

1 **Sıçanlarda Kadmiyumla Oluşturulan Böbrek Toksisitesine Karşı**

2 **Quercetin'in Koruyucu Etkisi**

3 **Kısa Başlık: Böbrek Toksisitesinde Quercetin'in Etkisi**

4 **Amaç:** Çalışmamızda kadmiyumla (Cd) oluşturulan böbrek toksisitesine karşı quercetin'in
5 (QE) koruyucu etkinliğini göstermeyi amaçladık.

6 **Gereç ve Yöntemler:** Çalışmada 24 adet Wistar albino cinsi erişkin erkek sıçan kullanıldı.
7 Sıçanlar her grupta 8 adet olmak üzere; kontrol, Cd ve Cd+QE olmak üzere 3 gruba ayrıldı.
8 Cd grubuna her gün 1 mg/kg Cd, 2 ml/kg serum fizyolojik içerisinde çözüldürüldükten sonra
9 CdCl₂ şeklinde 30 gün boyunca subkutan enjeksiyon olarak uygulandı. Cd ile birlikte QE
10 tedavisi verilen gruba, Cd enjeksiyonundan 2 gün önce başlanarak 15 mg/kg QE, deney süresi
11 boyunca intraperitoneal olarak uygulandı.

12 **Bulgular:** Böbrek dokularının histolojik olarak değerlendirilmesi sonucu, kontrol grubuyla
13 karşılaştırıldığında Cd verilen sıçanlarda mezengial hücrelerde artış, kapsüller, glomerüller ve
14 tübüler basal membranlarda kalınlaşma ile birlikte periyodik asit Schiff (PAS)-pozitif
15 alanların artışı gözlemlendi. Cd ile birlikte QE tedavisi verilen grupta sadece birkaç glomerüldeki
16 genişleme dışında, Cd'ye bağlı böbrek yapısında oluşan değişikliklere karşı QE'nin belirgin
17 koruyucu bir etkisinin olduğu saptandı. Bulgularımız, Cd ile birlikte QE tedavisi verilen
18 grupta böbrek kortikal dokularında TdT-(terminal deoksinukleotidil transferaz)- aracılı
19 deoksiuridin trifosfat işaretleme (TUNEL) aktivitesinde anlamlı bir azalma ile birlikte
20 proliferatif hücre nükleer antijeninin (PCNA) ekspresyonunda da artış olduğunu
21 göstermiştir.

22 **Sonuç:** Bu sonuçlar QE'nin Cd ile oluşturulan böbrek toksisitesini azaltabileceğini
23 göstermiştir.

24 **Anahtar sözcükler:** Böbrek toksitesi; immünohistokimya; kadmiyum; quercetin; TUNEL.

25 **Protective **Effect** of Quercetin Against Renal Toxicity**
26 **Induced by Cadmium in Rats**

27 **Running Title: Effect of Quercetin in Renal Toxicity**

28 **Objectives:** The aim of the present study was to examine the protective **effect** of quercetine
29 (QE) against cadmium (Cd)-induced renal toxicity.

30 **Materials and Methods:** A total of 24 male Wistar albino rats were divided into three
31 groups: control, Cd-treated and Cd-treated with QE; each group contain 8 animals. The Cd-
32 treated group was injected subcutaneously with CdCl₂ dissolved in **saline** in the amount of 2
33 ml/kg/day for 30 days, resulting in a dosage of 1 mg/kg Cd. The rats in QE treated groups
34 were given QE (15 mg/kg body weight) once a day intraperitoneally starting 2 days prior **Cd**
35 injection during the study period.

