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ORIGINAL ARTICLE

Zinc Supplementation Attenuates Cadmium-Induced Jejunal Injury in Adult Male Albino Rats: Histopathological and Biochemical Study

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ABSTRACT

Background: Cadmium is one of the hazardous heavy metals, broadly spread as water and food-borne toxicant. The first organ to be targeted by food-borne cadmium is the gastrointestinal tract, causing intestinal damage and deficits in metal trace elements. In contrast, Zinc is one of the most vital nutritional minerals motivating other heavy metals metabolism, such as cadmium. **Objective:** The purpose of this study was to investigate the histopathological and biochemical effects of cadmium on intestinal wall, through the investigation of possible oxidative and inflammatory pathways, moreover, to determine whether zinc may have a preventive function against this potential toxicity. **Methods:** Rats were divided to 4 groups (control, zinc, cadmium, and zinc/cadmium). After 14 days of cadmium supplementation (6 mg/kg) ± 500 mg zinc chloride, rats were weighted and jejunal samples were obtained for histological and biochemical analysis. **Results:** The administration of cadmium caused decrease in rats' body weight, increase in the activities of inflammatory markers (TNF-α and IL-1β levels) and intestinal oxidant/antioxidant imbalance (increase in malondialdehyde level, plus decrease in SOD and GPx activity). Moreover, cadmium triggered intestinal structural damage (short blunt or long desquamated villi tip, distorted enterocytes, vacuolations, hemorrhage and inflammatory cell infiltrations), decrease goblet cells number and index, inflammatory reaction with overexpression of NF-kb and low expression of anti-apoptotic protein Bcl-2. **Conclusions:** This study has revealed that zinc chloride supplementation can improve the cadmium-induced inflammatory reaction and villus pathology, mitigate oxidative stress and the weight loss through inhibition of oxidative stress and inflammatory markers.

Keywords:
Cadmium; Zinc; Jejunal pathology; Oxidant stress; Bcl-2; NF-kB.

INTRODUCTION

Cadmium (Cd) is regarded as a hazardous heavy metal. It is commonly spread in the environment, and in many industries including batteries, paint, fertilizers, and electroplating [1]. The major sources of cadmium exposure are drinking water and eating certain foods [2].

Although it affects various organs and tissues, the first organ to be targeted by food-borne cadmium is the gastrointestinal tract. It causes intestinal damage and metal trace element deficits [3]. Cd exposure induces an inflammatory response, state of oxidative stress, epithelial cell death, and tight junction damage in the intestines, causing the intestinal barrier to

be disrupted and Cd absorption to be amplified [4].

Cd toxicity was explained by its competitive interference with the metabolism of essential divalent metals like manganese and zinc, as well as protein structural disruption caused by Cd binding to sulfhydryl groups [5]. Moreover, inflammatory intestinal response to cadmium toxicity includes macrophage inflammatory protein-2 (MIP-2) chemotactic cytokine production and subsequent neutrophil infiltration [6].

Cadmium additionally triggers oxidative harm to cell organelles through increasing the production of reactive oxygen species (ROS). As a result of these ROS reacting with cellular biomolecules, lipid peroxidation, membrane protein destruction, DNA damage, altered gene expression, and apoptosis are all initiated [7]. When ROS-mediated effects are not counteracted by repair processes, affected cells undergo apoptosis or necrosis [8].

Increased nuclear factor- kB (NF-kB) immunoreactivity in the adrenal cortex validated cadmium's inflammatory action [9]. The activation of NF-kB has been found to be a risk factor for cancer, a crucial step in the activation of cyclooxygenase-2 (COX-2) in the lipopolysaccharide-exposed macrophages, as well as it affects the expression of inducible nitric oxide synthase (iNOS) [10].

Zinc (Zn) is the most abundant trace element found within cells. It participates in a wide range of biological functions and cellular processes, including cell proliferation, reproduction, immunological function, and free radical defense [11].

Certainly, there is mounting evidence that zinc plays an important antioxidant role in protecting cellular components from oxidation. Zn is one of the most important dietary nutrients in influencing heavy metal metabolism and toxicity, including Cd [12]. Cd displaces Zn once it enters the body by interfering with ionic and covalent bonds to sulphur, oxygen, and hydrogen [13], resulting in a considerable drop in systemic Zn and malfunction of many zinc-essential enzymes [14].

As a result, increasing Zn consumption may help to prevent Cd accumulation and toxicity. Therefore, this study was conducted to investigate the histopathological and biochemical effects of cadmium on the intestinal wall, through investigation of possible oxidative and inflammatory pathways, moreover, to determine whether zinc may have a preventive function against this potential toxicity.

METHODS

Chemicals:

Cadmium chloride in high priority salt formula, were purchased from Sigma (St. Louis, MO, USA (439800)). Zinc chloride in high priority salt formula, were purchased from Sigma (St. Louis, MO, USA (429430)).

Animals:

Eighty male adult (12 week) albino rats (body weight 250±20 gm) were obtained from the laboratory animal house of Faculty of Medicine, Zagazig University, Egypt. The experimental rats were kept at a 12-hour light/dark cycle and a temperature of 24°C. The rats were fed a standard pelleted diet and given free access to water. They were adapted for two weeks prior to any experimental processes to ensure their physical health, avoid diseased rats, and adjust to their new surroundings.

Ethical Approvals:

All rats were cared for in accordance with the National Institutes of Health (NIH) Animal Care Guidelines, and the experiment was approved by the Institutional Animal Care and Use Committee at Zagazig University, Egypt (ZU-IACUC/3/F/196/2021).

