Antioxidant, Antigenotoxic, and Hepatic Ameliorative Effects of Quercetin/Zinc Complex on Cadmium-Induced Hepatotoxicity and Alterations in Hepatic Tissue Structure

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Abstract: Applications of medicinal uses of metals and their complexes have been gaining major clinical significance, especially during the COVID-19 pandemic. The ligation behavior of quercetin (Q), a flavonoid, and Zn metal, i.e., the Zn/Q complex, was fully characterized based on molar conductance, infrared (IR) spectra, elemental analysis, electronic spectra, thermogravimetric analysis, proton nuclear magnetic resonance (1H-NMR), and transmission electron microscopy (TEM) in our lab. Hepatotoxicity was induced by cadmium (CdCl2). A total of 40 male albino rats were randomly distributed into the following four groups: Control, hepatotoxic group (CdCl2), Zn/Q-treated group, and group treated with a combination of CdCl2 and Zn/Q. Serum hepatic enzymes (AST, ALT, and LDH), total protein, and enzymatic and nonenzymatic antioxidant levels were determined. Histology and TEM for hepatic tissues, in addition to the gene expression of SOD as an antioxidant enzyme in the hepatic tissues, were evaluated. The Q/Zn treatment demonstrated potent protective effects against CdCl2-induced sever oxidative stress and suppressed hepatic toxicity, genotoxicity, liver enzyme disturbances, and structural alterations. In conclusion, the Zn/Q complex produced a high potent antioxidant effect against the oxidative injury and genotoxicity induced by CdCl2 and could be considered to be a potent ameliorative hepatoprotective agent against CdCl2 hepatotoxicity, which could be beneficial during the COVID-19 pandemic.

Keywords: quercetin; zinc; antioxidant agents; liver functions; oxidative stress; genotoxicity

1. Introduction

Cadmium (Cd) is a widespread heavy metal [1]. Cd has been listed among the toxic chemicals recognized by the WHO [1]. Mclellan et al. [2] demonstrated that Cd absorption by humans occurs very slowly after oral exposure, thus generating cumulative hepatotoxicity. Studies have reported that Cd mainly accumulates in hepatic tissues [3].

Several studies have demonstrated that exposure to CdCl2 results in large amounts of excessive free radical generation, thus eliciting high oxidative stress in almost all the body systems [4,5].

Cadmium has the ability to elevate both p53 and Bax gene levels and decrease the gene expression of Bcl-2, thus elevating the cellular apoptosis [6].

Excessive sources of Cd output such as coatings, alloys, cadmium batteries with nickel, chemical stabilizers, plastics, and ceramics are causing Cd pollution to rise year after year [3,4].
Cd is also found in cigarette smoke, which contributes to smokers’ addiction. The biological half-life of Cd, which is long, is known to be the most harmful impact because of the prolonged accumulation of Cd in biological organs over long periods of time [7]. Consequently, The International Agencies for Cancer Research listed Cd as a carcinogenic compound because of its cumulative impact and involvement in the progression of cancer [8,9].

A previous study demonstrated that Cd could cause severe hepatic damage, including the increase of free radicals, thus triggering oxidative stress, hepatic hemorrhage, edema, necrosis, and hepatic toxicity in addition to a decline hepatic cellular viability [10].

The proposed cellular mechanism that is primarily responsible for Cd-induced organ toxicity occurs through a variety of pathways, including cellular degeneration, inhibition of apoptotic mechanisms, and the blocking of DNA-repair mechanisms [11]. Additionally, the major molecular cytotoxicity induced by Cd is due to the increased of oxidative stress and the production of reactive oxygen species (ROS).

Quercetin (Q) is a flavonoid that has recently gained prominence as a potent antioxidant compound. It is mainly found in vegetables [12]. Studies have shown that Q is the most potent antioxidant agent due to its high ability to reduce free radicals [13,14]. Therefore, Q is considered to be an effective antioxidant supplement.