36 **Results:** The renal histology in Cd-treated rats showed mesangial expansion, thickening of
37 capsular basement membranes, glomerular basement membranes and tubular basement
38 membranes, characterized by an increase in **periodic acid Schiff (PAS)-positive** area as
39 compared with control animals. With the QE treatment, **despite the presence of only few**
40 **swollen glomeruli, we noticed a marked prevention in renal structure when compared with the**
41 **Cd-treated rats.** Furthermore, QE pretreatment resulted in increased proliferating cell nuclear
42 antigen (PCNA) immunoreactivity and decreased the activity of **Terminal Transferase dUTP**
43 **Nick End Labeling (TUNEL).**

44 **Conclusion:** These findings suggest that QE may attenuate Cd-induced renal toxicity.

45
46 **Key words:** Cadmium; immunohistochemistry; quercetin; renal toxicity; TUNEL.

47
48
49

50

51

INTRODUCTION

52 Cadmium (Cd) is a heavy metal that is present in air, water, soils, and sediments. ^[1,2] Cd
53 accumulates largely in the liver and kidney and produces pulmonary, liver, and renal tubular
54 diseases. Chronic intake of Cd in food or in air produces organ dysfunction as a result of cell
55 death. ^[3] Cd is widely used in pigments, plastic stabilizers, electroplating, alloys, nickel-Cd
56 batteries, and welding in industry and is also present in tobacco. ^[4,5] Natural sources of Cd are
57 spread to the atmosphere by volcanic activity, forest fires, and wind-blown transport of soil
58 particles. Other sources are phosphate fertilizers, which may contain high concentrations of
59 Cd, depending on the origin of the rock, and the application of contaminated sewage sludge as
60 a soil additive. ^[1] It has been demonstrated that the kidney is one of the most critically
61 affected organs after long-term environmental and occupational exposure to Cd. ^[1,6] Cd-
62 induced nephropathy is characterized functionally by various signs of tubular dysfunction,
63 such as low molecular weight proteinuria, glucosuria, and aminoaciduria, and pathologically
64 by chronic interstitial nephritis. ^[6]

65 Flavonoids are a group of naturally occurring compounds widely distributed as secondary
66 metabolites in the plant kingdom. They have been recognized for having interesting clinical
67 properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral
68 activities. ^[7] One of these flavonoids, quercetin (QE) (3,5,7,3',4'-pentahydroxyflavone),
69 prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen
70 radicals, ^[8] protecting against lipid peroxidation ^[9] and chelating metal ions ^[10].

71 The aim of the present study was to examine the protective effect of QE against Cd-
72 induced renal toxicity.

73

MATERIAL AND METHODS

74

Animals

75 Twenty four healthy male Wistar albino rats (weighing 200-250 g and averaging 16 weeks
76 old) housed at the Trakya University Animal Care and Research Unit were used for this study.
77 Food and tap water were available ad libitum. In the windowless animal quarter automatic
78 temperature ($21\pm 1^{\circ}\text{C}$) and lighting controls (12 h light/12 h dark cycle) was performed.
79 Humidity ranged from 55% to 60%. All animals received human care according to the criteria
80 outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National
81 Academy of Sciences and published by the National Institutes of Health. In addition the
82 experimental protocol was approved by the Ethical Committee of Trakya University Medical
83 Faculty (approved date and number: 05/01/2011 and 2011.01.010). QE was obtained from
84 Sigma Chemical (St Louis, MO, USA) and dissolved in 0.5 ml of 60% ethanol just before
85 intraperitoneal (i.p.) injection (15 mg/kg). Control group was injected with the same volume
86 of saline as the Cd treated groups received.

87 **Experimental groups**

88 A total of 24 male Wistar albino rats were divided into three groups: control, Cd-treated and
89 Cd-treated with QE; each group contain 8 animals. Control animals received daily injections
90 of the saline vehicle alone. The Cd-treated group was injected subcutaneously with CdCl₂
91 dissolved in saline in the amount of 2 ml/kg/day for 30 days, resulting in a dosage of 1 mg/kg
92 Cd ^[11]. The rats in QE treated groups were given QE (15 mg/kg body weight) once a day
93 intraperitoneally starting 2 days prior Cd injection during the study period. At the end of the
94 study, all animals were anesthetized with i.p. injection of sodium thiopental (100 mg/kg,
95 Sigma, St. Louis, MO, USA). After twenty minutes, the anesthetized rats were sacrificed and
96 renal tissues were removed for histopathological investigation.