Experiment protocol:

The rats were divided into four groups at random (control, zinc, cadmium, zinc/cadmium). Each group has 20 rats as follows: Control group: which received daily administration of distilled water and balanced diet for 2 weeks. Zinc (Zn) group: which received distilled water supplemented with 500 mg Zn/L daily for 2 weeks according to El-

Refaiy and Eissa [15]. Cadmium (Cd) group: which received CdCl2 (6 mg/kg) by oral gavage tube daily for 2 weeks according to Xue et al., [16]. Cadmium/zinc (Cd/Zn) group: which received CdCl2 (6 mg/kg) as mentioned with distilled water supplemented with 500 mg Zn/l daily for 2 weeks.

On day 14 of the experiment, rats were evaluated for general health and body weight before being deeply anaesthetized intraperitoneally with thiopental (50 mg/kg) and sacrificed. Rats' abdomens were carefully dissected and jejunal samples were collected, labeled with a given code number for further blind examination. Half of samples were immediately immersed and fixed in buffered formalin 10% for 24 h, then prepared for histological examination, the other half of samples were frozen immediately on dry ice and stored at -80°C for tissue homogenate analysis.

Anthropometric measures:

Body weight was measured by placing each animal in a closed plastic container and weighing them the day before and the day after the experiment. The findings were recorded in a record for each labelled rat.

Homogenate tissue analysis:

All tissues were maintained at +4 ºC throughout the preparation, jejunal tissues were homogenized using 5 – 10 ml cold buffer (i.e, 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram tissue as the previously described technique by Owoeye et al., [17]. The homogenates were centrifuged (15 minutes at 50,000 rpm), then the clear upper supernatants were removed and stored at -80°C for analyses.

The preserved homogenate was used for colorimetric assessment of oxidative stress parameters: Lipid peroxidation products malondialdehyde (MDA) was estimated (nmol/g) using a Biodiagnostic kit (Giza, Egypt with a cat. No. MD 2529) following the kit instructions.

Glutathione peroxidase activity (GPx) was assessed (U/g protein) according to the commercially available Biodiagnostic kit. Superoxide dismutase activity (SOD) activity was assessed in (U/g protein) according to a commercially available Biodiagnostic kit.

The inflammatory markers: Tumor necrosis factor-alpha (TNF-α) was assessed in (pg/ml) according to a commercially available MyBiosource ELISA kit. Interleukin-1β (IL-1β) was assessed in (pg/g tissue) by ELISA specific experimental kits (MyBioSource, ELISA Test Kits).

H&E histological and morphometrical analysis:

Samples of jejunum, moreover, the degree paraffin blocks, were cut up into 4-5µm slides using a microtome. Corresponding to Hegazy and Hegazy [18], sections were stained with hematoxylin and eosin (H&E) to evaluate general tissue histology. Sections were assessed at 100 magnifications as follows: 1. Villi heights (from the tip of the villus to the villus-crypt junction); 2. Total mucosa thickness in µm (from the top of the villus to the muscularis mucosa); 3. Crypt depths in µm (the subtraction of villus height from total mucosa thickness) using ImageJ software, 50 villi from 10 non overlapped fields from each rat were evaluated.

Moreover, the degree of intestinal pathology was graded using the method explained by Chen et al., [19]: Normal mucosa and villi, grade 0; Grade 1: The epithelial gap has widened, and blood vessels have become congested. In grade 2, the epithelial gap has clearly expanded, and the epithelium and lamina propria have separated. Grade 3: Mucosal epithelium and part of the villi tip peeling off; Grade 4: Villi peeling off, lamina propria exposed, and dilated blood vessels exposed. In grade 5, the lamina propria shows signs of decomposition, bleeding, and ulceration.

Moreover, the periodic acid-Schiff (PAS) reaction was used to detect carbohydrates. At 100 magnifications, goblet cells per 100 µm of villus epithelium were counted, moreover evaluation the PAS-reflected rate of goblet cells: The goblet cell index was defined as the ratio of caliciform cells to enterocytes in each villus "the goblet cell index = (number of caliciform

cells/total number of cells in the villus) x100" as described by Cirilo et al., [20].

Immunohistochemical and morphometrical analysis:

Other sections were prepared for immunohistochemical staining according to Hu et al., [21]. They were immersed in xylene twice for 20-30 minutes before being rinsed with a series of alcohol (70-99 percent), followed by 3 percent H2O2 for 20 minutes. After washing in phosphate buffered saline (PBS), the sections were placed in citrate buffer solution and heated in an 800-Watt microwave in 5-minute intervals four times before being incubated in secondary blocking agent for 20 minutes.

After that, each preparation was incubated for 75 minutes at room temperature in anti-Bcl-2 antibody (Sigma-Aldrich, MFCD00162586) and anti-NF-kB (nuclear factor-kappa) antibody (Sigma-Aldrich, ab16502, MFCD00802846) with the appropriate dilution (1/200) of primary antibodies. The chromogen was diaminobenzidine (DAB) solution, and the contrast coloring was Mayer's hematoxylin staining. For the negative controls, PBS was used. The preparations were photographed after they had been covered with the appropriate closing materials.

The degree of anti-NF-kB and anti-Bcl-2-stained sections were scored from 3 sections of each rat. Coded figures facilitated to blind examination and assessment according to Hu et al., [21], IHC scores were calculated in five non-intersected fields (x400) for each section by adding together (1) the percentage of positively stained cells was graded from 0 to 4 (0; no positive cells, 1; 25% of cells stained positive, 2; 26–50% of cells stained positive, 3; 51–75% of cells stained positive, and 4; >75% of cells stained positive) (2) staining intensity was graded from 0 to 3 (0; no positive cells, 1; 25% of cells stained positive, 2 (0; no staining, 1; light yellow, 2; yellowish brown, and 3; brown). Values are presented as the mean and standard deviation (ranged from 0 to 7).

Statistical Analysis:

All data were presented as the mean±standard deviation (SD), and statistically evaluated using the SPSS (Statistical Package for Social Science) version 18.0 program. The mean values of different groups were compared using one-way ANOVA. Multiple comparisons were estimated using the post-hoc test; a $p<0.05$ value was statistically significant, a $p<0.001$ value was highly statistically significant, and a $p>0.05$ value was non-statistically significant.

