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Original research article

Effects of quercetin and vitamin C on pathohistology changes of cadmium-induced organ damage in rat

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ABSTRACT

The study aimed to evaluate the effects of quercetin and vitamin C on cadmium (Cd)-induced toxicity in rats using a sub-chronic, orally exposed model. The products of lipid peroxidation, malondialdehyde, were monitored as biomarkers of oxidative stress. In addition, tissue Cd and histopathological changes in the kidney, liver and testis were evaluated. In Cd-treated rats, accumulation of Cd in the tissues was increased in a dose-dependent manner, in which co-administration with either quercetin or vitamin C had no effect. The higher dose of Cd treatment resulted in a significant increase in MDA levels in the kidney, suggesting an organ under a greater degree of oxidative stress. In the presence of quercetin or vitamin C, the kidney MDA levels were attenuated, particularly in the rats receiving 100 *ppm* Cd. In the liver, the MDA levels were not markedly altered among Cd-treated groups; nonetheless, treatment with vitamin C resulted in a noted reduction in MDA levels. Interestingly, administration of either quercetin or vitamin C appeared to increase the oxidative stress biomarkers in the testis. Histopathological examinations also revealed damages in the kidney, liver and testis upon Cd exposure. Supplemented with quercetin slightly reduced the liver damage; conversely, co-administration with vitamin C led to tissue damage. In conclusion, it was suggested that Cd induced a marked increase in oxidative stress and pathological changes in critical organs, particularly the kidney and testis. Quercetin showed beneficial activities in the liver regarding reducing the oxidative stress marker and tissue damage.

Keywords: cadmium, quercetin, vitamin C, oxidative damage

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1. Introduction

The International Register of Potentially Toxic Chemicals of the United Nations Environment Program and the WHO identified the heavy metal cadmium (Cd) as a potentially toxic substance at the global level. Cd occurs widely in nature at low concentrations as contaminants in the sulfide of copper, lead, and zinc. Additional sources of Cd arise from human activities, particularly mining residues from zinc and lead mines as a by-product from the manufacture of nickel-cadmium batteries, pigments in plastic and glasses, stabilizers in PVC plastics, fertilizers, electroplating as well as from the combustion of fossil fuels.[1,2] Cd is present in measurable amounts in almost everything we eat, drink and breath. Exposure to Cd by the oral route, *i.e., via* contaminated food, is a significant health risk among the general, non-smoking population.[2]

Evidence from both human and animal studies have revealed that Cd is toxic to primary targets such as kidneys, liver, cardiovascular, and skeletal system.[2-5] Cd-induced harmful effects on the reproductive system have also been described in males and females.[6,7] In the male reproductive system, some lines of evidence have indicated that oxidative stress and apoptotic pathway could be mainly involved in Cd-induced testicular damage.[8-10]

Dose, route and duration of Cd exposure could differentially affect the characteristics of toxic responses observed in animal models.[7] Rats supplemented with antioxidants, such as ascorbic acid (vitamin C), vitamin E, and zinc, have been shown to protect against Cd-induced testicular damages.[9-11] Other antioxidants, such as α- lipoic acid,[12] N- acetylcysteine,[13] selenium[14] and quercetin[15-17] have also been demonstrated to ameliorate the toxic effects of Cd in several in vitro studies and in vivo models.

Several flavonoids have been shown to possess strong radical scavenging properties.[18,19] Among them, quercetin, found in many plants and widely distributed in edible fruits and vegetables, is known as a metal chelator and antioxidant.[20] Meanwhile, vitamin C is a water-soluble antioxidant that reduces sulfhydryl, scavenges free radicals and protects against endogenous oxidative DNA damage.[21] It has been demonstrated that vitamin C can prevent elevated lipid peroxidation resulting from cadmium toxicity.[22] Vitamin C has a protective effect against growth rate depression and helps reduce Cd absorption from the gastrointestinal tract.[11] All of these study models, however, do not mimic the water contamination of Cd in the environments. Moreover, the effects of oral supplements of quercetin and vitamin C in sub-chronic Cd toxicity have not been evaluated. This study, therefore, aimed to assess the effects and histopatho-logical changes of quercetin and vitamin C on Cd-induced toxicity. A rat model was employed to evaluate the hypothesis that sub-chronic, orally exposed Cd could mediate renal, hepatic and testicular damages, through producing oxidative stress. In addition, the antioxidative effects of quercetin and vitamin C on reducing the toxic effects of Cd was also investigated.