97 **Histopathologic evaluation**

98 The renal tissues were individually immersed in Bouin's fixative, dehydrated in alcohol and
99 embedded in paraffin. Sections of 5 µm were obtained, deparaffinized and stained with
100 hematoxylin and eosin (H&E) and periodic acid Schiff (PAS). The renal tissue was examined
101 and evaluated in random order under blindfold conditions with standard light microscopy.

102 The thickening of the renal basement membrane was scored in a semiquantitative manner
103 in order to determine the differences between the control group and the experimental groups.
104 The thickening of the renal basement membrane was recorded as normal (+), thick (++), very
105 thick (+++) and more thick (++++). These analyses were performed in two sections from each
106 animal at x400 magnification in at least ten different regions for each section.

107 **Immunohistochemistry**

108 The harvested renal tissues were fixed in Bouin's, embedded in paraffin and sectioned at 5
109 µm thickness. Immunohistochemical reactions were performed according to the ABC
110 technique described by Hsu et al. ^[12] The procedure involved the following steps: (1)
111 endogenous peroxidase activity was inhibited by 3% H₂O₂ in distilled water for 30 min; (2)
112 the sections were washed in distilled water for 10 min; (3) non-specific binding of antibodies
113 was blocked by incubation with normal goat serum (DAKO X 0907, Carpinteria, CA) with
114 phosphate-buffered saline (PBS), diluted 1:4; (4) the sections were incubated with specific
115 mouse monoclonal anti-PCNA antibody (Cat. # MS-106-B, Thermo LabVision, USA),
116 diluted 1:50 for 1h at room temperature; (5) the sections were washed in PBS 3 × 3 min; (6)
117 the sections were incubated with biotinylated anti-mouse IgG (DAKO LSAB 2 Kit); (7) the
118 sections were washed in PBS 3 × 3 min; (8) the sections were incubated with ABC complex
119 (DAKO LSAB 2 Kit); (9) the sections were washed in PBS 3 × 3 min; (10) peroxidase was
120 detected with an aminoethylcarbazole substrate kit (AEC kit; Zymed Laboratories); (11) the
121 sections were washed in tap water for 10 min and then dehydrated; (12) the nuclei were

122 stained with hematoxylin; and (13) the sections were mounted in DAKO paramount. All
123 dilutions and thorough washes between steps were performed using **PBS** unless otherwise
124 specified. All steps were carried out at room temperature. As a negative control, primary
125 antibody was replaced with PBS.

126 **TUNEL assay**

127 The TUNEL method, which detects fragmentation of DNA in the nucleus during apoptotic
128 cell death in situ, was employed using an apoptosis detection kit (TdT-FragelTM DNA
129 Fragmentation Detection Kit, Cat. No. QIA33, Calbiochem, USA). All reagents listed below
130 are from the kit and were prepared following the manufacturer's instructions. **5- μ m-thick**
131 **renal** sections were deparaffinized in xylene and rehydrated through a graded ethanol series as
132 described previously. They were then incubated with 20 mg/ml proteinase K for 20 minutes
133 and rinsed in TBS. Endogenous peroxidase activity was inhibited by incubation with 3%
134 hydrogen peroxide. Sections were then incubated with equilibration buffer for 10–30 minutes
135 and then TdT-enzyme, in a humidified atmosphere at 37 °C, for 90 minutes. They were
136 subsequently put into pre-warmed working strength stop/wash buffer at room temperature for
137 10 minutes and incubated with blocking buffer for 30 minutes. Each step was separated by
138 thorough washes in TBS. Labelling was revealed using DAB, counter staining was performed
139 using **methyl green**, and sections were dehydrated, cleared and mounted.

