 3 mg/kg;
G6: received 0.1 ml of $ZnCl_2$ solution at concentration 10 mg/kg, and after 30 mints, received 0.1 ml of $CdCl_2$ solution at concentration 3 mg/kg.

2.5. Body weight and relative liver index determination

Mice were weighed and slaughtered under anesthetic at the end of the experiment. The livers were removed, weighed to determine organ index, and then homogenized in phosphate buffer saline for biochemical investigations (Samir and Zine 2013). The supernatants were used after centrifugation for 15 minutes at 4 °C at 13000 rpm. The following formula was used to determine the liver index:

Relative liver index (%) = (liver weight (g) / body weight (g)) × 100

2.6. Metal concentration in the liver determination

The concentration levels of zinc and cadmium in the livers of mice were measured using inductively coupled plasma-mass spectrometry (ICP-MS) according to Lossow et al (2020). Briefly, the ground dried-freeze livers (0.5 g) were digested in an acid solution containing 65% HNO_3, Suprapure, Merck, and 30% H_2O_2. Samples were heated to 200 °C for 10 minutes using a microwave digestion system (CEM, Kamp-Lintfort, Germany). Then analyses were carried out by ICP-MS.

2.7. Liver metallothionein determination

According to Richards and Steele (1987), metallothionein was separated and quantified using anion-exchange high-performance liquid chromatography coupled (HPLC). Before being injected into the HPLC system, the liver homogenate samples were centrifuged at 3000 rpm for 15 min, and the separation was performed using a Shimadzu model 10-AT vp LC with a binary delivery pump (model LC-10A Shimadzu, a UV-Vis 10A-SPD) spectrophotometer to monitor the diluted peaks, and an injector (Rheodyne, Model 7125, USA).

2.8. Malondialdehyde determination

Lipid peroxidation was estimated according to Guidet and Shah (1989). Briefly, a mixture of 1 ml of 17.5% trichloroacetic acid and I ml of 0.6% thiobarbituric acid was added to 1 ml supernatant of liver homogenized. After being incubated in a water bath at 100 ºC for 15 minutes, the samples were cooled, and 1 ml of TCA (70%) was added and permitted to remain at room temperature for 30 minutes. Following centrifugation, the absorbance was observed at 532 nm.

2.9. Caspases-3 activity determination

Following the manufacturer's instructions, caspase-3 activity in liver homogenate was measured using an ELISA kit to evaluate the apoptotic effect of cadmium chloride on liver tissue.

2.10. Quantification of DNA damage with the comet assay

DNA damage was determined using the comet assay described by Jackson et al (2013). Briefly, homogenate liver tissue suspension was mixed with low melting point agarose (20 µl) and loaded on slides covered with normal melting point agarose. The slides then left for 1 h in ice-cold lysis buffer, pH=10 (2500 mM NaCl, 100 mM Na2EDTA, 0.01MTris, 1% Triton X-100, 10% DMSO, adjusted to pH 10 with NaOH). Electrophoresis was carried out in alkaline buffer (pH = 13, 1 mM Na2EDTA) with a voltage of 38 V and current of 294 mA. Cells were then neutralized with 400 mM Tris-HCL (pH = 7.5) neutralization buffer for 5 min and followed by fixation with ethanol 96%, then stained with SYBRGreen (100 ng/ml) for 30 min and covered with a coverslip. Damage DNA was visualized using fluorescence microscopy (Novel 40X-1600X Halogen lamp Trinocular, Movel Scientific Instrument Co. Ltd., China). Quantitative score of DNA damage was presented as % Tail of DNA of cells performed using Image J analysis software

2.11. Statistics

SPSS version 23.0 (SPSS Inc. Chicago, IL, USA) software was used for statistical analysis. A One-way ANOVA followed by LSD was considered. Significant was considered at $P \leq 0.05$.

3. Results

3.1. Eco-toxicity test (LD50)

A toxicity test was done to obtain the lethal dose of 50 (LD_{50}) in mice; 1,2,4,8,16 mg/kg concentrations of $CdCl_2$ were exposed to mice via IP for 48 h. and 12.50, 25, 50, 100, 200 mg/kg BW concentrations of $ZnCl_2$, The LD50 of $CdCl_2$ was calculated to be 6.023 mg/kg as shown in Table 1 and Figure 1. The LD50 of $ZnCl_2$ was estimated to be 89.05 mg/kg BW as shown in Table (2) (Figure 2). The results revealed a dose-response relationship, and the experiment was repeated two times (in triplicate each).