2. Materials and Methods

2.1 Animals and Experimental design

Seventy-two male rats of Wistar strain (2-month-old with initial body weight about 200 gm.) from the National Laboratory Centre were delivered to and housed at the research facility at Faculty of Veterinary Science, Mahidol University. All animals were housed two per cage in a room maintained under conventional conditions at a temperature of 22 ± 1°C, with a relative humidity of 50 ± 10% and a 12-h/12-h light/dark cycle. They had *ad libitum* access to rat chow and drinking water. Cages and feeders were sanitized on a weekly basis.

After an acclimation period of 4 days, the rats were randomized allotted to 9 experimental groups (6 rats/group) as follows: Control group: rats did not receive Cd with

or without 50 mg/kg quercetin or 50 mg/kg vitamin C intragastrically 5 times/week for 8 weeks; Cd-treated group: rats received $CdCl_2$ 10 and 100 *ppm* in drinking water for 8 weeks; Cd-treated group with quercetin; rats received $CdCl_2$ 10 and 100 *ppm* in drinking water with 50 mg/kg quercetin intragastrically 5 times/week for 8 weeks; and Cd-treated group with vitamin C: rats received $CdCl_2$ 10 and 100 *ppm* in drinking water with 50 mg/kg vitamin C intragastrically 5 times/week for 8 weeks.

The body weights and general health status of all rats were monitored and recorded at the beginning and once a week throughout the 8-week-period. At the end of 8 weeks, they were subjected to deep CO_2 anesthetization. Prior to euthanasia, blood was collected by cardiac puncture. Afterwards, the kidney, liver, and testis were collected for further analysis. Tissue sample collection was performed between 8, and 10 am to avoid any possible cyclic daily variation in antioxidant levels. The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Science, Mahidol University (MU-FVS-ACUC 2015-51). The study procedures involving the animals and their care conform to the institutional guidelines, in compliance with national and international laws and guidelines for the Use of Animals in Biomedical Research.

2.2 Measurement of tissue cadmium levels

Approximately 1 g sub-sample was excised from semi-thawed kidney, liver and testis and digested in 5 ml concentrated nitric acid and 1 ml hydrogen peroxide in a microwave digestion system for 1 hour (Milestone, Ethos Plus). Digested samples were allowed to cool, then transferred to a volumetric flask and diluted to 25 ml with ultrapure water. The sample solutions were analyzed for Cd by inductively coupled plasma mass spectrometry (ICP-MS; Agilent-7500CX). Samples with Cd levels >14 mg/ml were re-analyzed and confirmed with inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin-Elmer the Optima 4300DV). The analytical process was conducted under strict quality assurance. Analytical recovery determined using spiked samples (n=5-8 for each batch of analysis) ranging from 93%-99%. The relative percent difference (RPD) between duplicates was less than 10%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.002 and 0.004 mg/kg, respectively. All results are presented on a wet weight basis. This analytical laboratory has participated in the Proficiency Testing (PT) annually with FAPAS (part of The Food and Environment Research Agency, Department for Environment, Food and Rural Affairs, UK) and Department of Medical Sciences, Ministry of Public Health, Thailand.

2.3 Determination of oxidative stress biomarker: A lipid peroxidation product

The malondialdehyde-thiobarbituric acid reactive substances (MDA-TBARS) assay was employed to quantify oxidative stress by measuring lipid peroxidation product, malondialdehyde (MDA), which subsequently reacted with 2-thiobarbiyuric acid (TBA) under high temperature and acidic conditions. This reaction generated a chromogen that could be monitored spectrophotometrically at 532 nm.[23] The concentration of lipid peroxides is expressed as nmol/mg protein. The total protein was determined by the Coomassie Plus (Bradford) Protein Assay (Sigma-Aldrich Company, Ltd.).

2.4 Histopathological studies

Slices of kidney, liver and testis were dissected and preserved for routine histology by fixation in 10% formaldehyde. The fixed samples were embedded in paraffin, sectioned to 5 µm hematoxylin/eosin for morphological analysis. The sections were stained with haematoxylin and eosin (H&E) and assessed by light microscopy on a Nikon ECLIPSE E200 (Tokyo, Japan).

2.5 Data and statistical analysis

The data are presented as mean ± SEM. Statistical comparisons between groups were analyzed using the general linear model.

Analysis of variance (ANOVA) was used to determine whether the differences between means were significant. The level of statistical significance was $p < 0.05$ (SPSS software 19.00 version).

3. Results

3.1 Organ and body weights

No significant difference among treatment groups was observed for the body weight gain *per* week (Fig.1). In rats receiving low dose Cd and vitamin C, the weekly body weight gain tended to be higher than the control and quercetin-treated groups. The weights of the kidneys, liver and testis in the control group at the end of the study were 2.1±0.01, 10.6±0.04 and 3.9±0.01 g, respectively. The weights of the organs in rats receiving 10, 100 *ppm* Cd, including those treated with quercetin and vitamin C, were not different significantly different.