140 The number of **PCNA and** TUNEL positive cells, in each specimen was also scored. Ten
141 randomly selected area were scored for each specimen in every experiment as follows: 0 = no
142 positive response; 1 = less than 10% of cells; 2 = 11–20% of cells; 3 = 21–40% of cells; 4
143 =more than 40% of cells.

144 **Statistical analysis**

145 All statistical analyses were carried out using SPSS statistical software (SPSS for windows,
146 version 11.0). All data were presented in mean (\pm) standard deviations (S.D.). Differences in
147 measured parameters among the three groups were analyzed with a nonparametric test
148 (Kruskal-Wallis). Dual comparisons between groups exhibiting significant values were
149 evaluated with a Mann–Whitney *U*-test. These differences were considered significant when
150 probability was less than 0.05.

151 RESULTS

152 Histopathological findings

153 Normal structure of the renal cortical tissue was observed in control rats (Fig. 1a). The
154 animals exposed to Cd showed severe changes in the renal tubules and glomeruli.
155 Hypertrophy and degeneration of renal tubules epithelia with infiltration of mononuclear
156 cells, increased amounts of mesangial matrix, dilatation of glomeruli were evident in all
157 animals treated with Cd (Fig. 1b). With the QE treatment, despite the presence of only few
158 swollen glomeruli and tubuli, we noticed a marked prevention in renal structure when
159 compared with the Cd-treated rats (Fig. 1c). Moreover, the renal histology in Cd-treated rats
160 showed an enlargement of the glomeruli, mesangial expansion, thickening of capsular
161 basement membranes (CBMs), glomerular basement membranes (GBMs), and tubular
162 basement membranes (TBMs), characterized by an increase in PAS-positive area as compared
163 with control animals (Fig. 2a, b). Treatment of QE reduced the glomerular size, thickening of
164 CBMs, GBMs and TBMs as compared with Cd-treated group (Fig. 2c; Table 1).

165 Immunohistochemical findings

166 PCNA-positive cells were strongly detected in the renal cortical tissues of the control rats
167 (Fig. 3a). However, the signal density of positive cells was significantly higher in Cd-treated

168 group (Fig. 3b). Treatment of QE markedly increased the reactivity of PCNA in the renal
169 cortical tissues (Fig. 3c; **Table 2**).

170 **TUNEL findings**

171 The number of TUNEL-positive cells in the control group was negligible (Fig. 4a). When
172 kidney sections were TUNEL stained, there was a clear increase in the number of positive
173 cells in the Cd-treated rats in the renal cortical tissues (Fig. 4b). Treatment of QE markedly
174 reduced the reactivity and the number of TUNEL positive cells (Fig. 4c, Table 2).

175 **DISCUSSION**

176 Cd is a toxic industrial and environmental metal. ^[2,5,13,14] The major environmental source of
177 Cd to humans and animals is food and drinking water. ^[15] With the increasing production and
178 utilization of cd, not only industrial workers, but also the general population are also exposed
179 to the toxic effects of cd. It has been found that cd produces various pathological conditions,
180 including hepatic and renal dysfunctions, testicular damage, and respiratory and nervous
181 system disorders. ^[13,14,16,17] Cd induced chronic toxicity has been shown as histological and
182 ultrastructural by various investigators in experiments using various species of animals. ^[18,19]
183 During acute exposure, Cd-induced necrosis and cellular damage are produced in kidney,
184 liver and testicular tissue; chronic exposure results in damage to the kidney, pancreas, and
185 bone. ^[20]

186 Cd injection in rats also affects the genito-urinary system. The kidney is a critical target
187 organ following Cd exposure. ^[14] Renal metabolism is perturbed in both acute and chronic
188 exposure. Adaptive mechanisms counteract renal tubular acidosis during chronic, but not
189 following, acute exposure. Following chronic exposure, the kidney also shows an alteration in
190 lipid content, possibly caused by mitochondrial proliferation. ^[20] The renal effects are
191 generally considered to be mainly tubular. Scott et al. ^[11] showed cellular damage in the