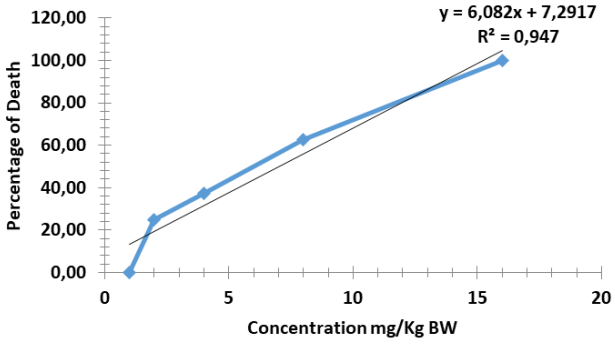

Figure 1 Cadmium chloride median lethal dose (LD50) on male mice via intraperitoneal injection.

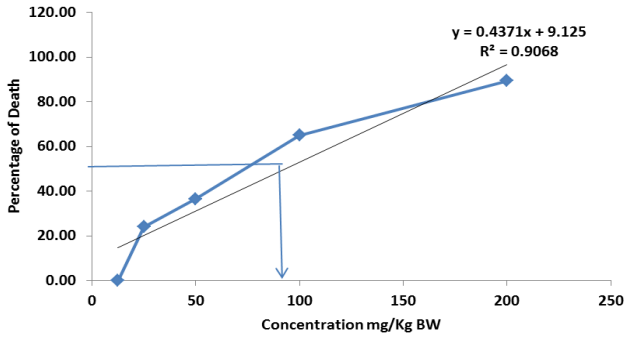

Figure 2 Zinc chloride median lethal dose (LD50) on male mice via intraperitoneal injection.

Table 1 LD50 of $CdCl_2$ on male mice (Acute toxic effect with log concentration).

Treatment groups	Cd (mg/kg)	Number of animals	After 48 h of treatment		Death (%)
			live	death	
Control (G1)	0	8	8	0	0
Zn (G2)	1	8	8	0	0
Cd (G3)	2	8	6	2	25
(G4)	4	8	5	3	37
(G5)	8	8	3	5	62
(G6)	16	8	0	8	100

3.2. The body weight of the animals

By the experiment's end, the animal's body weight was calculated. In the control group (G1) and the zinc group (G2), mice gained some body weight (significantly increased $P \leq 0.05$ during the experiment (4.781 ± 0.045 and 3.807 ± 0.040 gm) respectively referenced to their weight at the start of the experiment. Cadmium alone decreased the animal's body weight in both concentrations used (groups G3 and G5); a highly significant difference was observed in G5 with (6.306 ± 0.038 gm) body weight loss and (2.683 ± 0.047gm) body weight loss in the group (G3). The group (G6), which received a combination of zinc and cadmium doses, showed a significant ($P < 0.05$) decrease in the animal's body weight loss with (3.742 ± 0.038 gm). While in the group (G4), the loss in body weight of treated animals was (1.20 0± 0.01 gm).

Adding the zinc significant reduced the body weight loss caused by cadmium in the (G4) group experiment, as shown in Figure 3.

3.3. The relative liver weight

The results of relative weight, as shown in Figure 4, revealed a significant increase ($P \leq 0.05$) in relative liver weight of experimental groups G3, G5, and G6 (8.146 ± 0.041, 7.082 ± 0.02, 6.985 ± 0.03%) respectively compared with control (G1, 5.946 ± 0.039%). No significant increase in the relative liver weight was recorded in G2 and G4 compared with the control.

3.4. Concentration of cadmium and zinc in mice liver

3.4.1 Cadmium concentration

The results indicated a significant increase ($P \leq 0.05$) in cadmium concentration in experimental groups G3, G4, G5, and G6 (76 ± 0.58, 90 ± 0.9, 138 ± 0.7 and 189 ± 0.6), respectively, compared to the control group (G1, 0.0 3 ± 0.001) as shown in Figure 5. The treated groups showed a significant increase in all experimental groups compared with the Zn group. The highest level was in group G6, indicating concentration depending on aspect.