3.2 Tissue Cd levels

Cd accumulation in the kidney, liver and testis was increased in a dose-dependent manner in the Cd-treated rats. Co-treatment with quercetin or vitamin C did not affect Cd accumulation in the kidney, liver and testis (Fig.2).

3.3 Lipid peroxidation levels

The MDA levels in the kidney were increased in a positive correlation with Cd concentrations. The kidney MDA levels were decreased in rats receiving 100 *ppm* Cd and supplemented with quercetin or vitamin C (Fig.3). In the liver, the MDA levels were not markedly altered among Cd-treated groups. The treatment with vitamin C resulted in a marked reduction in MDA levels ($p<0.05$, Fig.3). Co-administration with either quercetin or vitamin C appeared to elevate the oxidative stress biomarkers in the testis of 100 ppm Cd-exposed rats, but a significant difference was not found (Fig.3).

3.4 Histopathological findings in kidney

In the control group, the cortex and medulla of the kidneys had a typical structure with a rare glomerular dilation and tubular necrosis (Fig.4 a). Tubular necrosis and glomerular widening were evident in the kidneys of all Cd-exposed animals (Fig.4 b-c). In rats receiving the higher dose of Cd, the tubular injury was more extensive. The tubular necrosis and glomerular dilation were not ameliorated by quercetin or vitamin C (Fig.4 d-f, g-i).

3.5 Histopathological findings in liver

Light microscopic examination showed a typical structure of the liver in the control groups with rare chromatin condensation and nucleus fragmentation (Fig.5a). Exposure to Cd-induced degenerative changes in this organ. Enlargement of the cell sizes was observed. The chromatin was condensed and more compacted in many hepatocytes. Necrosis of single cells with contracted and pycnotic nuclei and condensed chromatin were also found in the Cd-exposed group. With the quercetin supplement, a decrease in necrosis of single hepatocytes was observed (Fig.5 d-f). The livers of the rats that received vitamin C showed more chromatin condensation and pycnotic nuclei (Fig.5 g). The liver of rats after exposure to co-administration of Cd and vitamin C exhibited severe mononuclear cells infiltrations (Fig.5 i).

3.6 Histopathological findings in testis

Histological observation in the control rat testis revealed well defined seminiferous tubules. Basement membranes were regular and intact spermatogonia were concentrically organized. The Leydig cells of the interstitial tissue were polygonal and had a large spherical nucleus with one eccentric nucleolus. Tubules were normal and contained sperms (Fig.6 a). Following a low dose of sub-chronic Cd exposure (Cd10), necrosis and degeneration of seminiferous tubules were found. The seminiferous tubules appeared as isolates, and the Leydig cells were lost. Tubule lamina had disappeared or was irregular and contained necrotic material (Fig.6 b).

Histopathological findings in the testes of rats exposed to quercetin (Fig.6 d) or co-administration of Cd and quercetin

(Fig.6 d-f) were similar to those of rats treated with Cd alone at the high dose (Cd100, Fig.6 c). In vitamin C-treated rats (Fig.6 g) and Cd plus vitamin C groups (Fig.6 g-i), hemorrhagic areas were markedly noted in testicular tissues. Furthermore, in the testis of the rats exposed to both Cd and vitamin C (Fig.6 i), extensive areas of edema were observed, especially in the testis of rats treated with Cd-high doses and vitamin C.

4. Discussion

Cd is one of the most abundant non-essential elements due to its extensive usage in various industrial applications. The health risk to humans from acute and chronic Cd exposure has been well documented. Cd increases oxidative stress that contributes to pathogenesis because of its long retention in target tissues such as the kidney, liver and testis. Cd mainly accumulates in the liver and kidney. It is therefore suggested that antioxidant therapy could be of benefit from intervention with Cd toxicity.[16,17]

The levels of Cd (10 and 100 *ppm* used in this study are considered environmentally relevant and designated as low and high levels of contamination, respectively. The concentration of quercetin, 50 mg/kg, has been previously reported in a rat model of Cadmium-induced nephrotoxicity,[16,17] while 50 mg/kg of vitamin C has been suggested to be effective in several models.[9-11] Exposure via the oral route is relevant as the main route of exposure in the general, non-smoking population.

In the present study, Cd clearly mediated kidney, liver and testis damages at a dose as low as 10 *ppm* when administered for eight weeks. The enhanced oxidative stress, as determined by the MDA-TBARS assay was evident, especially in the kidney. It has been reported that Cd exerts its toxicity *via* different mechanisms. It induces oxidative stress by the depletion of glutathione and increasing lipid peroxidation levels.[24] The products of lipid peroxidation may lead to changes in biological membranes resulting in serious cellular injury. The severity of tissue damages appeared to correlate with the levels of tissue Cd accumulation since it has been shown that Cd could disturb membrane integrity[14,25] and induce the generation of cytotoxic and inflammatory mediators,[26] resulting in severe structural changes to the organs.