Previous studies have shown that Q significantly diminished several types of toxicity in rats exposed to CdCl₂ by reducing the rate of oxidative injury [15,16]. Although there have been several reports about the amelioration effects of Q on different types of toxicity in male rats, it remains intriguing, especially in the case of complexation of this potent antioxidant (Q) with metals such as (Zn), which may increase its effectiveness and minimize any induced toxicity as reported in our previous study [17].

Due to its therapeutic relevance and reported antioxidant activities, complexation of flavonoids with metals has gained attention [17]. Based on the expression of Zn gene “ZnT8,” there was a correlation between changes in Zn levels and metabolic dysfunction [18]. In addition, Zn was reported to be a beneficial ion in the treatment of inflammatory diseases [19]. A previous study found that supplementation with Zn prevented a variety of diseases. Lowering Zn ion intake also decreased tolerance to a variety of infections [20].

In the current study, we investigated the impact of Cd exposure on the liver, as well as the potential ameliorative effect of a novel Zn/Q complex (a strong antioxidant) on Cd-induced hepatic toxicity.

2. Materials and Methods

2.1. Chemicals and Analyses

The chemicals used in this study (Q and ZnCl₂) were purchased from Sigma-Aldrich (Steinheim, Germany). The Q complex with zinc (II) was synthesized according to the following methodology [17].

2.2. Synthesis of Zn/Q Complex

According to our previously published study and as demonstrated by Refat et al. [17], we added 30 mL of methanol to 1 mmol Q dissolved in 1 mmol of Zn(NO₃)₂·6H₂O. The yield product was continuously stirred for about 4 h at 25 °C. A greenish yellow product resulted after continuous stirring, which was filtered and evaporated at 25 °C and then washed with MeOH. Chemical characterization has been confirmed by Refat et al. [17]. We confirmed the stability and chemical formula of our novel Zn/Q complex by IR, UV, TEM, and H¹NMR.

2.3. Experimental Animals

In total, 40 three-month-old adult male Wistar albino rats with weights ranging from 180 g to 200 g were obtained from faculty of the pharmacy at Zagazig University (Zagazig, Egypt). Rats were kept in metal cages under pathogenic-free conditions. Extensive efforts
were made to minimize suffering of the animals. The experimental protocol, as shown in Figure 1, was ethically approved (Zu-IACUC/1/f/41 I2018) and followed the animal international guidelines.

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2.4. Experimental Protocol

After 2 weeks of acclimatization, the male albino rats were randomly divided into 4 groups. Group I (control group) was orally administrated physiological saline, group II (CdCl₂ group) was orally treated with CdCl₂ (5 mg/kg⁻¹) [21], group III was orally treated with the novel Zn/Q complex (30 mg/kg) [14], and group V was orally treated with CdCl₂, followed with Zn/Q complex at the same previous doses.

2.5. Blood Sample Collection

The rats were sacrificed under light doses of ketamine/xylazine anesthesia, and efforts were made to reduce stress and pain to the rats. Blood samples were collected from the eye plexus under light anesthesia. The samples of blood were continuously for 20 min centrifuged at 5000 rpm for the biochemical analyses. The male rats were decapitated immediately ethically, and the hepatic tissues were preserved at −80 °C for the molecular examination.

2.6. Preparation of Homogenate of the Hepatic Tissues

Small pieces of the hepatic tissues were used for assessment of the antioxidant enzymes and estimating of the marker of lipid peroxidation (MDA). Hepatic tissues were continuously homogenized in buffer saline in cold medium (approximately 5 mL) and centrifuged at approximately 6000 r.p.m for 1/2 h. The supernatant was used for analyses of the antioxidant enzymes.
2.7. Determination of the Oxidative Stress Biomarkers

The hepatic tissue homogenates were used for estimation of GPx according to Sedlak and Lindsay (1968) Approximately 100 µL of each hepatic tissue homogenate was mixed with 1.5 mL of 20% glacial acetic acid, 1.5 mL of 0.8% thiobarbituric acid, 200 µL of 8.1% SDS, and 600 µL of dist. H₂O. The mixture was heated at 95 °C for 1 h in a water bath. The absorbance was measured at 532 nm using a UV-spectrophotometer (PerkinElmer, Waltham, MA, USA). [22]. MDA content was determined using thiobarbituric acid of ≥99% purity (Sigma Chemical Co., St. Louis, MO, USA) as described previously according to Ohkawa et al. (1979) [23]. CAT levels were estimated according to Beers and Sizer (1952) [24] by assaying the hydrolysis of H₂O₂ and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. Before determining the CAT activity, samples were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein. SOD activity was determined according to previous methodology by assaying the autoxidation of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused the inhibition of 50% pyrogallol autoxidation. SOD activity is expressed as U/mg protein. Three replicates were measured per sample and the final concentrations are presented/g tissue.