Table 2 LD50 of $ZnCl_2$ on male mice (Acute toxic effect with log concentration).

Treatment groups	Cd (mg/kg)	Number of animals	After 48 h of treatment		Death (%)
			live	death	
Control (G1)	0	8	8	0	0
(G2)	12.50	8	8	0	0
(G3)	25	8	6	2	25
(G4)	50	8	5	3	37.5
(G5)	100	8	3	6	75
(G6)	200	8	1	7	87.5

3.4.2. Zinc concentration in mice liver

As shown in Figure 6, all treated groups revealed significant increases compared with G1 after being treated with $ZnCl_2$ in liver mice. Notably, administration with ($CdCl_2$ 1.5 mg/kg) and ($CdCl_2$ 3mg/kg) significantly enhanced the concentration of zinc in the liver. On the other hand, groups treated with $ZnCl_2$ + $CdCl_2$ showed a significant increase in zinc concentration with 40 ± 0.700 and 45 ± 0.850 for G4 and G6 groups, respectively, for the control group.

3.5. Liver metallothionein determination

As shown in Figure 7, $Zncl_2$ and $CdCl_2$ treated alone or in a combination significantly increased MT concentration in liver mice compared to the G1 group. Also, co-administration of ($Zncl_2$ 10 + $CdCl_2$ 3 mg/kg) in G6 showed significant decreased in MT concentration compared with G5. Moreover, a significant difference was observed between G5 and G6 and other treatment groups.

3.6. Liver malondialdehyde (MDA) determination

The current study result of MDA levels in liver mice showed a significant increase in G3, G5, and G6 groups (52.673 ± 5.940, 74.923 ± 4.686, and 60.673 ± 4.537 nmol/ml, respectively) compared to the control group (25.692 ± 2.321 nmol/ml); however, G2 and G4 showed no difference (27 ± 3.148 and 25.955 ± 2.798 nmol/ml respectively) compared to G1. Interestingly, the mice treated with $ZnCl_2$ alone or in a combination preserved or decreased MDA levels influenced by $CdCl_2$ (Figure 8).

3.7. Caspases-3 level determination

As shown in Figure 9, the concentrations of caspase 3 did not change significantly in the group (G2) compared to the control (G1). Still, they significant increased in the cadmium-treated groups (G3 and G5) with (26 ng/ml) and (35 ng/ml), respectively, compared to the G1. Both co-treatment groups had significantly lower caspase 3 after treatment with Zn and Cd, with (18 ng/ml) and (29 ng/ml), respectively.

3.8. Quantification of DNA damage with the comet assay

Strand breaks of DNA of mice livers were evaluated using the comet assay. Cellular analysis was performed using Image J software. The head, tail length, and tail intensity data were illustrated in Table 3. Treatment with cadmium significantly increased the tail length, whereas the head length decreased significant compared to the control (G1). Co-treatment with zinc reduced these parameters compared to those measured in cells treated with cadmium alone (Figure 10).

4. Discussion

Cadmium toxicity in biological systems is well known; however, the mechanisms of toxicity are yet unknown. The route of exposure, the dose provided, and the period of exposure all influence the dose of cadmium that enters the biological system (Das and Al-Naemi 2019). Cadmium, as heavy metal, is physically and structurally similar to zinc. Thus, researchers have been interested in looking at the interaction between Cd and Zn as Cd has the potential to

affect Zn-dependent pathways in biological systems (Tang et al 2016).

In the current work, the results demonstrated that mice treated with cadmium at 1.5 and 3 mg/kg showed a significant loss in body weight. In contrast, treatment of mice with zinc resulted in an improvement in body weight. Here, we observed that body weight loss was associated with Cd accumulation in liver tissue; however, Zn concentration was not altered in tissue. Oxidative stress and inflammation induced by Cd can alter the metabolic function and antioxidant activity resulting in tissue toxicity and weight loss. It has been found that Cd-induced toxicity via hypoxic stress in rats results in decreased O2 consumption in tissue. To avoid Cd toxicity, the metabolic compensation type shifted from cell air-respirations to an air- respirations (Brzoska et al 2016; Poli et al 2022). The elevated MDA levels found in this study support this hypothesis. Previous animal studies found that i.p. treatment with $CuCl_2$ caused oxidative damage in the liver, resulting in a significant increase in the level of MDA and degenerative hepatocytes (Al-Baqami and Hamza 2021). On the other hand, Previous researchers reported that supplementation with natural products, minerals and/or vitamins could reduce Cd-induced toxicity in the tissue (Pałka et al 2022).