Cd accumulation in many organs of humans and animals, liver and kidney are recognized as a primary target organ of Cd-induced toxicity. Cd has a very low rate of excretion. Its long biological half-life over time affects the functions of these organs. The susceptibility to Cd toxicity may be different depending on the concentration of Cd and cellular antioxidant mechanism. Although Cd accumulation in the testis was very low, it seemed to be highly sensitive than kidney and liver in Cd toxicity.

Investigations concerning Cd toxicity have shown that, compared to other organs, rodent testes are extremely susceptible to Cd.[27] It has been well established that testicular oxidative stress commonly induces patho-physiological change leading to male infertility.[28,29] Although the testicular tissue expresses several antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, to counteract the oxidative stress, their levels could be significantly diminished upon Cd exposure.[9,10]

Vitamin C (ascorbic acid) is a water-soluble antioxidant that reduces sulfhydryl, scavenges free radicals and protects against endogenous oxidative DNA damage.[30] Although previous evidence suggests vitamin C as an effective antioxidant in several oxidative stress models, including Cd-induced oxidative damages in the kidney, liver and testis.[9-11] In this study, supplementation with 50 mg/kg vitamin C, intragastrically 5 times/week caused a higher degree of testis damage as evidenced by increased lipid peroxidation and changes in histopathological characteristics. On the other hand, vitamin C reduced the MDA levels in the kidney and liver of Cd-treated rats.

Vitamin C plays a switch from being

an antioxidant in physiologic conditions to a pro-oxidant under pathological conditions. Previous studies reported that vitamin C could act as a pro-oxidant *in vivo* with the help of reducing transition metals, driving a Fenton reaction.[31-33] The beneficial or damaging effect of a nutrient depends on the bimodal characteristic conditional on the inorganic chemistry of the cell.[34] The evidence for antioxidant protection of lipids by vitamin C, in both iron and non-iron supplementation, was also reported,[29] suggesting vitamin C as a potential antioxidant under physiologic conditions. In the presence of low levels of Cd, vitamin C may act as pro-oxidant by enhancing the Fenton reaction. Therefore, the dose of vitamin C should be considered in this setting situation.

Several flavonoids have been shown to possess strong radical scavenging properties.[35] Among them, quercetin, found in many plants and is widely distributed in edible fruits and vegetables, is known as a metal chelator and antioxidant.[16,17] Murota[36] suggested, however, that quercetin might be poorly absorbed from the digestive tract and may not provide significant protection against oxidative stress.[28] In the present study, it was observed that quercetin slightly reduced lipid peroxidation and could attenuate Cd-induced liver damages. It should be noted that supplements with quercetin or vitamin C in Cd 100 ppm-treated rats reduced the increased levels of serum ALT and AST, supporting the benefit of quercetin and vitamin C in liver damage. Morales et al. demonstrated that quercetin could also modulate Cd-induced renal tubular necrosis. Animals treated with the combination Cd plus quercetin showed well-preserved cell structures and organelles in most kidney samples.[16,17] However, it was shown in the present study that quercetin could not provide benefit in rescuing damages in renal tissues upon Cd treatment.

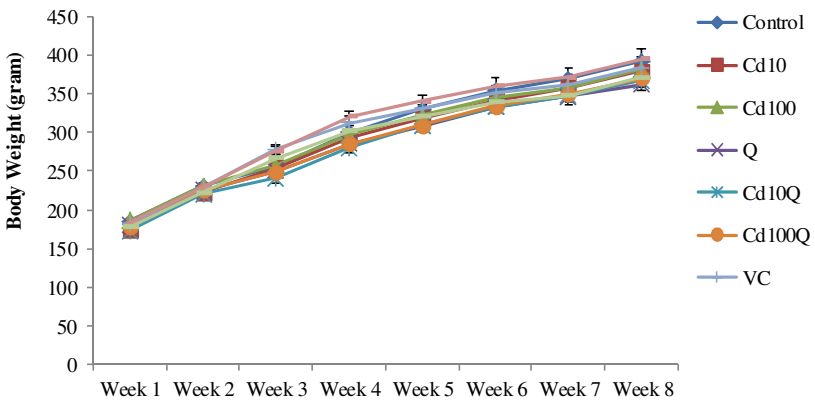

Fig. 1. The weekly body weight (g) of control, and rats treated with cadmium, quercetin, vitamin C, or their combination, over a period of treatment (8 weeks), presented as mean ± SEM.