2.8. Transmission Electron Microscopic Study (TEM)

Liver portion was fixed in 2.5% glutaraldehyde for the ultrastructural examination. The liver specimens were washed in slightly basic buffer phosphate for about 20 min, followed by further processing (Hayat, 1986) [25]. Ultrathin sections were mounted on Cu- grids and examined by transmission electron microscope (JEOL JEM-1200 EX II, Tokyo, Japan).

2.9. Comet Assay of the Hepatic Tissues

Hepatic tissues were placed in a Petri dish with lysing solution, and the cellular viability was determined as described by Singh et al. [26]. For each sample, 3 slides were prepared, and random cells were selected for imaging using the fluorescence microscope.

2.10. Histological Analysis of Liver Tissues

Hepatic tissues were kept in buffered formalin (10%). After the fixation step, the hepatic tissues were embedded in paraffin and then stained for further processing.

2.11. RNA Isolation and Semiquantitative Polymerase Chain Reaction (PCR)

Total RNA was isolated from the hepatic tissues using TRIzol reagent, followed by the addition of RNase-free DNase. Amplification data were analyzed using the 2^-ΔΔCt method. The isolated RNA was dissolved in diethyl pyrocarbonate-treated water and then stored at −80 °C until use. The observed values were comparable to those of β-actin. PCR of the liver β-actin, SOD, CAT and GPx were performed by using specific primers of rat’s SOD and -actin genes purchased from "Macrogen" (Beotkkot-ro, Gasan-dong, Geumcheon-gu, Seoul) (Korea). The sequences of the primers are listed in Table 1.

Table 1. PCR primers and methods for the tested genes.

| Gene Name | Primer Sequence (5′–3′) | Product Size | Accession Number |
|-----------|--------------------------|--------------|-----------------|
| SOD       | Forward—GGAGAGGCCGCTCTGTCG
Reverse—TGTGTATGAGTTGCTGCCCTTGC | 631 bp | BC066063.1 |
| β-actin   | Forward—TATACACGCGCCGACA
Reverse—ATGCTACGTACATGGCTGG  | 516 bp | NM_007393 |
| GPx       | Forward—CGGTTCCTCGCACTACTG
Reverse—ACACCGGGACCCAAAATGAGTC | 670 bp | NM_017006 |
| CAT       | Forward—GCAATGCGAACTTACCCGTCG
Reverse—AGAATGCTGACACTAGTGACCTG  | 532 bp | NM_012520 |
The PCR conditions were as follows: 95 °C for about 5 min, followed by cycles of 95 °C for 1 min, an annealing temperature step at 60 °C for 1 min, and 72 °C for 1 min [20].

2.12. Statistical Analysis

The statistical analysis was performed using SPSS software version 27 (IBM, 2020) and Open Epi version 2.3.1 [27]. Data were summarized as mean and standard deviation. The Shapiro–Wilk test was used to determine the distribution characteristics of variables and variance homogeneity [28].

3. Results

3.1. Characterization and Interpretations of (Zn/Q)

Analytical analysis was performed to confirm Zn/Q novel complex structure of (Figure 2). The Zn/Q complex was soluble in DMSO. The value of the molar conductivity (\(\Lambda_m\)) novel Zn/Q complex was “15 ohm\(^{-1}\).cm\(^2\).mol\(^{-1}\),” and the Zn/Q was non-electrolytic in nature [17].