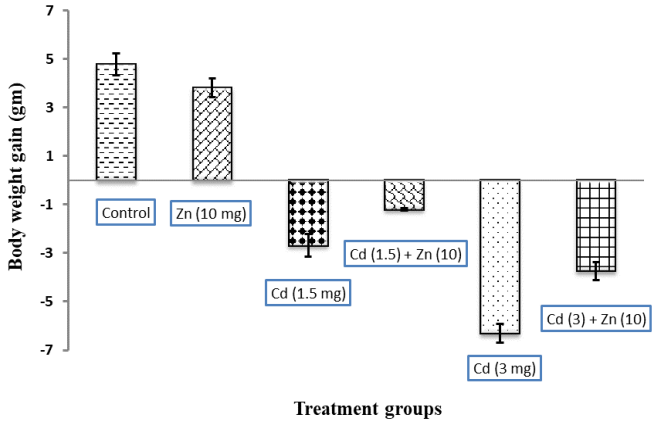

Figure 3 Body weight gain (gm) in male mice treated with zinc, cadmium, zinc, and cadmium compared to the control group. Values represent mean ± SD, n = 6, (*) Significant differences ($P < 0.05$).

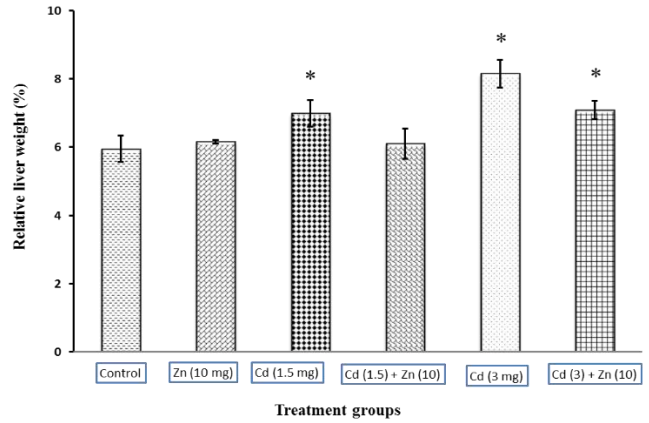

Figure 4 Relative liver weight (%) of male mice treated with zinc, cadmium, zinc and cadmium compared to the control group. Values represent means ± SD, n = 6, (*) Significant differences vs. control ($P \leq 0.05$).

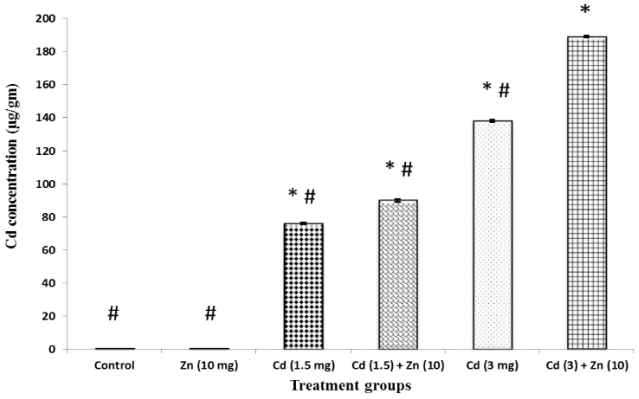

Figure 5 Cadmium concentration (µg/gm wet tissue) in the liver of mice, after being treated with zinc, cadmium, zinc and cadmium, compared to control, determined by ICP-AES. Values represent mean ± SD, n = 6, (*) Significant differences vs. control ($P < 0.05$), (#) significant differences vs. Zn group ($P < 0.05$).