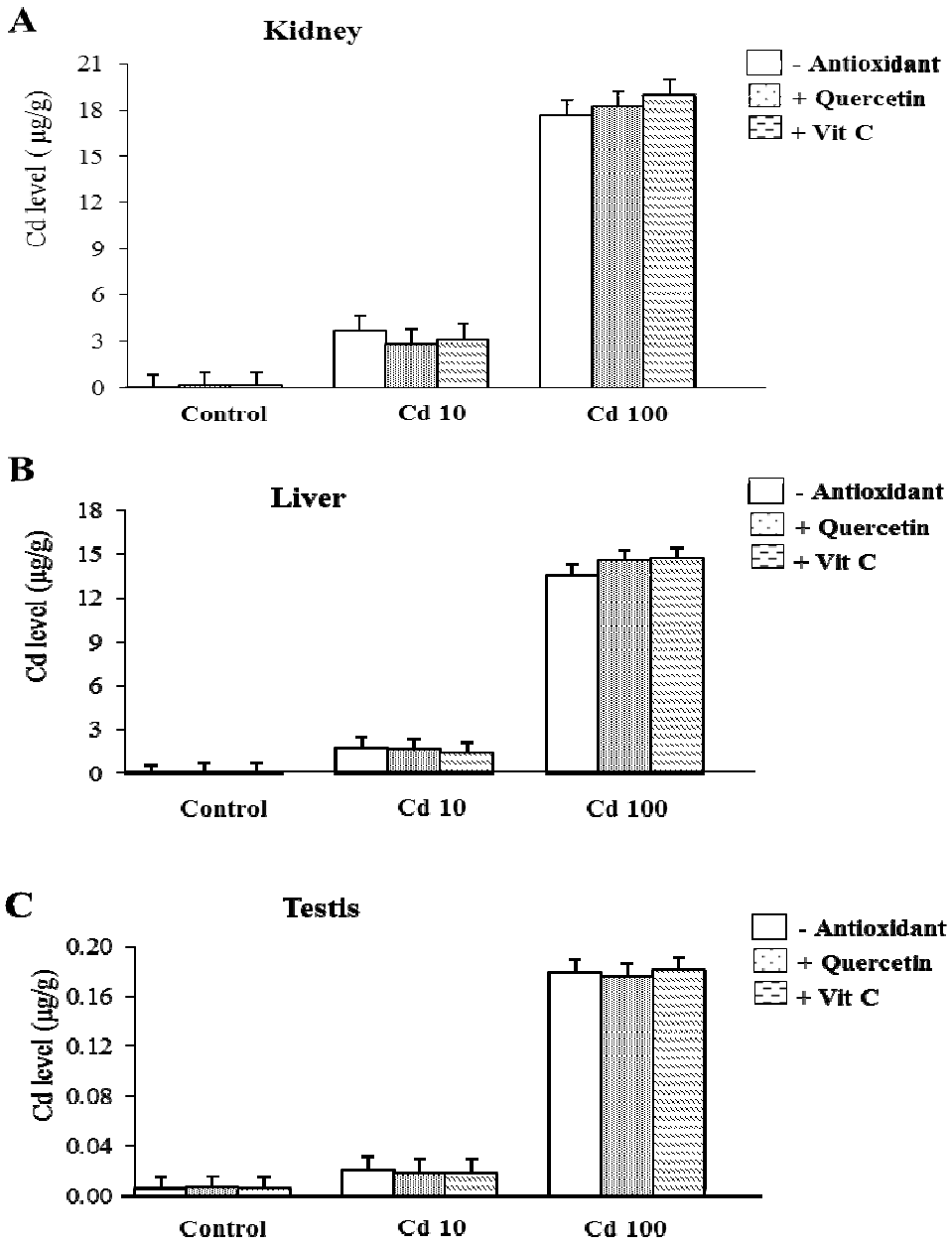

Fig. 2. Cd accumulation in (A) kidney, (B) liver and (C) testis after 8 weeks of treatment in control and Cd-treated rats, with and without quercetin and vitamin C. The data are expressed as mean ± SEM (n=6).