192 glomeruli with changes in glomerular capillaries by light microscopy. Chronic Cd exposition
193 caused a nephropathy with peculiar damage of the renal proximal tubule. Atrophy and
194 degeneration of proximal tubules with vacuolization of tubular cells were the most striking
195 lesions seen. ^[21] Cd exposure has been shown to cause large cytoplasmic vacuoles containing
196 membranous material in proximal tubular lining cells. Light microscopy shows focal areas of
197 necrosis and interstitial fibrosis within the renal cortex. These findings are associated with
198 renal Cd levels. ^[22] With increasing Cd doses, the size of nuclei and nucleoli in the cells of
199 proximal tubule shows significant enlargement and also an increase in the number of nucleoli
200 on light microscopy. In the present study, the renal histology in Cd-treated rats showed
201 mesangial expansion, thickening of capsular basement membranes, glomerular basement
202 membranes and tubular basement membranes, characterized by an increase in PAS-positive
203 area as compared with control animals. With the QE treatment, despite the presence of only
204 few swollen glomeruli and tubuli, we noticed a marked prevention in renal structure when
205 compared with the Cd-treated rats. These findings are in agreement with the results of above
206 mentioned investigators on Cd-induced renal toxicity in rats.

207 A recent study ^[23], PCNA-immunoreactive cells were preferentially detected in the
208 degenerating proximal tubular lesions observed histopathologically in animals treated with
209 CdCl₂ at 200 and 600 ppm. Significantly increases in PCNA were also found in these high-
210 dose Cd-treated groups. In experiment, the severity of proximal tubular degeneration and the
211 mitotic figures in such degenerating tubules correlated well with both renal Cd-concentrations
212 and PCNA in the proximal tubular epithelium. To date, no PCNA expression on Cd-induced
213 renal toxicity in rats by QE treatment have been reported. In the present study, the signal
214 density of positive cells was significantly higher in Cd-treated group. Treatment of QE
215 markedly increased the reactivity of PCNA in the renal cortical tissues.

216 Both necrotic and apoptotic mechanisms have been implicated in the pathways leading to
217 death of proximal tubule epithelial cells during exposure to Cd. High levels of Cd are clearly
218 capable of causing proximal tubule necrosis. ^[22,24] However, other studies indicate that the
219 early stages of Cd nephrotoxicity primarily involve apoptosis of proximal tubule epithelial
220 cells, with little evidence of necrosis. ^[25,26] Moreover, results of several recent studies suggest
221 that the early stages of Cd-nephrotoxicity may involve changes in cell adhesion molecule
222 function and cytoskeletal organization that occur before the onset of either necrotic or
223 apoptotic death of proximal tubule epithelial cells. ^[27,28]

224 Recently, Iwai and Matsuno ^[29] have shown that chronic Cd exposure induces
225 apoptosis and subsequent regeneration of the renal tubular epithelium in rats and beagle dogs,
226 respectively. Apoptosis, a distinctive form of cell death, which occurred in various tissues
227 under certain physiological or pathological conditions ^[30], differs obviously from necrosis.
228 Thus, since apoptosis may be one of the main features of Cd intoxication, it seems necessary
229 to conduct more effective studies on the phenomenon in the tubular epithelium after exposure
230 to higher doses of Cd. In a study ^[26], the basement membrane was intact and no macrophages
231 infiltration was found in tubular epithelium. Furthermore, since the regenerating tubular cells
232 actively proliferated and replaced the epithelium, the dead cells seemed to be pushed out into
233 the tubular lumen. The cells interfaces between the regenerating cells and the dead cells were
234 simple without any cytoplasmic infoldings or junctional complexes. In vitro experimental
235 studies using proximal tubular cells have shown that Cd damages F-actin and disrupt
236 intercellular junctions. ^[31] These junctional changes might result in easy detachment of dead
237 cells from the tubular lining.