RESULTS

Effect of Cd ± Zn on body weight:

On the day before the experiment, rats' weights showed no significant difference between different study groups. However, rats treated with cadmium (Cd group) showed a highly significant difference in body weight (201.3±10.4 gm) vs 274.5±34.5 gm and 246.6±42.3 gm in control and Cadmium/zinc groups, respectively (Table 1, Figure 1).

Effect of Cd ± Zn on Inflammatory Markers:

Evaluation of cadmium effects on TNF-α and IL-1B marker in jejunal tissue revealed that Cd significantly increased TNF-α (126.7±13.5) vs 92.6±8.7 in control group. Furthermore, IL-1B levels were significantly increased in Cd group (78.3±13.5) vs 46.6±9.3 in control group. Nevertheless, after zinc supplementation in Cd/Zn group, there were significant differences against Cd group in TNF-α and IL-1B levels, despite that TNF-α in Cd/Zn group still showed significant difference with control group (Table 1, Figure 1).

Effect of Cd ± Zn on Oxidative Stress Markers:

Evaluation of cadmium effects on Malondialdehyde (MDA) as a lipid peroxidation marker in jejunal tissue revealed that cadmium significantly increased MDA up to 11.3±3.8 vs 14.8±3.1 in control group. Moreover, Cd effects on superoxide dismutase (SOD) and glutathione peroxidase (GPx) as an antioxidant marker in jejunal tissue revealed that Cd treatment significantly decreased SOD activity 1.2±0.6 vs 2.3±0.4 in control group and decreased GPx activity 21.4±4.6 vs 41.1±5.2 in control group. However, after zinc supplementation in Zn

group revealed increase in antioxidant activity (both GPx and Sod levels), in Cd/Zn group, there were significant differences against both Cd and control groups in MDA, GPx, and SOD levels (Table 1, Figure 1).

Effect of Cd ± Zn on H&E histological examination of jejunal wall:

Control and Zn groups showed the same results of normal jejunal histology; consisted of mucosa (formed of villi and crypts), submucosa and muscularis externa. Jejunal villi were long and slender with pointed tip (finger-like projections) and lined by tightly packed columnar cells (enterocytes) with basal oval vesicular nuclei and prominent continuous brush borders with goblet cells in between. Jejunal crypts (crypts of Lieberkühn) at base of villi were well organized, lined by columnar cells and surrounded by narrow layer lamina propria which extended to core of villi. (Figure 2: A-D).

In Cd group, jejunal villi were either short with blunt or in average length but with desquamated tip and distorted enterocytes, which had darkly stained nuclei and lost brush borders. Goblet cells were destructed. The lining epithelial cells of crypts had vacuolations and pyknotic darkly stained nuclei. Lamina propria had wide spaces, vacuolations, hemorrhage and inflammatory cell infiltrations. Also, there were submucosal separation and abundant epithelial exfoliations in jejunal lumen (Figure 2: E-I).

Regarding Cd/Zn group, some of the jejunal villi restored their length but still had blunt tip, other were short with some desquamations. Their lining columnar epithelium had either vesicular or darkly stained nuclei, some of them gained brush borders and others still lost them. Few epithelial exfoliations were present in jejunal lumen. Lamina propria had small areas of hemorrhage. Inflammatory cell infiltrations were negligible. Goblet cells, submucosa and muscularis externa were apparently normal. (Figure 2: M-P).

Morphometrical image analysis revealed pathology grading scores indicating severe pathological damage in Cd group (3.5±0.75), this pathological score was alleviated by zinc supplementation in Cd/Zn group. Moreover, the villus height, mucosal thickness and crypt depth was significantly reduced in the Cd group when compared to the control group's corresponding values. When Zn and Cd were co-treated, all parameters improved when compared to the control and Cd groups (Table 2, Figure 2).

Effect of Cd ± Zn on PAS histological examination of jejunal wall:

Control and Zn groups showed same results, there was a strong positive PAS staining in the goblet cells and continuous brush borders of enterocytes. While Cd group exhibited apparent weaken intensity of PAS staining of goblet cells and lost brush borders of most enterocytes. Regarding Cd/Zn group, there was a relatively strong positive goblet cells' PAS staining and most of brush borders of enterocytes were preserved and well-stained (Figure 3). Morphometrical image analysis revealed that goblet cells count/100 μm and The Cd group's goblet cell index was significantly lower than the control group's corresponding values. The combination of zinc and Cd restored goblet cell parameters to levels comparable to those observed in the control group (Table 2, Figure 3).

Effect of Cd ± Zn on NF-kB immunoreactivity:

Crypt-lining cells of control and Zn groups showed a little positive nuclear immunoreactivity for NF-kB, which was apparently increased in Cd group and moderately scattered in Cd/Zn group (Figure 4). Moreover, morphometrical image analysis for NF-kB score in Cd group, there was highly significant increase (5.3±1.6) vs control (2.3±0.7) and Zn (2.4±1.1) groups, while Cd/Zn group (3.7±0.9) showed a significant increase vs control group and a highly significant decrease vs Cd group (Table 2, Figure 4).

Effect of Cd ± Zn on Bcl-2 immunoreactivity:

Positive immune-stained cytoplasm for Bcl-2 (anti-apoptotic protein) showed normal expression in crypt-lining cells in both control and Zn groups; otherwise, Cd group displayed sparse expression. Moreover, Cd/Zn group

demonstrated an improvement of the Bcl-2 (Figure 5). Moreover, morphometrical image analysis for Bcl-2 score in Cd group, there was a highly significant decrease (3.2±1.2) vs control (6.1±0.8) and Zn (5.9±0.7) groups, while Cd/Zn group (4.3±1.1) showed a highly significant decrease vs control group and a highly significant increase vs Cd group (Table 2, Figure 4).

Table 1: Effect of Cd ± Zn on body weight, oxidative stress, and inflammatory markers in different studied groups.