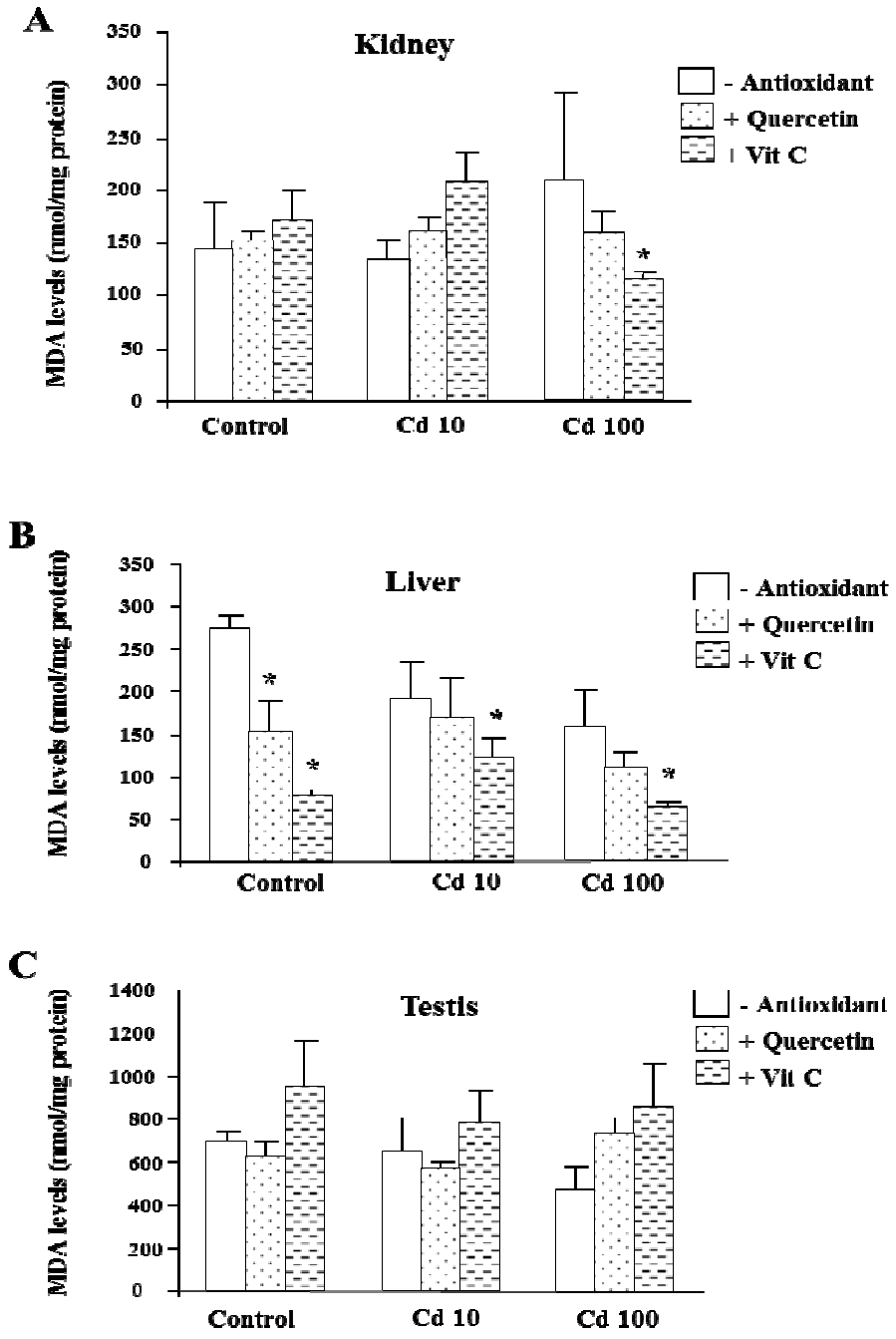

Fig. 3. Lipid peroxidation (MDA) levels in (A) kidney, (B) liver and (C) testis after 8 weeks of treatment in control and Cd-treated rats, with and without quercetin and vitamin C. The data are expressed as mean ± SEM (n = 6). * $p<0.05$ versus Cd-treated group of the respective concentration.