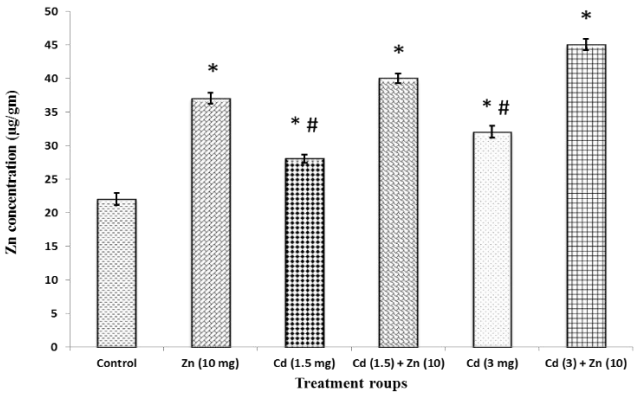

Figure 6 Zinc concentration (µg/gm wet tissue) in the liver of mice, after being treated with zinc, cadmium, zinc and cadmium, compared to control, determined by ICP-AES. Values represent means ± SD, n = 6, (*) Significant differences vs. control ($P < 0.05$), (#) significant differences vs. all other groups ($P < 0.05$).

Two principal theories have been suggested to explain the toxicity of cadmium. Firstly, Cadmium-induced excessive production of reactive oxygen species (ROS) causes oxidative and cellular damage (Patra et al 2011). Secondly, cadmium can bind to macromolecules, causing them to lose function via attaching to cryptic binding sites and reducing protein function (Permyakov 2021); however, these hypotheses have little concrete evidence. Also, cadmium attaches to proteins in physiological binding sites for another metal, such as zinc, copper, or calcium, displacing the physiological metal and lowering protein function.

Different tissue responds differently to Cd exposure. In the current study, treatment with zinc in the (G4) and (G6) groups improved body weight. This suggests that zinc treatment may cause a decrease in the effect of cadmium by binding to biomolecules. Also, it has been found that chronic exposure of female rats to Cd causes important bio-element to redistribute in the body organs, resulting in the retention of Zn and Cu in the liver and kidneys and a decrease in their amounts in the serum (Borowska et al 2017). Our findings unequivocally show that intraperitoneal Cd injection led to a metal buildup in liver tissue and changed Zn levels.

Table 3 Comet assay of liver cells from different treatment groups of male mice representing of head and tail lengths (micrometers), head and tail intensity (% DNA), and extent tail moment (arbitrary units=tail length×tail % DNA).

Groups	No Damage % (Mean±SD)	Low Damage % (Mean±SD)	Medium, Damage % (Mean±SD)	High Damage % (Mean±SD)
Control	42.49±0.564^{B}	42.383±0.47^{B}	7.626±0.414^{D}	7.502±0.47^{D}
G2	45.08±0.617^{A}	45.082±0.455^{A}	4.811±0.616^{E}	5.027±0.293^{E}
G3	35.8±0.813^{D}	37.116±0.929^{D}	13.427±0.556^{B}	13.657±1.216^{B}
G4	29.144±1.537^{E}	29.6±2.23^{E}	19.84±2.07^{A}	21.421±1.942^{A}
G5	39.553±0.582^{C}	39.265±0.663^{C}	10.248±0.43^{C}	10.934±0.74^{C}
G6	36.854±0.57^{D}	36.502±1.176^{D}	13.289±0.638^{B}	13.355±1.01^{B}
P-value	0.0003	0.0000	0.0005	0.00034
LSD	1.27019	1.72358	1.4510	1.61858

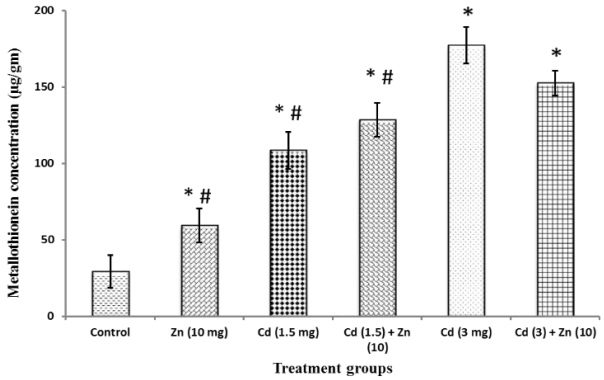

Figure 7 Metallothionein concentration (µg/gm wet tissue) in the liver of male mice, after being treated with zinc, cadmium, zinc and cadmium, compared to control, determined by (HPLC) with (AAS). Values represent means ± SD, n = 6, (*) Significant differences vs. control ($P \leq 0.05$), (#) significant differences *vs*. G5 and G6 ($P \leq 0.05$).