	Control group (n20)	Zn group (n20)	Cd group (n20)	Cd/Zn group (n20)	P value
Body weight *	274.5±34.5	281.4±22.5	201.3±10.4[b]	246.6±42.3[ad]	< 0.001
Inflammatory Markers					
TNF-α	92.6±8.7	87.5±9.1	126.7±13.5 [b]	105.4±10.7[ad]	< 0.001
IL-1β	46.6±9.3	42.4±6.7	78.3±13.5 [b]	53.4±10.4[d]	< 0.001
Oxidative Stress Markers					
MDA	14.8±3.1	11.3±3.8	32.1±3.6 [b]	26.4±7.8 [bc]	< 0.001
GPx	41.1±5.2	48.5±3.4 [a]	21.4±4.6 [b]	31.6±7.2 [bd]	< 0.001
SOD	2.3±0.4	2.9±0.3 [a]	1.2±0.6 [b]	1.8±0.6[ad]	< 0.001

*At last day, MDA: malondialdehyde (nmol/g), SOD: Superoxide dismutase (U/g protein), GPx: glutathione peroxidase (U/g protein), TNF-α: Tumor Necrosis Factor Alpha (pg/ml), IL-1β: Interleukin-1β (pg/g tissue). One-way ANOVA, and least significant difference (LSD) test, P > 0.05: no significant differences, P < 0.05: significant differences, P < 0.001: highly significant differences.

[a] significant vs control group, [b] highly significant vs control group, [c] significant vs Cd group, [d] highly significant vs Cd group.

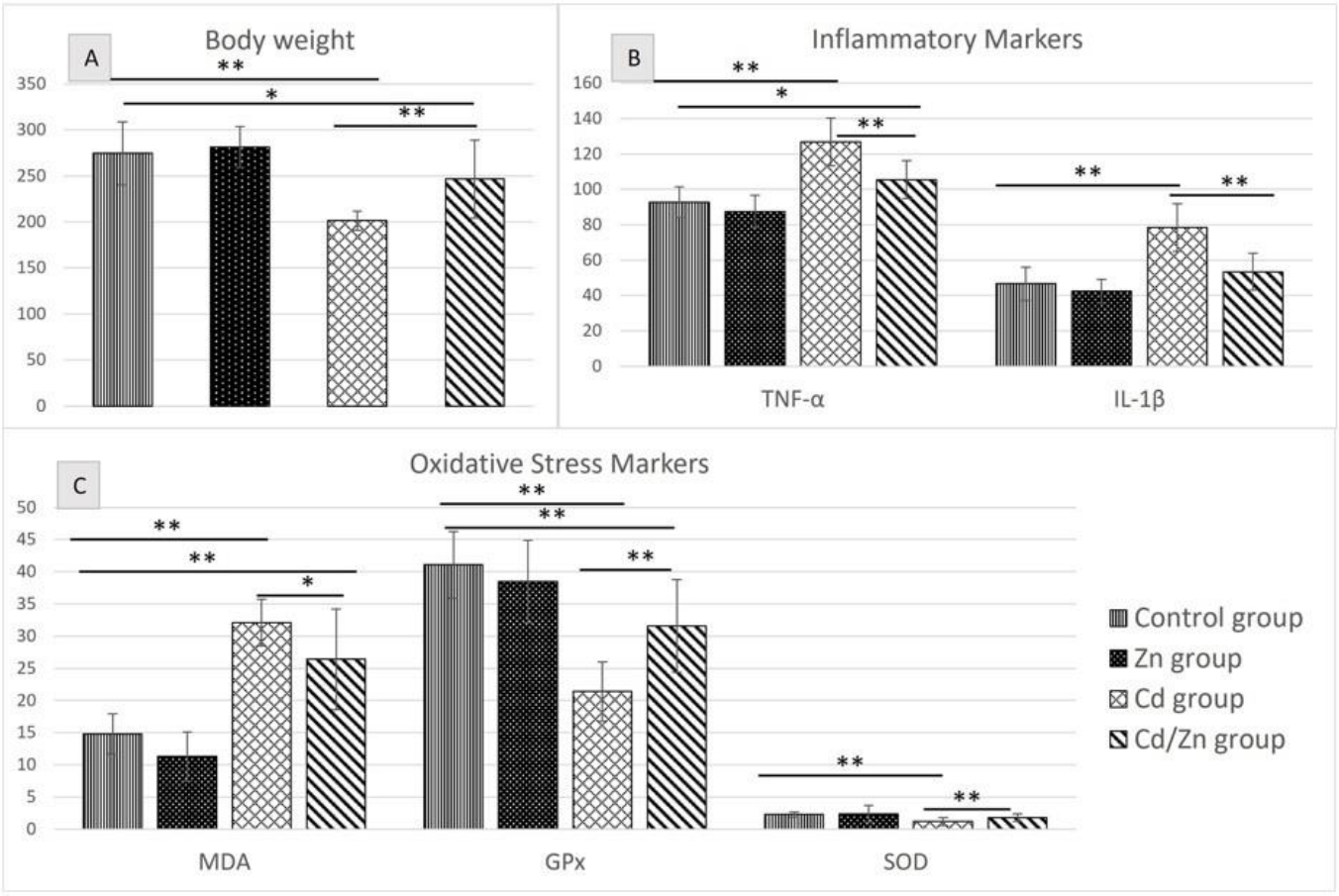

Figure 1: A: Body weight; B: Inflammatory markers (TNF-α and IL-1B marker); C: Oxidative stress markers (MDA, GPx & SOD) in different studied groups.

Table 2: Effect of Cd ± Zn on histological and immunohistochemical morphometrical parameters of jejunal wall in different studied groups.