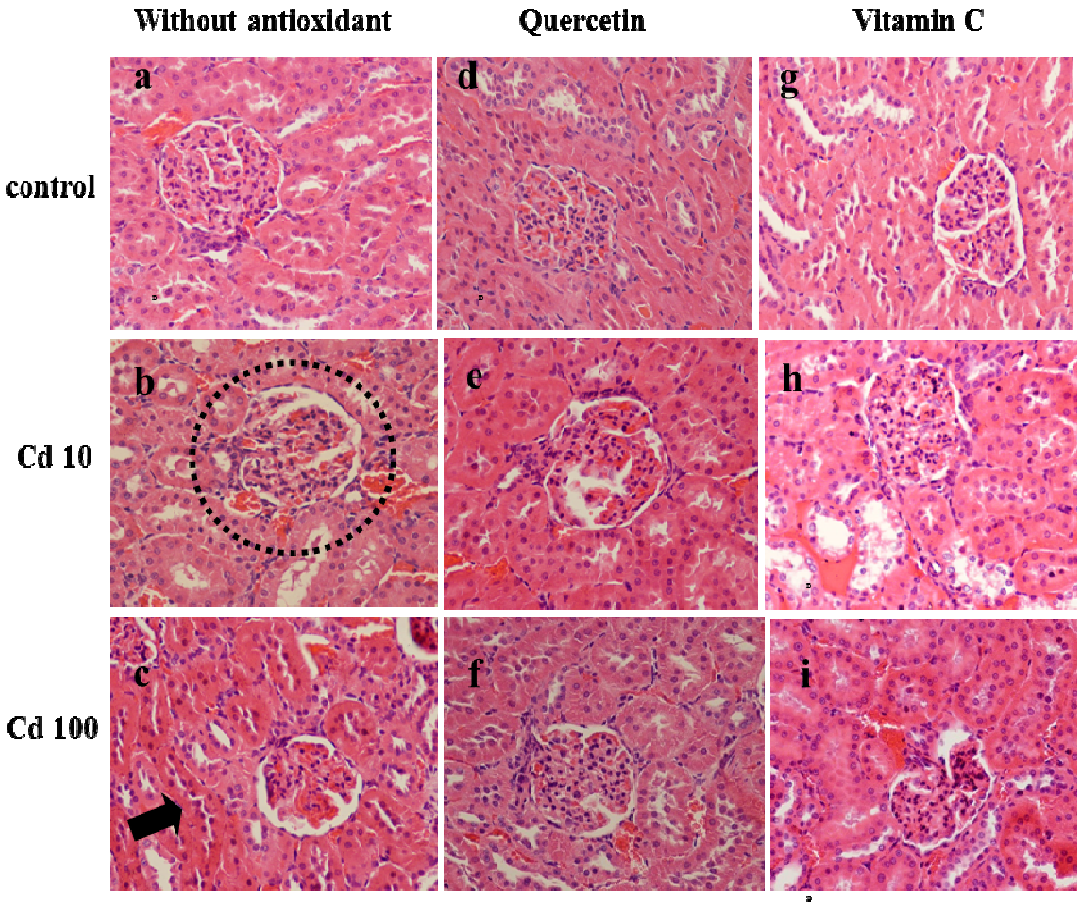

Fig. 4. Representative photomicrographs of H&E staining of the kidney in the control and Cd-treated groups in the presence and absence of antioxidant. The images were taken at 400x original magnification. Tubular necrosis (➡). Glomerular basement membrane degeneration (◌).