![Chemical structure of the Zn/Q complex](image)

**Figure 2.** Chemical structure of the Zn/Q complex [17].

The complexity of the prepared Zn/Q was confirmed by its C, H, and N analysis as follows: MF: C\(_{15}\)H\(_{23}\)NO\(_{17}\)Zn; molecular weight: 554.73 g/mol; experimental analysis (%): Zn 11.37, C 32.13, N 2.45, and H 4.09. The final product of the Zn/Q complex was approximately 75%. The melting point of the Zn/Q complex was higher than 250 °C.

3.2. TEM Examination of Zn/Q Complex

Zn/Q complex, transmission electron microscopy (TEM) was clearly demonstrated in our previous study [17]. The uniform matrix was clear and confirmed that the novel Zn/Q complex had black and spherical spots.

3.3. Hepatic Effect of (Zn/Q) Complex

The male rats treated with CdCl\(_2\) had significantly higher serum AST, ALT, and LDH levels than the control group. In addition, when CdCl\(_2\) was combined with the novel Zn/Q complex, serum ALT and AST enzymes were significantly higher than in the control group. In contrast to the CdCl\(_2\)-treated population, CdCl\(_2\) combined with Zn/Q caused substantial reductions in ALT and AST levels (Table 2).

On the one hand, compared with the control group, the results for the CdCl\(_2\)-treated group showed a significant reduction in total protein levels. On the other hand, the result for the group treated with CdCl\(_2\) and Zn/Q showed a small reduction in total protein levels as compared with the control group (Table 2).

Compared with the control group, the results for the group treated with CdCl\(_2\) only showed a significant increase in LDH serum levels. Whereas the CdCl\(_2\) and Zn/Q group showed a substantial increase (\(p \leq 0.05\)) in LDH levels as compared with the control group, LDH levels in the CdCl\(_2\) and Zn/Q group were significantly lower than in the CdCl\(_2\)-treated group (Table 2).
Table 2. Hepatic enzymes of treated groups with CdCl₂, Q/Zn, and their combinations.

| Groups               | ALT (U/mL)       | AST (U/mL)       | LDH (U/L)       | Total Protein (g/dL) |
|----------------------|------------------|------------------|-----------------|----------------------|
| 1—Control group      | 12.42 ± 1.08     | 14.16 ± 1.75     | 130.28 ± 4.58   | 8.16 ± 1.82          |
| 2—CdCl₂ group        | 162.06 ± 4.65 ab | 182.28 ± 4.05 ab | 897.19 ± 5.25 ab| 4.19 ± 0.25 ab       |
| 3—Zn/Q group         | 12.52 ± 0.49     | 13.49 ± 1.15     | 127.39 ± 3.02   | 8.75 ± 1.55          |
| 4—CdCl₂ + Zn/Q       | 25.25 ± 2.65 a   | 32.45 ± 4.02 a   | 147.16 ± 4.58 a | 7.48 ± 1.69          |
| LSD                  | 1.9              | 1.4              | 4.3             | 1.7                  |

\(a\) Statistically significant different compared to control group. \(b\) Statistically significant different compared to other treatment groups. LSD: Least significant difference.

3.4. Enzymatic and Nonenzymatic Antioxidant Biomarkers

The antioxidant enzymes in the hepatic tissues of the groups treated with CdCl₂, Zn/Q, and their combinations were measured (Table 3). The MDA levels of the CdCl₂-treated group markedly increased with a significant reduction in the antioxidant enzymes (SOD, CAT, and GPx). Treatment of the male rats with CdCl₂ in combination with Zn/Q reduced the MDA levels significantly and elevated the antioxidant capacities more than that of CdCl₂ alone (Table 3).