	Control group (n20)	Zn group (n20)	Cd group (n20)	Cd/Zn group (n20)	P value
H&E morphometry (in µm)					
Pathological score	0.5±0.25	0.75±0.5	3.5±0.75 [b]	1.5±0.25 [bd]	<0.001
Villus height	578.4±71.4	573.3±83.5	494.8±85.9 [a]	550.3±99.5	0.0107
Thickness of the mucosa	1032.4±84.7	1021.4±79.2	638.7±64.6 [b]	950.9±95.3 [ad]	<0.001
Crypt depth	512.2±90.2	459.6±83.1	281.5±46.8 [a]	473.4±62.8 [d]	<0.001
PAS morphometry					
Goblet cells count/100 µm	5.2±0.8	4.8±0.9	2.7±1.2 [b]	3.9±1.4[ac]	<0.001
Goblet cell index	19.4±3.6	21.3±2.7	11.6±3.2 [b]	17.4±2.5 [d]	<0.001

Immunohistochemical morphometry					
Bcl2 score	6.1±0.8	5.9±0.7	3.2±1.2 [b]	4.3±1.1 [bc]	<0.001
NF-kB score	2.3±0.7	2.4±1.1	5.3±1.6 [b]	3.7±0.9 [ad]	<0.001

The goblet cell index = (number of caliciform cells/total number of cells in the villus) x100. One-way ANOVA, and least significant difference (LSD) test, P > 0.05: no significant differences, P < 0.05: significant differences, P < 0.001: highly significant differences, a significant vs control group, b highly significant vs control group, c significant vs Cd group, d highly significant vs Cd group.

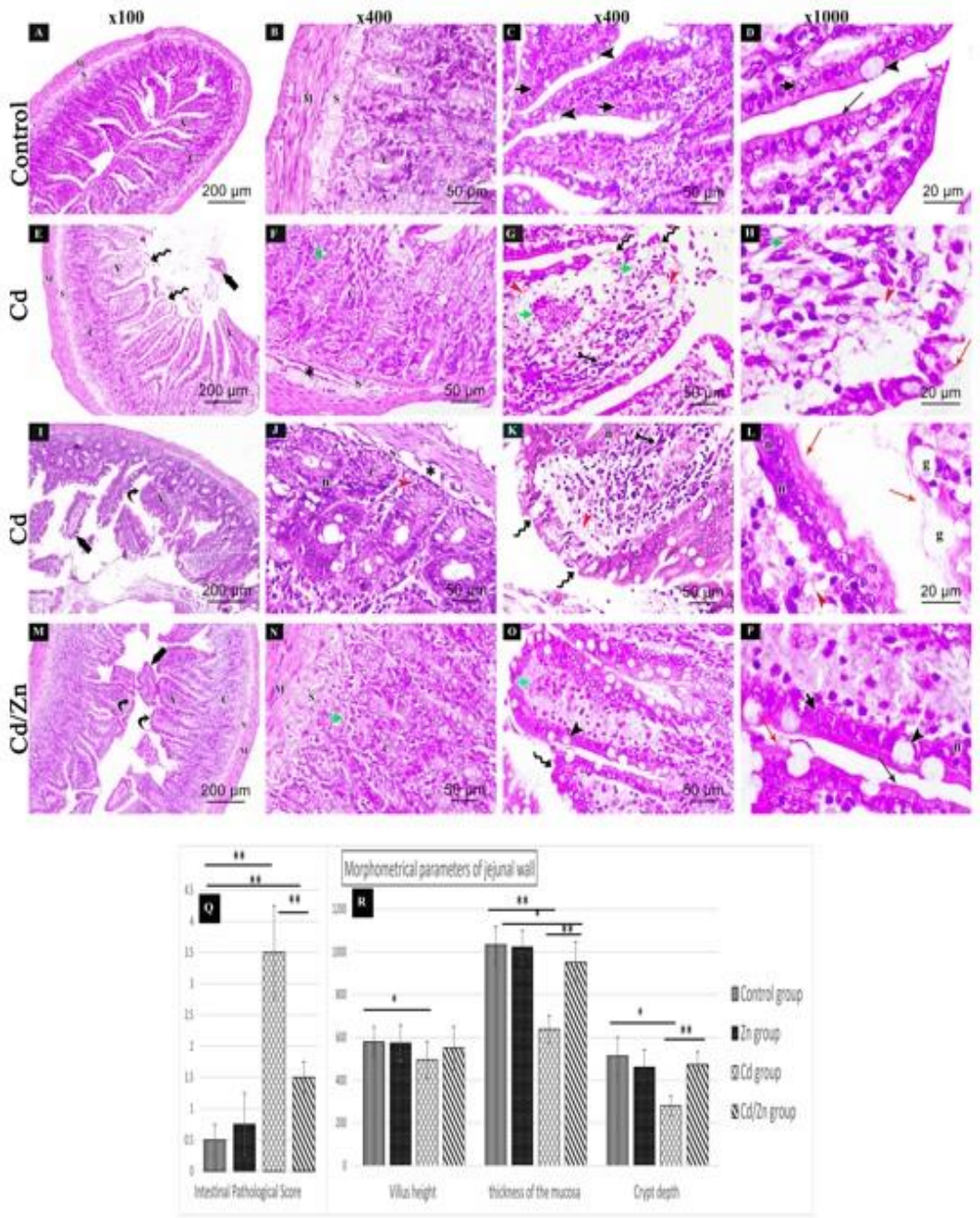

Figure 2: H&E-stained sections of Control and Zn groups (A-D) are showing long slender jejunal villi (V) lined by tightly packed columnar cells with basal oval vesicular nuclei (short arrows) and prominent