Cadmium affects several aspects of tissue function, most notably through metallothionein protein (MT). MT synthesis is one of the tissue protection mechanisms against Cd toxic effects in the tissue, cells with an overabundance of MTs are resistant to the toxicity of cadmium (Genchi et al 2020). MT is a thiol-rich, strongly regulated protein with a low subatomic weight (Isani et al 2014). Results of this study indicate that Cd exposure induces MT synthesis in the liver of mice, and co-treatment with Zn promoted a reduction in Cd accumulation in the liver. Taken together, the results suggested that the protection mechanism of Zn on Cd effects could be through increasing specific antioxidant pathways via increasing MT level and decreased lipid peroxidation. This is in accordance with the findings of Stanevieiene et al (2008), who observed the effects of low doses of injected $CdCl_2$ at 1.4 µM and/or $ZnSO_4$ at 4.8 µM on the translation machinery and cell death in mouse liver. They reported that Zn pretreatment could protect the translation machinery against Cd-induced inhibition but did not impact liver cell death in mouse liver exposed to Cd ions.

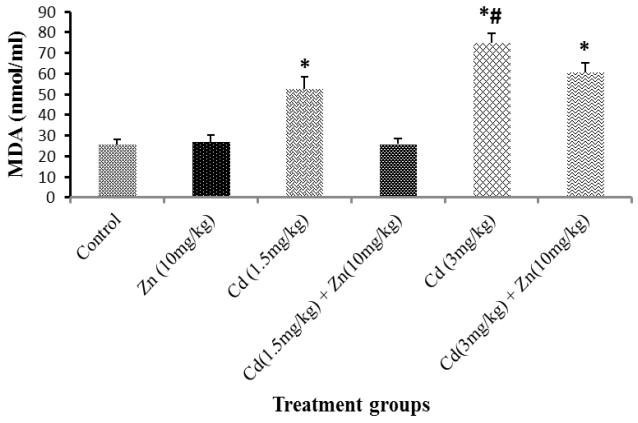

Figure 8 Malondialdehide concentration (nmol/ml) in the liver of male mice, after being treated with zinc, cadmium, zinc and cadmium, compared to control, determined by the thiobarbituric acid reaction. Values represent means ± SD, n = 6, (*) Significant differences ($P < 0.05$) compared with control, Zn, Cd (1.5) + Zn, (**) significant differences ($P < 0.05$) Significant difference between Cd(3mg/ml) compared with Cd (1.5), Cd(3)+Zn.

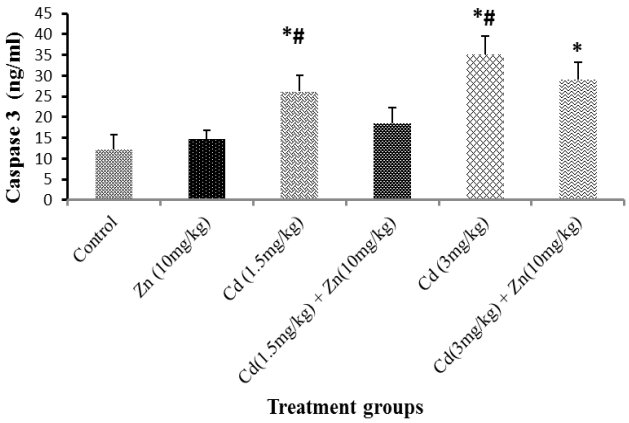

Figure 9 Caspase 3 concentration (ng/ml) in the liver of male mice, after being treated with zinc, cadmium, zinc and cadmium, compared to control, determined by (ELIZA). Values represent means ± SD, n = 6, (*) Significant differences vs. control ($P < 0.05$), (#) significant differences vs. all other groups ($P < 0.05$).