Table 3. Hepatic enzymes of treated groups with CdCl₂, Q/Zn, and their combinations.

| Groups               | SOD (U/g Tissue) | CAT (U/g Tissue) | GPx (mg/g Tissue) | MDA (nmol/g Tissue) |
|----------------------|------------------|------------------|-------------------|---------------------|
| 1—Control group      | 54.36 ± 3.52     | 29.36 ± 2.03     | 15.36 ± 1.66      | 8.36 ± 1.02         |
| 2—CdCl₂ group        | 10.69 ± 1.25 ab  | 8.36 ± 1.36 ab   | 2.36 ± 0.25 ab    | 140.36 ± 4.69 ab    |
| 3—Zn/Q group         | 60.9 ± 3.65 a    | 30.25 ± 1.69     | 16.58 ± 1.25      | 6.25 ± 2.36 a       |
| 4—CdCl₂ + Zn/Q       | 44.69 ± 2.69 a   | 24.03 ± 1.69 a   | 12.05 ± 1.69 a    | 30.36 ± 1.02 a      |
| LSD                  | 3.3              | 1.7              | 1.6              | 1.7                 |

\(a\) Statistically significant different compared to control group. \(b\) Statistically significant different compared to other treatment groups. LSD: least significant difference.

3.5. Histological Examination

Histological examination (Figure 3) showed that Control group showing normal hepatic structure and normal nuclei. Meanwhile, D-galactose-treated group showing experimental rat liver after treatment with CdCl₂ with marked toxicity in the form of dilatation, elongation, irregularity of the portal vein, hypertrophy of hepatocytes with the appearance of hydropic degeneration of hepatocytes with vesicular nuclei with new bile duct formation, appearance of mummified cells, and infiltration by inflammatory cells. Zn/Q-treated group showing normal hepatic structure with normal nuclei. CdCl₂ and Zn/Q-treated group showing great improvement and restoration of hepatic structures with mild changes in the form of hypertrophy of hepatocytes with granular eosinophilic cytoplasm and vesicular nuclei with a slightly dilated central vein lined by endothelial cells. The histological index for scoring the hepatic structural alterations is shown in Table 4.

Table 4. Histopathological index in the hepatic tissues of rats treated with either CdCl₂ and/or Q/Zn.

| Findings               | Control Group | CdCl₂ Group | Zn/Q Group | CdCl₂+Zn/Q |
|------------------------|---------------|-------------|------------|------------|
| Normal hepatic tissues | ++++          | - - - -     | +++        | +++        |
| Detached hepatic tissues | - - - -     | +++         | - - - -    | - - - -    |
| Normal sized nuclei    | +++           | - - - -     | +++        | +++        |
| Inflammatory cells     | - - - -       | +++         | - - - -    | - - - -    |

- - - - Absence of the change in the animals of the studied group. ++++ A change was observed in 90% of the group. +++ A change was observed in 80% the group. + + A change was observed in 50% of the group. - - A change was observed in 25% of the group.
3.5. Histological Examination

Histological examination (Figure 3) showed that Control group showing normal hepatic structure and normal nuclei (H&EX400). (B) D-galactose-treated group showing experimental rat liver after treatment with CdCl₂ with marked toxicity in the form of dilatation, elongation, irregularity of the portal vein (►), hypertrophy of hepatocytes with the appearance of binucleated hepatocytes and hydropic degeneration of hepatocytes with vesicular nuclei (►), Ductular reaction with new bile duct formation (►), appearance of mummified cells (►), and infiltration by inflammatory cells (►) (H&EX400). Figure (C): Zn/Q-treated group showing normal hepatic structure with normal nuclei (H&EX400). Figure (D): CdCl₂ and Zn/Q-treated group showing great improvement and restoration of hepatic structures with mild changes in the form of hypertrophy of hepatocytes with granular eosinophilic cytoplasm and vesicular nuclei and appearance of some binucleated cells (►), with a slightly dilated central vein lined by endothelial cells and dilated congested blood sinusoids (►), some of which have been infiltrated by mononuclear inflammatory cells (►) (H&EX400). The histological index for scoring the hepatic structural alterations is shown in Table 4.