continuous brush borders (thin arrow) with goblet cells (arrow heads) in between. Intestinal crypts (C) at base of villi are well organized. Normal continuous submucosa (S) and muscularis externa (M) are observed. While in Cd group (E-H) and (I-L), jejunal villi (V) are either short blunt (curved arrow) or in average length but with desquamated tip (zigzag arrows), distorted enterocytes which have darkly stained nuclei (n) with loss of their brush borders (thin red arrows) and destructed goblet cells (g) in between. The lining epithelial cells of crypts (C) have vacuolations (red arrowhead) and pyknotic darkly stained nuclei (n). Lamina propria have wide spaces, vacuolations (red arrowhead), hemorrhage (short green arrow) and inflammatory cell infiltrations (crossed arrow). Also, there are submucosal (S) separation (*) and abundant epithelial exfoliations (thick arrow) in jejunal lumen. In Cd/Zn group (M-P), some of the jejunal l villi (V) are restoring their length but still have blunt tip (curved arrow), other are short with some desquamations (zigzag arrow). Their lining columnar epithelium has vesicular (short arrow) or darkly stained (n) nuclei, some of them gaining brush borders (thin arrow) and other still losing them (red thin arrow). Few epithelial exfoliations (thick arrow) are present in jejunal lumen. Lamina propria has small areas of hemorrhage (short green arrow). Goblet cells (arrowhead) and submucosa (S) and mucularis externa (M) are apparently normal. Moreover, (Q, R) charts show morphometrical analysis of pathological score besides villus height, thickness of mucosa and crypt depth in different study groups. * Significant difference. ** Highly significant difference.

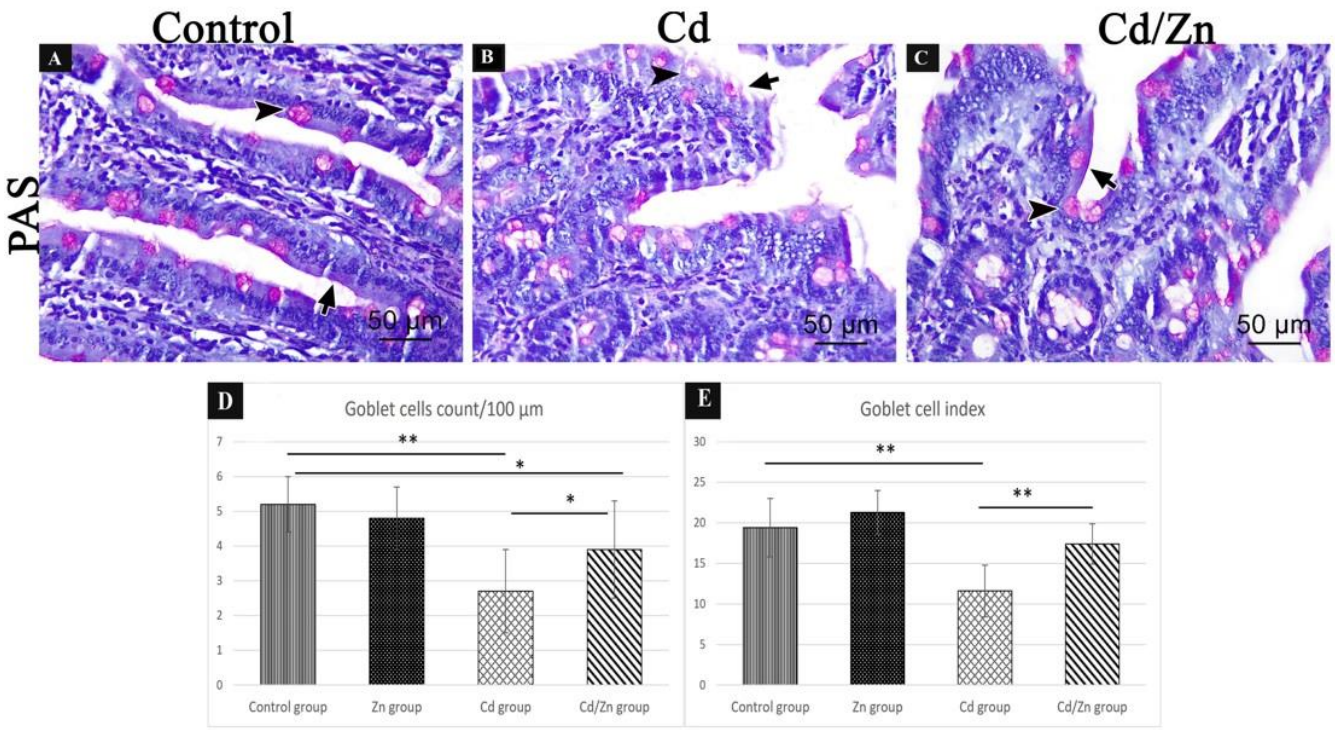

Figure 3: PAS staining of the goblet cells (arrowhead) and brush borders of enterocytes (short arrow) of jejunal villi. A: control group; B: Cd group; C: Cd/Zn group; D: Morphometrical comparing of goblet cell count/100µm. E: Morphometrical comparing of goblet cell index. * Significant difference. ** Highly significant difference.

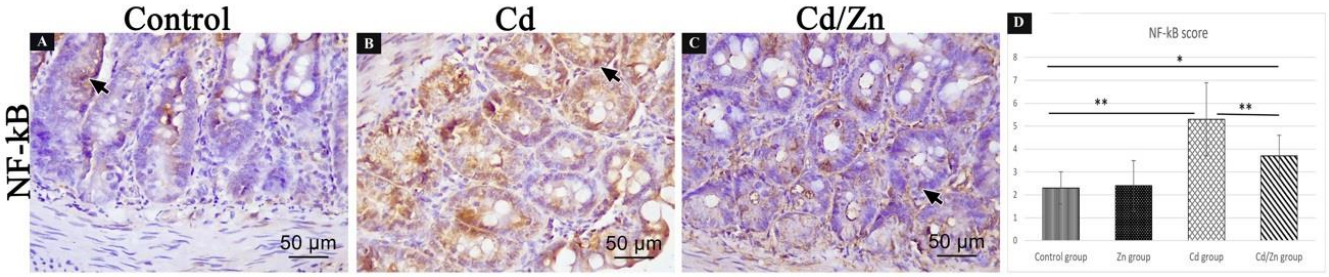

Figure 4: Immunoreactivity for NF-kB in jejunal crypts: positive stained nuclei are taking brown color (short arrow). A: control group; B: Cd group; C: Cd/Zn group; D: Morphometrical comparing of NF-kB score. * Significant difference. ** Highly significant difference.

