

Supplemental for
Effects of quercetin on cadmium-induced toxicity in rat urine using
metabonomics techniques

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Supplementary Table S1. The water consumption of rats at each time point.

Time	water consumption (ml)					
	C	Q1	Q2	D	DQ1	DQ2
Week 1	37.07 ±2.77	35.26 ±3.84	36.34 ±2.54	36.80 ±3.89	38.21 ±3.15	38.09 ±3.61
Week 3	43.00 ±2.76	40.87 ±2.68	41.29 ±2.38	42.73 ±4.12	43.91 ±3.61	43.26 ±4.69
Week 5	46.79 ±2.48	46.43 ±2.40	45.53 ±2.31	45.43 ±3.00	47.51 ±2.09	47.32 ±4.12
Week 7	49.57 ±2.36	48.64 ±2.12	48.71 ±1.98	48.92 ±2.28	50.39 ±1.58	50.10 ±1.91
Week 9	50.09 ±1.47	50.01 ±2.37	49.49 ±1.65	49.87 ±2.54	50.34 ±2.29	49.74 ±2.23
Week 11	49.53 ±2.31	49.73 ±1.09	50.16 ±2.44	49.55 ±1.16	49.72 ±1.42	50.07 ±1.32
Week 12	50.10 ±2.21	50.28 ±1.54	50.82 ±1.96	50.32 ±2.08	50.72 ±1.43	51.20 ±1.12

C: control group; Q1: low-dose quercetin-treated group; Q2: high-dose quercetin-treated group; D: Cd-treated group; DQ1: low-dose quercetin plus Cd-treated group; DQ2: high-dose quercetin plus Cd-treated group. Values expressed as mean ± SD (n=10).

Supplementary Table S2. The rat body weights at each time point.

Time	Body weight (g)					
	C	Q1	Q2	D	DQ1	DQ2
Week 0	201.40 ±5.95	203.15 ±12.17	204.05 ±10.03	204.25 ±9.46	203.55 ±12.49	206.20 ±7.67
Week 4	331.39 ±21.33	321.31 ±21.86	330.19 ±21.94	318.60 ±19.20	319.73 ±17.18	315.19 ±8.71
Week 8	481.29 ±33.33	459.38 ±36.33	481.00 ±37.56	464.40 ±23.18	457.00 ±21.56	461.05 ±19.49
Week 12	557.58 ±36.01	550.98 ±34.82	562.93 ±32.51	542.70 ±20.54	545.25 ±34.38	539.00 ±31.36

C: control group; Q1: low-dose quercetin-treated group; Q2: high-dose quercetin-treated group; D: Cd-treated group; DQ1: low-dose quercetin plus Cd-treated group; DQ2: high-dose quercetin plus Cd-treated group. Values expressed as mean ± SD (n=10).

Supplementary Table S3. The results from CV-ANOVA of the positive PLS-DA model

	SS	DF	MS	F	P	SD
Total corrected	59	59	1			1
Regression	39.1215	8	4.89019	12.5462	9.57639e-010	2.21138

CV: cross-validation; ANOVA: analysis of variance; SS, sum of squares of deviation from mean; DF, degrees of freedom; MS, mean square; SD, standard deviation.

Supplementary Table S4. The results from CV-ANOVA of the negative PLS-DA model

	SS	DF	MS	F	P	SD
Total corrected	59	59	1			1
Regression	42.4968	8	5.3121	16.4161	1.05253e-011	2.3048

CV: cross-validation; ANOVA: analysis of variance; SS, sum of squares of deviation from mean; DF, degrees of freedom; MS, mean square; SD, standard deviation.

Supplementary Table S5. Summary of intensity values of biomarkers identified in the rat urine in positive and negative modes.

Group	Taurine	Phosphocreatine	L-Carnitine	LPC	UA	GSA
C	12427.3±1071.1	4293.3±176.1	8405.3±884.8	403.1±38.4	231.48±11.87	1478.6±141.4
Q1	12424.3±843.5	4330.8±98.4	8437.2±624.3	398.2±36.1	232.76±10.64	1462.4±75.1
Q2	12430.1±697.7	4263.0±179.2	8400.3±485.0	391.5±34.9	230.65±9.91	1472.0±119.5
D	9056.5±535.5**	3658.0±157.2**	4961.6±479.2**	521.6±51.5**	87.08±8.95**	1908.2±138.3**
DQ1	9115.7±577.7**	3682.3±217.9**	5394.6±350.1**	514.7±38.8**	90.90±5.75**	1876.1±86.7**
DQ2	11112.0±749.9**, ##	4006.8±335.9**, ##	7119.3±503.3**, ##	448.4±37.2*, ##	155.92±13.48**, ##	1702.7±167.2**, ##

C, control group; Q1, low-dose quercetin-treated group; Q2, high-dose quercetin-treated group; D, Cd-treated group; DQ1, low-dose quercetin plus Cd-treated group; DQ2, high-dose quercetin plus Cd-treated group. Values expressed as mean ± SD (n = 10).