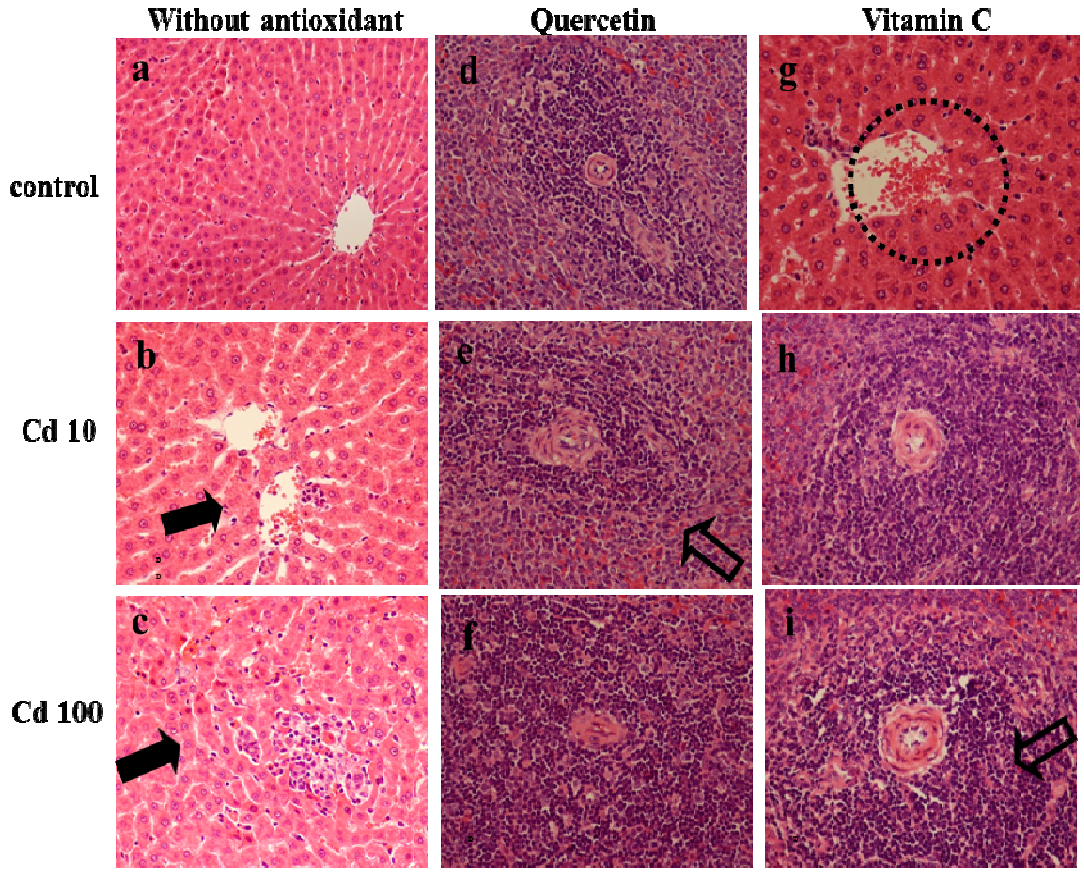

Fig. 5. Representative photomicrographs of H&E staining of rat livers in the control and Cd-exposed groups in the presence and absence of antioxidants. The images were taken at 400x original magnification. Hepatocytes necrosis (➡), chromatin condensation and pycnotic nuclei (◌) mononuclear cells infiltrations (⇨).

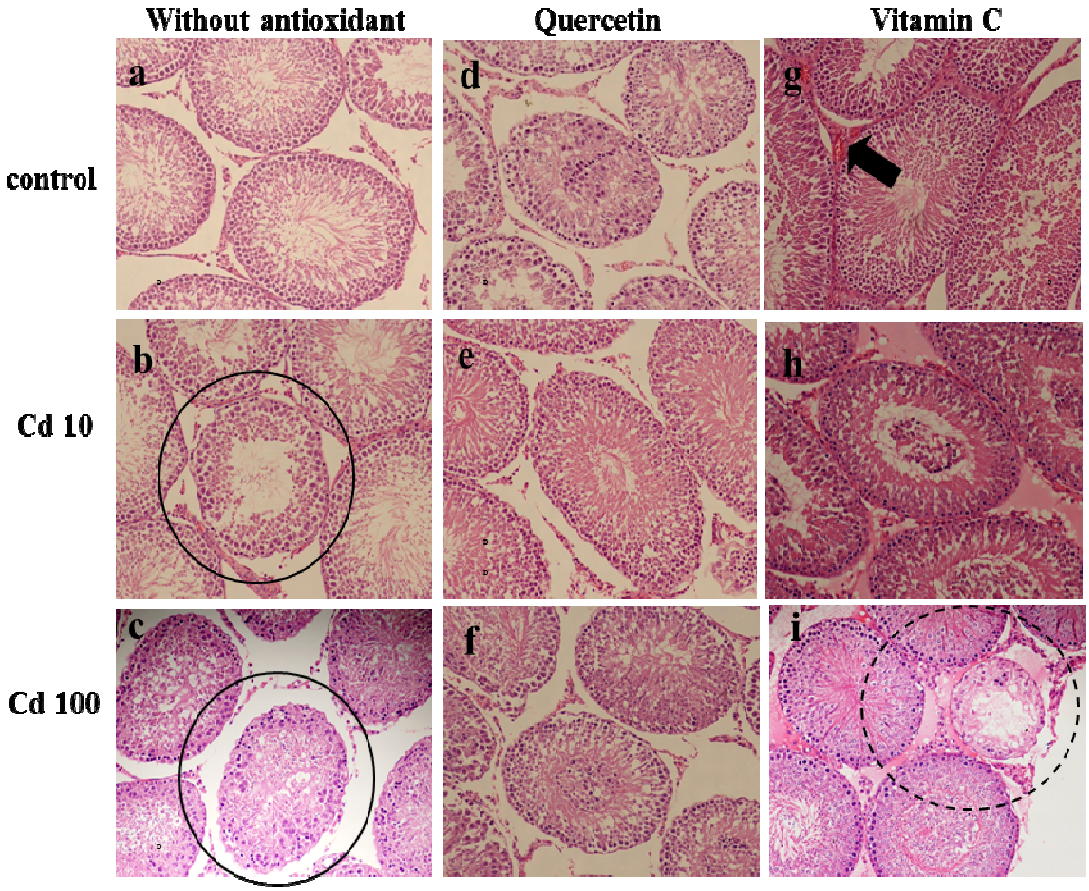

Fig. 6. Representative photomicrographs of H&E staining of rat testes in the control and Cd-exposed groups in the presence and absence of antioxidants. The images were taken at 400x original magnification. Degeneration of seminiferous tubules (➡), hemorrhagic areas (⇨), areas of edema (⬚)

4. Conclusion

It was suggested that Cd induced a marked increase in oxidative stress and pathological changes in critical organs, particularly the testis. Co-treatment with quercetin had benefits regarding reducing oxidative stress marker and tissue damages, especially in rat liver.

Acknowledgements

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