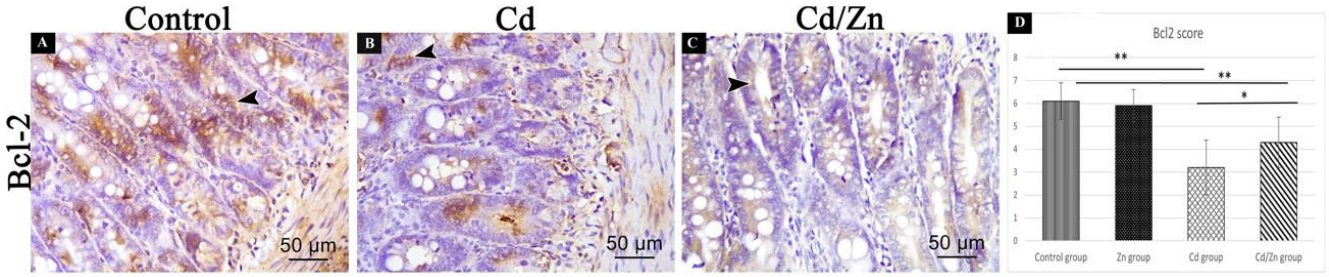

Figure 5: Immunoreactivity for Bcl-2 in jejunal crypts: positive stained cytoplasm is taking brown color (arrowhead). A: control group; B: Cd group; C: Cd/Zn group; D: Morphometrical comparing of Bcl2 score. * Significant difference. ** Highly significant difference.

DISCUSSION

Cadmium is a well-known industrial and environmental pollutant that can be found in water, air, soil, food, and cigarette smoke [1-3]. Cd exposure can result in a wide range of short- and long-term effects [22]. To rule out the impact of hormonal changes in female animals' estrous cycles, we conducted the experiment on male rats rather than females according to [23]. Changes in body weight serve as a sensitive indicator of an animal's overall health status. Consequently, by the end of our study, the harmful effect of cadmium was indicated by a significant decrease in body weight of Cd-treated rats in relation to their corresponding in control group, and this was in concordance with the results of Kim et al., [24].

Eriyamremu et al., [25] attributed decreased weight in Cd-treated rats to severe malabsorption, nutrients loss and to low probability to decrease food intake. Other studies have found that heavy metals, such as cadmium, have toxic effects on the gut microbiota, having a significant impact on both food digestion and host health [26].

Regarding Cd/Zn group, a significant improvement in the rat weight was noticed in comparison to the Cd-treated rats, and this was in the same line with Kouadria et al., [27] who explained that to the beneficial effect of Zn as an antioxidant against the oxidative stress caused by Cd. Also, these findings were consistent with Ni et al., [3], who stated that Cd-polluted diet leads to lowering of piglets' weight gain, while the animal weight gain increased during nutritional supplementation with Zn (100 or 200 mg/kg) and Cd together, reflecting a valuable effect of Zn on maintaining normal growth process.

Since the alimentary mucosa is the first tissue exposed to Cd, it triggers an inflammatory response which provides crucial defense against infections and toxins [28]. As shown in this work, the inflammatory cytokines (TNF-α and IL-1B) are significantly increased in Cd-treated group in comparison to control group. This

agreed with Ninkov et al., [4] and Lag et al., [28], who suggested that Cd possesses pro-inflammatory properties, which could elevate inflammatory factors' level. Additionally, increased IL-1b and TNF production may be related to the capacity of intestinal epithelium to produce variety of innate immunity cytokines or due to intestinal epithelial necrosis [29].

While during co-administration of Zn with Cd, there was a significant decrease in the mean levels of these cytokines, this was in concordance with Bonaventura et al., [30]. Also, Choi et al., [31] demonstrated that zinc preparations decrease various inflammatory cytokines expression, including interleukin (IL)-1beta, IL6, IL-8, and TNF-α.

In contrary, Choi et al., [31] suggested that Cd could be an anti-inflammatory factor, and also Ni et al., [3] revealed that Cd treatment lowered relative expressions of IL-10, IL-12, TNF-, and IFN- in the piglets' jejunum when compared to control. Moreover, co-treatment of Cd and Zn raised relative expressions of IL-8, IL-10, IFN-, MCP1, and TNF- in comparison to Cd treatment alone.

The inflammatory effect of cadmium was confirmed in this work by an increase in NF-kB immunoreactivity in intestinal tissue of the Cd-treated group in comparison to control group. This is in line with Imam et al., [9], who found a significant increase in adrenal NF-kB immuno-expression in cadmium receiving rats. Conversely, Breton et al., [33] revealed that 3 weeks of exposure to low dose of Cd salts (5ppm) possibly have useful effect on a chronic inflammatory condition through activation of antioxidant paths and decrease of NF-κB and pro-inflammatory cytokine levels.

Considering Zn/Cd, nuclear immune-expression of NF-κB was significantly lowered. This is supported by Choi et al., [30], who revealed that some zinc preparations inhibit the activity of NF-κB. Liu et al., [34] declared that NF-κB plays as a key moderator of inflammatory responses and provokes numerous pro-inflammatory genes expression, including those encoding chemokines and cytokines, furthermore it shares in inflammasome regulation. Liang at al., [35] added that NF-kB is also involved in inflammation, immune response, and apoptosis.

It was documented that one of the mechanisms for Cd-induced toxicity is oxidative stress. Cd not only stimulates the generation of reactive oxygen and nitrogen species, but it also inhibits the activity of antioxidant enzymes in vivo [36]. In support, we found that Cd exposure was associated with increased levels of lipid peroxidation product MDA in jejunal tissue and at the same time decreased activity of antioxidant GPx and SOD, this was consistent with Xue et al., [37].