3.6. TEM Examination of the Hepatic Tissues of the Treated Groups

The hepatic tissues showed normal structure of the nuclei (Ns) with normal-sized mitochondria (M) (scale bar = 5 μm) (Figure 4A), while the CdCl₂-treated group showed severe toxicity in hepatic tissues with the degeneration of most hepatic structures and nuclear components (►►) and the appearance of a fatty change (green arrow) (scale bar = 5 μm) (Figure 4B). There was a significant improvement and normal hepatic structures in the group treated with Zn/Q (scale bar = 5 μm) (Figure 4C). However, in the group treated with CdCl₂ and Zn/Q, there were hepatic tissues with a near restoration of hepatic structures (scale bar = 5 μm) (Figure 4D).
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3.6. TEM Examination of the Hepatic Tissues of the Treated Groups

In the group treated with Zn/Q, there were hepatic tissues with a near restoration of hepatic structures (scale bar = 5 µm) (Figure 4C). However, in the group treated with CdCl2 and Zn/Q, there were hepatic tissues with marked toxicity in the form of dilatation, elongation, irregularity of the portal vein, hypertrophy of hepatocytes with granular eosinophilic cytoplasm and vesicular nuclei with a slightly dilated central vein lined by endothelial cells. The histological index for scoring the hepatic structural alterations is shown in Table 4.

3.7. Comet Assay of the Hepatic Tissues

A comet assay of the hepatic tissues was assessed. On the one hand, the control group showed intact round nuclei without large tail hallows (Figure 5A). On the other hand, the CdCl2 group showed a high degree of damage, with large apoptotic cells and tail hallows (Figure 5B). The Zn/Q group showed intact nuclei (Figure 5C). The group treated with CdCl2 and Zn/Q showed less DNA damage (Figure 5D).

3.8. Gene Expression in the Liver

A semiquantitative PCR technique was performed to assess the inhibitory effect of Zn/Q on the oxidative injury in hepatic tissues. The relative expression of mRNA of the SOD enzyme was lower in the CdCl2-treated group than that in the Zn/Q and control groups (Figure 6). In the CdCl2 group treated with Zn/Q, the relative SOD mRNA levels were significantly elevated as compared with the CdCl2 group (Figure 6).

Figure 4. Photomicrographs of hepatic tissues (A) Normal hepatic tissues and normal nucleus (N) and normal mitochondria (M) (Scale bar = 5 µm). (B) CdCl2 group showing large detached hepatic tissues (¶) with the degeneration of nuclear contents (**) and reduced mitochondrial size (scale bar = 5 µm). (C) Zn/Q group showing normal appearance of the hepatic structures, normal round echo-chromatic nucleus (N), and normal sized mitochondria (M) (scale bar = 5 µm). (D) CdCl2+Zn/Q group showing normal hepatic structures with the appearance of normal nucleus (N) and mitochondria (M) (scale bar = 5 µm).
Figure 5. (A) Control group showed normal nuclei. (B) CdCl₂-treated group showed greater DNA damage. (C) Zn/Q group showed normal intact DNA. (D) CdCl₂ and Zn/Q group showed less DNA damage with almost restoration of normal cellular boundaries.

Figure 6. The genes expression of mSOD and mβ-actin in hepatic tissues. 1: Control group; 2: CdCl₂ group only; 3: Zn/Q group; and 4: CdCl₂+Zn/Q group.

4. Discussion

The hepatotoxic effects of CdCl₂ have been previously demonstrated [29]. We confirmed that CdCl₂ exposure significantly elevated hepatic enzymes (AST and ALT) and hepatic damage markers (LDH). CdCl₂ exposure also produced excessive free radicals that triggered oxidative injury in the hepatic tissues and induced genotoxicity. As observed in our previous investigation, the Zn/Q complex has potent antioxidant agents that significantly alleviate oxidative stress and free radical scavenging activities, ameliorate blood glucose levels, and enhance the immune system in diabetics [17]. Therefore, we aimed to use this novel Zn/Q complex against hepatotoxicity and oxidative injury induced by CdCl₂ in treated rats.

Previous reports have shown that CdCl₂ exposure causes hepatic and renal toxicity, thus affecting the biochemical functions and cellular vitality of the body [29]. Cd adminis-
tation induced adverse effects on hepatic functions and significantly elevated the hepatic enzymes AST, ALP, ALT, and LDH with a reduction in total protein levels.