* Significantly different from the control group at $p < 0.05$ (one-way ANOVA). ** Significantly different from the control group at $p < 0.01$ (one-way ANOVA).

Significantly different from the Cd-treated group at $p < 0.05$ (one-way ANOVA). ## Significantly different from the Cd-treated group at $p < 0.01$ (one-way ANOVA).

Supplementary Table S5. (continue) Summary of intensity values of biomarkers identified in the rat urine in positive and negative modes.

Group	DA	Alpha-CEHC	Allantoic acid	Quercetin 3-O-glucuronide
C	1758.5±106.6	1248.4±86.4	33238.2±1404.0	-
Q1	1778.8±112.3	1232.5±101.5	33052.0±1528.4	374.9±35.2 ^{**, ##}
Q2	1777.4±106.1	1237.1±110.5	33086.6±997.1	504.5±53.0 ^{**, ##}
D	2258.6±120.6 ^{**}	1794.8±102.0 ^{**}	36030.2±1643.2 ^{**}	-
DQ1	2172.8±111.6 ^{**}	1758.1±116.8 ^{**}	35827.6±1206.3 ^{**}	363.0±34.0 ^{**, ##}
DQ2	1955.0±160.2 ^{**, ##}	1363.13±82.36 ^{*, ##}	34461.5±1203.9 ^{*, ##}	519.1±36.8 ^{**, ##}

C, control group; Q1, low-dose quercetin-treated group; Q2, high-dose quercetin-treated group; D, Cd-treated group; DQ1, low-dose quercetin plus Cd-treated group; DQ2, high-dose quercetin plus Cd-treated group. Values expressed as mean ± SD (n = 10).

* Significantly different from the control group at $p < 0.05$ (one-way ANOVA). ** Significantly different from the control group at $p < 0.01$ (one-way ANOVA).

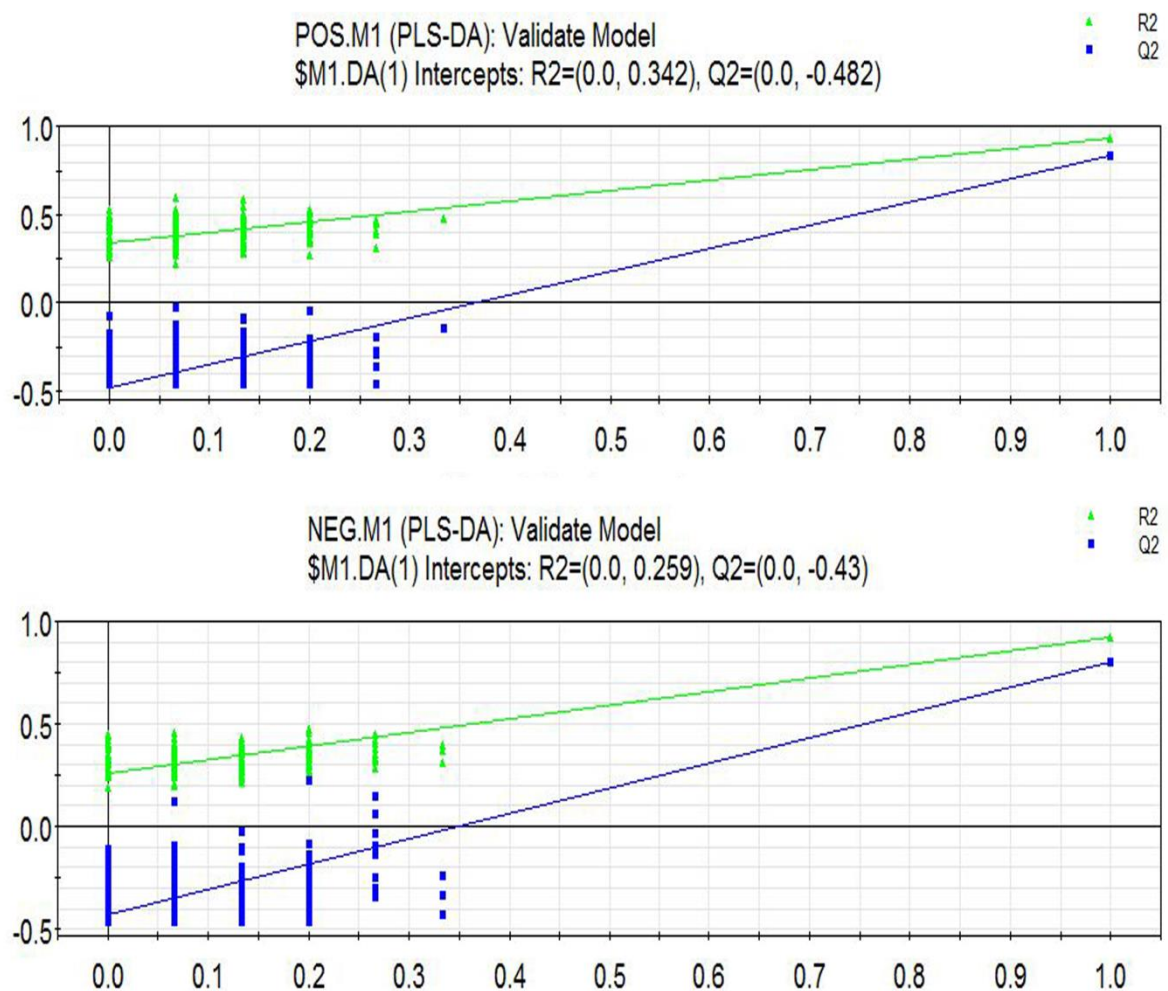
Significantly different from the Cd-treated group at $p < 0.05$ (one-way ANOVA). ## Significantly different from the Cd-treated group at $p < 0.01$ (one-way ANOVA).