238 In a more recent study, Aoyagi et al. ^[25] noted an increase in the number of TUNEL-
239 labeled cells in the renal cortex of Cd treated rats after 4 and 5 weeks of exposure, but that the
240 level of apoptotic labeling was much less pronounced after 6 and 8 weeks of exposure.

241 However, no quantitative data was included in their analysis. In our present study, the number
242 of TUNEL positive cells was semi-quantitatively higher in Cd-treated group than control
243 group. Treatment of QE, markedly reduced the reactivity and the number of TUNEL positive
244 cells. In conclusion, these findings suggest that QE may attenuate Cd-induced renal toxicity.

245 **REFERENCES**

- 246 1.Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of
247 cadmium carcinogenesis. *Toxicology* 2003;192:95–117.
- 248 2.Koçak M, Akçil E. The effects of chronic cadmium toxicity on the hemostatic system.
249 *Pathophysiol Haemost Thromb* 2006;35(6):411–416.
- 250 3.Kim SC, Cho MK, Kim SG. Cadmium-induced nonapoptotic cell death mediated by
251 oxidative stress under the condition of sulfhydryl deficiency. *Toxicol Lett* 2003;144:325–
252 336.
- 253 4.Ohta H, Yamauchi Y, Nakakita M, Tanaka H, Asami S, Seki Y, et al. Relationship between
254 renal dysfunction and bone metabolism disorder in male rats after long-term oral
255 quantitative cadmium administration. *Industr Health* 2000;38:339–355.
- 256 5.Washington B, Williams S, Armstrong P, Mtshali C, Robinson JT, Myles EL. Cadmium
257 toxicity on arteriols vascular smooth muscle cells of spontaneously hypertensive rats. *Int J*
258 *Environ Res Public Health* 2006;3(4):232–238.
- 259 6.Bernard A, Lauwerys R. Effects of cadmium exposure in humans. In *Handbook of*
260 *Experimental Pharmacology* (E. C. Foulkes, Ed.), Springer-Verlag, Heidelberg 1986;135–
261 177.
- 262 7.Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. *Adv*
263 *Exp Med Biol* 1998;439:175–182.
- 264 8.Inal ME, Akgun A, Kahraman A. Radioprotective effects of exogenous glutathione against
265 whole-body gamma-ray irradiation: age- and gender-related changes in malondialdehyde

- 266 levels, superoxide dismutase and catalase activities in rat liver. *Methods Find Exp Clin*
267 *Pharmacol* 2002;24:209–212.
- 268 9. Laughton MJ, Evans PJ, Moroney MA, Hoult JR, Halliwell B. Inhibition of mammalian 5-
269 lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives.
270 Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem Pharmacol*
271 1991;42:1673–1681.
- 272 10. Afanas'ev IB, Dorozhko I, Brodskii AV, Korstyuk VA, Potapovitch A. Chelating and free
273 radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid
274 peroxidation. *Biochem Pharmacol* 1989;38:1763–1769.
- 275 11. Scott R, Aughey E, Sinclair J. Histological and ultrastructural changes in rat kidney
276 following cadmium injection. *Urol Res* 1977;5(Suppl 1):15–20.
- 277 12. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in
278 immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP)
279 procedures. *J Histochem Cytochem* 1981;29:577-80.
- 280 13. Yiin SJ, Chern CL, Sheu JY, Lin TH. Cadmium-induced lipid peroxidation in rat testes
281 and protection by selenium. *Biometals* 1999;12:353–359.
- 282 14. Yiin SJ, Chern CL, Sheu JY, Tseng WC, Lin TH. Cadmium-induced renal lipid
283 peroxidation in rats and protection by selenium. *J Toxicol Environ Health* 1999;57(Suppl
284 6):403–413.
- 285 15. Ohta H, Cherian MG. Gastrointestinal absorption of cadmium and metallothionein.
286 *Toxicol Appl Pharmacol* 1991;107:63–72.
- 287 16. Nigam D, Shukla GS, Agarwal AK. Glutathione depletion and oxidative damage in
288 mitochondria following exposure to cadmium in rat liver and kidney. *Toxicol Lett*
289 1999;106:151–157.