The results of the current study are consistent with those found by the authors of [10], who demonstrated a reduction in total protein levels in mice treated with CdCl_{2}. Similar hypoproteinemia was recorded in the CdCl_{2} group, which may have been due to protein synthetic machinery impairment or to excessive excretion upon Cd oral administration [30]. The reduction in total protein levels may have been due to inflammation of the hepatic tissues resulting from CdCl_{2} exposure, which disturbs and reduces protein biosynthesis [30].

In accordance with the present results, Gaskill et al. [31] demonstrated that hepatic injury was produced after CdCl_{2} administration. In addition, they reported that continuous administration of CdCl_{2} and Q reduced the accumulation of free radicals in the serum, testis, and epididymis. These results confirm our finding of significant recovery after administration of the Zn/Q complex, which restored the hepatic tissues to normal states after severe toxicity induced by CdCl_{2}.

Previous studies have demonstrated that Q improved the renal functions in CdCl_{2}-exposed rats by lowering the rate of oxidative stress in the kidneys. Similar results were found in the current study, which showed more improvement in the hepatic tissues after administration of Zn/Q to treat Cd-induced toxicity in male rats [32,33].

Our data are similar to those by Gaskill et al. [31], who demonstrated the induction of severe hepatic damages represented by a marked increment in hepatic enzymes (AST, ALT, and ALP) in a CdCl_{2} group as compared with a control group. The present results are also in agreement with those found by Newairy et al. [34], who demonstrated that CdCl_{2} elevated the oxidative damage markers. This oxidative stress might be the first sign of CdCl_{2} inducing severe hepatotoxicity. Supporting these results, Renugadevi and Prabu [35] demonstrated CdCl_{2}-induced hepatotoxicity and oxidative damage in the hepatic tissues. Hepatic injury caused by CdCl_{2} treatment was clearly shown by elevated levels of hepatic enzymes, concurrently with increased lipid peroxidation markers. Lakshmi et al. [36] also reinforced the current results by revealing that CdCl_{2} exposure significantly enhanced the elevation of the hepatic enzyme levels and reduced total protein levels.

The present results are also in agreement with those found by Abu-AlZahab et al. [10], who demonstrated that CdCl_{2} induced reduction in antioxidant enzymes (SOD, CAT, and GPx), either in the tissue homogenates or gene expression of these enzymes. These results confirmed that heavy metals have adverse effects through the excessive production of free radicals and accumulation of reactive oxygen species such as H_{2}O_{2}, which cause cumulative and excessive oxidative injuries to lipid, nucleic acids, and proteins and eventually cellular death.

An important finding of the current study was the significant elevation of SOD and CAT mRNA expression in Zn/Q complex group, either alone or following treatment with CdCl_{2}, compared to that in CdCl_{2}-treated group. So, we hypothesized that the Q/Zn complex both improves CdCl_{2}-induced hepatotoxicity and oxidative stress complications, possibly by upregulating the expression of SOD, GPx, and CAT mRNAs. This could be due to antioxidant properties of the complex Q/Zn, because studies have suggested an association between chronic oxidative stress state and impaired cellular defense [17].

The toxic effect of CdCl_{2} on hepatic activity may work through the genotoxic pathway. Additionally, heavy metals such as Cd may infiltrate the blood barrier of the hepatic tissues to disturb the metabolic pathway and thus influence the genetic integrity [37,38].

As part of the toxic hepatic effects of CdCl_{2}, we evaluated the enzymatic antioxidants activities of SOD, CAT, and GPx and the final marker of lipid peroxidation, i.e., MDA. SOD catalyzes the dismutation of O_{2} to yield H_{2}O_{2} [39]. H_{2}O_{2} is converted to H_{2}O by the CAT or GPx enzyme without incidence of this mechanism, and H_{2}O_{2} accumulates and begins to destroy the cellular membranes, then releases high levels of MDA.

The observed reduction in antioxidant enzyme activities such as SOD, CAT, and GPx is consistent with previous studies [40–42]. The decreased level of antioxidant enzymes in the hepatic tissues after CdCl_{2} administration triggered a high concentration of hydrogen
peroxide and increments in lipid peroxidation. The increased production of reactive oxygen species in hepatic tissues following CdCl\textsubscript{2} administration may contribute to a reduction in antioxidant enzyme levels, which are effectively involved in the elimination of free radicals. It has been suggested that hepatic oxidative stress results from CdCl\textsubscript{2} exposure and that it may be responsible for alterations of hepatic structures and enzymes.