Supplementary Table S6. Mass fragment information of potential biomarkers.

Metabolite identification	Scan mode	Accurate mass (Da)	Fragmentation pattern (Da)
L-Carnitine	+	162.11	118.12, 102.09, 98.10,
Allantoic acid	+	177.06	160.04, 132.04, 131.06, 91.05
Alpha-CEHC	+	279.16	231.14, 165.09, 161.06, 127.01
Dopamine	+	154.09	138.06, 137.06, 136.08, 123.06
Taurine	+	126.00	108.99, 73.35, 62.41
Phosphocreatine	+	212.04	194.03, 116.05, 114.07, 98.00
Quercetin 3-O-glucuronide	+	479.08	433.08, 303.05, 301.03
Guanidinosuccinic acid	+	176.07	158.02, 130.06, 60.22
Uric acid	—	167.02	149.99, 124.01, 122.00, 96.02
LysoPC(18:2(9Z,12Z))	—	518.32	505.27, 504.27, 279.22

Supplementary Table S7 The information of potential biomarkers.

Metabolite	Function / Related Pathways	Referenced Website/Database
L-Carnitine	• Plays a critical role in energy metabolism • An important component in lipid metabolism	HMDB, KEGG
Allantoic acid	• Related to oxidative stress	KEGG
Alpha-CEHC	• Related to inflammatory in liver	KEGG
Dopamine	• Affects the nervous system	MetaboAnalysis/ HMDB, KEGG
Taurine	• Inhibits fatty acid-induced lipid accumulation • Maintains normal lipids • Affects energy metabolism	MetaboAnalysis/ HMDB, KEGG
Phosphocreatine	• Plays a critical role in intracellular energy transfer systems	MetaboAnalysis/ HMDB, KEGG
Quercetin 3-O-glucuronide	• Has protective effects on neurodegeneration or neurotoxicity	KEGG
Guanidinosuccinic acid	• Produces neural excitotoxicity • Causes renal failure	KEGG
Uric acid	• Increases the production of ROS • Activates NF-κB signalling in proximal tubular cells	MetaboAnalysis/ HMDB, KEGG
LysoPC(18:2(9Z,12Z))	• Induces inflammation • Promotes atherosclerosis • Induces the enhancement of oxidative stress	MetaboAnalysis/ HMDB, KEGG



Supplementary Figure S1. Permutation test result of the PLS-DA models in the positive (POS. M1) and negative modes (NEG. M1).

The goodness of fit (R^2Y) and predictive capability (Q^2) of the original model are indicated on the far right, which remain higher than those of the 200 permuted models to the left.