- 290 17. Newairy AA, El-Sharaky AS, Badreldeen MM, Eweda SM, Sheweita SA. The
291 hepatoprotective effects of selenium against cadmium toxicity in rats. *Toxicology*
292 2007;242(1–3): 23–30.
- 293 18. Asar M, Kayisli UA, Izgut-Uysal VN, Akkoyunlu G. Immunohistochemical and
294 ultrastructural changes in the renal cortex of cadmium-treated rats. *Biol Trace Elem Res*
295 2004;97:249–263.
- 296 19. Takaki A, Jimi S, Segawa M, Hisano S, Takebayashi S, Iwasaki H. Longterm cadmium
297 exposure accelerates age-related mitochondrial changes in renal epithelial cells.
298 *Toxicology* 2004;203(1–3):145–154.
- 299 20. Griffin JL, Walker LA, Shore R, Nicholson JK. Metabolic profiling of chronic cadmium
300 exposure in the rat. *Chem Res Toxicol* 2001;14(Suppl 10):1428–1434.
- 301 21. Gatta A, Bazzera G, Amodio P, Menon F, Angeli P, Schiaffino E, et al. Detection of the
302 early steps of cadmium nephropathy. Comparison of light and electron-microscopical
303 patterns with the urinary enzymes excretion. *Nephron* 1989;51:20–24.
- 304 22. Goyer RA, Miller CR, Zhu S, Victery W. Nonmetallothionein-bound cadmium in the
305 pathogenesis of cadmium nephrotoxicity in the rat. *Toxicol Appl Pharmacol*
306 1989;101:232–244.
- 307 23. Shibutani M, Mitsumori K, Niho N, Satoh S, Hiratsuka H, Satoh M, et al. Assessment of
308 renal toxicity by analysis of regeneration of tubular epithelium in rats given low-dose
309 cadmium chloride or cadmium-polluted rice for 22 months. *Arch Toxicol* 2000;74(10):571-
310 577.
- 311 24. Brzoska MM, Kaminski M, Supernak-Bobko D, Zwierz K, Moniuszko-Jakoniuk J.
312 Changes in the structure and function of the kidney of rats chronically exposed to
313 cadmium. I Biochemical and histopathological studies. *Arch Toxicol* 2003;77:344–352.

314 25.Aoyagi T, Hayakawa K, Miyaji K, Ishikawa H, Hata M. Cadmium nephrotoxicity and
315 evacuation from the body in a rat modeled subchronic intoxication. *Int J Urol*
316 2003;10:332–338.

317 26.Tanimoto A, Hamada T, Koide O. Cell death and regeneration of renal proximal tubular
318 cells in rats with subchronic cadmium intoxication. *Toxicol Pathol* 1993;21:341–352.

319 27.Jacquillet G, Barbier O, Cougnon M, Tauc M, Namorado MC, Martin D, et al. Zinc
320 protects renal function during cadmium intoxication in the rat. *Am J Physiol Renal Physiol*
321 2006;290:F127–F137.

322 28.Prozialeck WC, Vaidya VS, Liu J, Waalkes MP, Edwards JR, Lamar PC, et al. Kidney
323 injury molecule-1 (Kim-1) as an early biomarker of cadmium nephrotoxicity. *Kidney Int*
324 2007;72:985–993.

325 29.Iwai S, Matsuno K. An ultrastructural study on cadmium-induced damage of the renal
326 proximal tubules of rats. *J Med Soc Toho* 1991;137: 757-771 (in Japanese).