Oxidative stress correlates with organ damage. In one study, it was suggested that the high percentage of apoptosis observed in hepatic tissues may have been triggered by oxidative stress. The antioxidant enzyme (SOD) was downregulated in the group treated with CdCl\textsubscript{2} as compared with the control group [10].

Previous studies have demonstrated the upregulation of Bax in cadmium-exposed rats [43] and an increment in DNA oxidation [44]. The observed results confirmed that Cd increased the rate of cellular apoptosis and hence decreased antioxidant agents that prevented the incidence of lipid peroxidation, thus stabilizing the cellular membranes. The Zn/Q complex acted effectively to scavenge reactive oxygen species, thus preventing cellular damage. In the current study, CdCl\textsubscript{2} significantly reduced the activities of SOD, CAT, and GPx in the liver.

Refat et al. demonstrated that Zn/Q could protect the pancreas from oxygen free radicals and, consequently, support the pancreas in repairing damaged single-strand breaks induced in DNA “genotoxicity” [45]. Moreover, as observed in a histological study, rats that received Zn/Q for 1 h after CdCl\textsubscript{2} showed protected liver tissue and significantly decreased hepatic toxicity.

Zn/Q hinders the damaging effects of toxic compounds, drugs, and chemotherapeutic medications that induce free radical formation and oxidative injury to mitochondria [46]. Zn/Q can also modulate DNA damage, cellular proliferation induced by oxidative stress, and DNA single-strand breaks [47]. The protective effect of Zn/Q, owing to its ability to act as a free radical scavenger and eliminate the ROS triggered from Cd [48], prevents the induction of DNA damage.

Previous studies have shown that Q is considered as a potent antioxidant agent, owing to its ability to scavenge free radicals [12,49]. The antioxidant properties of Q may be due to its role as an antioxidant supplement. Previous studies have shown that Q significantly reversed hepatic toxicity in male rats exposed to Cd by lowering oxidative stress [12,49].

A key strength of the present study was the complexation between two potent antioxidants (Q and Zn), which act as enhancers for antioxidant enzymes and alleviate severe oxidative injury as previously confirmed by Refat et al. [17].

Zn deficiency is reported to disturb metabolic mechanisms. Thus, it may affect then cellular metabolism in hepatic tissues [50]. As a result, Zn/Q is a novel complex in alleviating the oxidative stress in the hepatic tissues, thus enhancing the metabolic state and the biochemical parameters and elevating the levels of antioxidant enzymes in the liver.

Olechnowicz et al. [51] demonstrated that Zn as a metal plays a major role in the development of metabolic syndromes and essentially involved in the expression of cytokines. Thus, Zn could share in suppressing inflammation. Zn is important to reinforce the antioxidant enzymes responsible for scavenging the reactive oxygen species by reducing oxidative stress.

Zn has important key roles in metabolic processes. Understanding the properties of Zn may help in the treatment of metabolic syndrome and thus may provide protection against any strokes. We support the very important and vital effect of the novel Zn/Q novel complex for protecting against oxidative injury and hepatic toxicity induced by CdCl\textsubscript{2}.

5. Conclusions

The current study revealed that hepatic tissues were significantly affected by the toxicity induced by CdCl\textsubscript{2} administration and clarified the potential antioxidant effect of a novel antioxidant agent, i.e., the Zn/Q complex, which elevated antioxidant enzymes, inhibited excessive free radicals, and reduced cellular inflammation as compared with CdCl\textsubscript{2} alone. The Zn/Q complex was confirmed to be highly efficient and safe for the
protection of hepatic tissues against the oxidative damage and genotoxicity induced by CdCl₂. The current results offer a new approach to clarify the role of new therapeutic strategies for preventing a series of oxidative stress complications, thereby providing new hope for alleviating severe oxidative stress.

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