Similar findings of an increase of MDA and a decrease of SOD were detected by Zhai et al., [38] in the mice liver in acute Cd intoxication. Many prior studies pointed out the ability of Cd to cause enhancement of lipid peroxidation, nucleic acids damage, and apoptotic processes, as well as limiting antioxidant protein action by binding them to sulfhydryl groups and disrupting calcium homeostasis [39]. Cadmium induced ROS results in activation of several signaling pathways which are involved in cell death [40]. Host tissue attempts to counteract ROS species and minimize damage by changing SOD activity [41].

The current work revealed the antioxidant role of Zn by an increase in the activity of GPx and decrease MDA level. This was also in concordance with Ni et al., [3], who displayed that Zn participates in building of antioxidative enzymes and mitigating Cd-induced oxidative impairment in the small intestine. Previous studies also reported the protective role of Zn against Cd-induced oxidative stress and toxicity in the liver and kidney tissues [42].

In this study, a marked degree of pathological damage of intestinal mucosa was detected in Cd-treated group, which were in the same line with Xue et al., [37]. Bearcroft et al., [43] described that intestinal damage occurs more specifically, in the villus apex and their distal endings. In support to our study, Cd

adversely affected intestinal epithelium in the form of loss of villus tip and desquamation of apical part of villi. Epithelial vacuolations and exfoliations, hemorrhage, and inflammatory cellular infiltrations in lamina propria with submucosal separation were also observed.

Similar intestinal histopathological findings were described by Xue et al., [37], after consumption of different doses of cadmium. Furthermore, Berzina et al., [44] described that the gut of cadmium-treated chicken was showing some fused villi with epithelial desquamation and vacuolization of villous apex. Mild to moderate inflammatory infiltration and lymphocytes mainly was detected in the lamina propria.

Artis [45] attributed changes in goblet cell and presence of inflammatory cells that infiltrated intestine is a trial from host to improve injured intestinal barrier, which is necessary for keeping immune-mediated gut homeostasis. Consequently, the values of intestinal villus height, crypt depth and mucosal thickness were considerably lowered in comparison with their corresponding values in control group which were consistent with Xue et al., [37]. Excessive secretion of different inflammatory mediators may be the cause of this epithelial damage [46].

Regarding Cd/Zn group, the intestinal epithelial tissue showed marked intestinal refinement with an improvement of all values of previous parameters to become nearly the same of the normal ones. These findings were in concordance with El-Refaiy and Eissa [15], who demonstrated that combination of Zn with Cd partially defended against Cd-induced tissue damage in the lung, liver, testis, and kidney.

This was explained by Liu and Jin [47], who revealed that Zn had a defensive effect on histological damage by retaining membrane integrity due to its direct action on free radicals. Moreover, Zinc's anti-Cd toxicity could be related to interaction between the two cations during the absorption and distribution stages. Brzoska and Moniuszko-Jakoniuk [48] added that increased Zn consumption may possibly diminish Cd absorption from the gastrointestinal tract and its accumulation in the body, potentially ameliorating the harmful effects of Cd.

Intestinal goblet cells are responsible for synthesis and secretion of mucin which acts as the first line of defense against different pathogens and plays important role in keeping mucosal homeostasis [49]. Many prior studies reported that the changes in the number of goblet cells and mucin secretion under various circumstances such as different diet, pathogens, altered microbiota or surgery [50].

In the current study, H&E and PAS-stained sections showed goblet cell degeneration and dispersion with a significant decrease in goblet cells count and index in Cd group. Conversely, Ninkov et al., [4] described that cadmium administration was related to up-regulation of the intra-epithelial goblet cells of villi compared to the control group. We also observed a significant increase in the previous parameters of goblet cells in Zn/Cd group in relation to Cd to reach control values. This agrees with Kalita et al., [51], who described an increase in goblet cells number and its secretion after zinc administration in weaned piglets.

Oxidative stress plays an important role in the induction of apoptosis by stimulating various pro-apoptotic proteins and down-regulating other anti-apoptotic proteins [52]. Bcl-2 is known to be an anti-apoptotic protein that reduces apoptosis [53]. It has a critical role in modulating the mitochondrial apoptosis pathway by impeding the cell death and inhibiting several molecules needed for caspases cleavage [54].

Our results showed that zinc supplements can alleviate apoptotic changes generated in the jejunal mucosa by cadmium exposure for 14 days, as demonstrated by the increase of Bcl-2 immuno-expression in intestinal tissues. In support, previous studies revealed that adding of Zn preparations (ZnSO4) protected the cells by the inhibition of the DNA degradation induced by Cd and prohibited apoptosis by preventing the Ca2+/Mg2+-dependant endonuclease [55], the apoptotic protease and caspase [56] or by

encouraging synthesis of DNA and activation of the anti-apoptotic proteins Bcl-2 [57].

Ni et al., [3] observed the role of Zn in reduction of intestinal apoptosis which is more obvious by increasing the amount of this additive and they attributed that to its antioxidant effect against Cd leading to stabilization of cell membrane and reduction of apoptosis.

CONCLUSIONS

In conclusion, our study found that zinc chloride treatment can reduce Cd-induced villus damage, and mucosal inflammatory reactions, as well as oxidative stress and weight loss. We also discovered that it has anti-inflammatory, anti-oxidative, and anti-apoptotic properties when it comes to Cd. We recommend decreasing cadmium contents in food and water, and to conduct more research on zinc effects.

Abbreviations:

Cd; Cadmium, ROS; reactive oxygen species, Zn; Zinc, MDA; malondialdehyde, GPx; Glutathione peroxidase activity, SOD; Superoxide dismutase activity, TNF-α; Tumor necrosis factor-alpha, IL-1β; Interleukin-1β, PAS; periodic acid-Schiff, anti-NF-kB; nuclear factor kappa.

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