Several different methods may mediate the induction of DNA damage. Reactive oxygen species are implicated in the DNA damage brought on by the carcinogenic metal ion (Nilsson and Liu 2020). Apoptosis, DNA synthesis and repair, cell cycle progression, cell proliferation and differentiation, and other biological processes can all be impacted by cadmium (Zhou et al 2013). In addition, Skippe (2016) reported that cadmium chloride decreases HepG2 cell viability and raises DNA damage, malondialdehyde activity, lactate dehydrogenase leakage, and antioxidant enzyme activity, and the GSH/GSSG ratio significantly decreased. Additionally, zinc participates in crucial activities, including transcription and DNA replication through zinc finger proteins, which can prevent oxidation and damage to DNA and other biological components (Powell 2000).

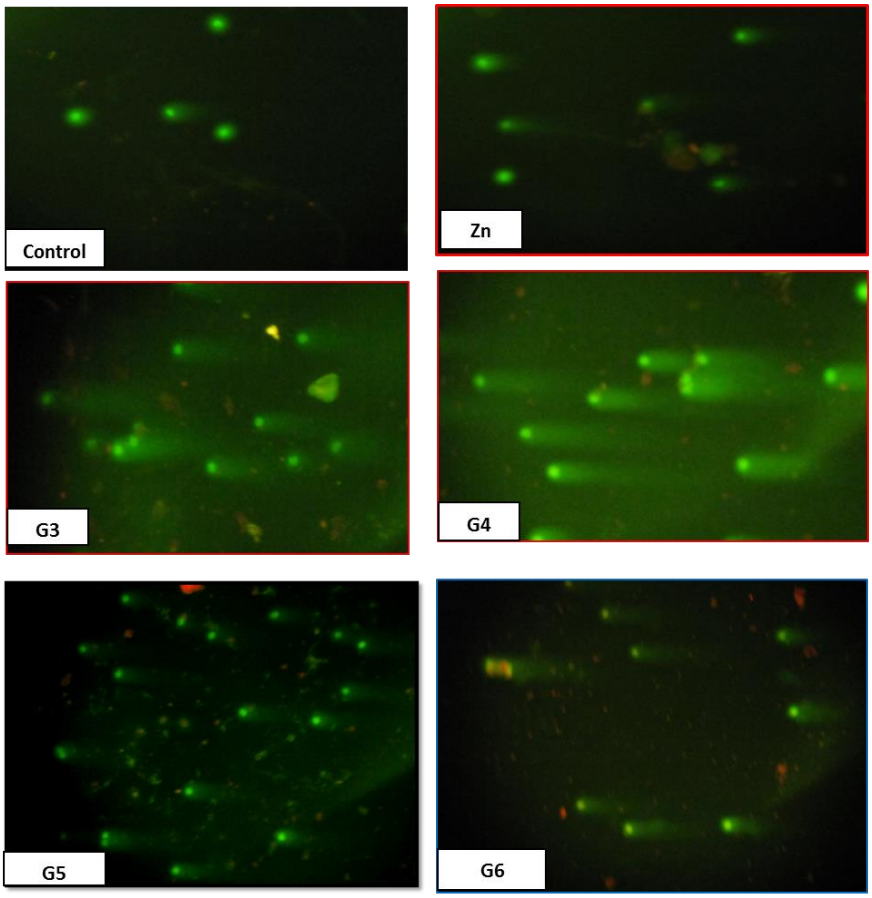

Figure 10 Comet assay of liver cells from different treatment groups of male mice representing: control, Zinc chloride (10 mg/kg), G3 = cadmium chloride (1.5 mg/kg), G4 = (1.5 cadmium chloride + 10 mg/kg Zinc chloride), G5 = cadmium chloride (3 mg/kg), G6 = (3 cadmium chloride + 10 mg/kg Zinc chloride) treatment groups.

5. Conclusions

This study's findings indicate that $CdCl_2$-treated mice showed alteration in metallothionein protein concentration, cytotoxicity, apoptosis, and genotoxicity of hepatocytes. The lipid peroxidation and caspase-3 may be taken as an index of hepatotoxicity and suggest that oxidative stress is involved along with apoptosis pathways induction, which has a regular time-and dose-response pattern.

Conflict of Interest

The authors declare no conflict of interest.

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