327 30.Walker NI, Harmon BV, Gobe GC, Kerr JFR. Patterns of cell death. *Meth Archiev Exp*
328 *Pathol* 1988;13:18-54.

329 31.Prozialeck WC, Niewcnhuis RJ. Cadmium (Cd²⁺) disrupts intercellularjunctions and actin
330 filaments in LLC-PKI cells. *Toxicol Appl Pliarmacol* 1991;107:81-97.

331
332
333
334
335
336
337
338
339
340
341

342 **Table 1.** Semiquantitative comparison of the thickening of renal basement membrane in
343 control, Cd-treated and Cd-treated with QE groups

	Control	Cd-treated	Cd-treated with QE
Basement membrane thickening	+	++++	++

344 The thickening of the renal basement membrane was recorded as normal (+), thick (++)
345 very thick (+++) and more thick (++++) (n: 8 for each group).

347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366

367 **Table 2.** Positive staining of **PCNA** and TUNEL cell numbers in renal cortical tissues for
368 each group. Control, Cd-treated and Cd-treated with QE

	Control	Cd-treated	Cd-treated with QE
PCNA	3.27 ± 0.42	0.96 ± 0.11^a	2.36 ± 0.14^c
TUNEL	0.08 ± 0.01	2.72 ± 0.17 ^b	0.86 ± 0.07 ^d

369

370 Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, n =
371 8 for each group.

372 ^a *P* < 0.001 compared with control group.

373 ^b *P* < 0.0001 compared with control group.

374 ^c *P* < 0.01 compared with control group.

375 ^d *P* < 0.01 compared with control group.

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391 **Figure 1.** Light microscopy of renal cortical tissues in different groups. (a) In control, normal
392 renal cortical tissue architecture was seen; (b) In Cd-treated group, increased amounts of
393 mesangial matrix, dilatation of glomeruli, tubuli and mononuclear cell **infiltration**; (c)
394 Treatment of QE reduced the amounts of mesangial matrix, dilatation of glomeruli, tubuli and
395 mononuclear cell infiltration (arrow head: mesangial matrix, thin arrows: glomerular
396 dilatation, thick arrows: tubular dilatation, asterisk: mononuclear cell infiltration), (H&E,
397 scale bar, 50 μ m).

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

413

414

415 **Figure 2.** Light microscopy of renal cortical tissues in different groups. (a) In control, normal
416 renal cortical architecture was seen; (b,c) Decreased the thickened CBMs, GBMs and TBMs
417 in Cd-treated with QE rats as compared with Cd-treated rats. (oblique arrows: CBMs PAS
418 positive area, thick arrows: TBMs PAS positive area, thin arrows: GBMs PAS positive area),
419 (PAS, scale bar, 50 μ m).

420

421

422

423

424

425

426

427

428

429

430

431

432

433

434

435

436

437

438

439

440

441 **Figure 3.** PCNA expression of renal cortical tissues in different groups. (a,b) PCNA-positive
442 cells were decreased in the renal cortical tissues in Cd-treated group as compared with control
443 group. (c) PCNA-positive cells were significantly increased in Cd-treated with QE group.
444 (Arrow: PCNA positive cells) (Immunoperoxidase, haematoxylin counterstain, scale bar, 50
445 μm).

446

447

448

449

450

451

452

453

454

455

456

457

458

459

460

461

462

463

464

465

466

467

468

469

470

471

472

473

474 **Figure 4.** TUNEL staining of renal cortical tissues in different groups. (a) In control group, a
475 few TUNEL-positive cells were observed in the renal cortical tissues; (b) The positive cells
476 were significantly higher in the renal cortical tissues of the Cd-treated group; (c) Treatment of
477 QE markedly decreased the reactivity of TUNEL. (Arrow: TUNEL positive cells), (TUNEL,
478 scale bar, 50 μ m).

479

480

481

482

483

484

485

486

487

488

489

490

491

492

493

494

495

496

497

498

499

500