Received: 17 July 2019 | Revised: 28 November 2019 | Accepted: 8 January 2020

DOI: 10.1002/jbt.22448

WILEY

Serum metabonomics analysis of quercetin against the toxicity induced by cadmium in rats

Siqi Jia | Tong Guan | Xia Zhang | Yajing Liu | Yanli Liu | Xiujuan Zhao

Department of Nutrition and Food Hygiene, Public Health College, Harbin Medical University, Harbin, China

Correspondence
Xiujuan Zhao, Harbin Medical University, 194 Xuefu Road, Harbin, 150081 Heilongjiang, China.
Email: xiujuan_zhao@sina.com

Abstract

This study aimed to investigate the protective effect of quercetin against the toxicity induced by chronic exposure to low levels of cadmium in rats by an ultra performance liquid chromatography mass spectrometer. Rats were randomly divided into six groups as follows: control group (C), low dose of quercetin group (Q1: 10 mg/kg·bw), high dose of quercetin group (Q2: 50 mg/kg·bw), cadmium chloride group (D), low dose of quercetin plus cadmium chloride group (DQ1), and high dose of quercetin plus cadmium chloride group (DQ2). Cadmium chloride ($CdCl_2$) was administered to rats by drinking water ad libitum in a concentration of 40 mg/L. The final amount of $CdCl_2$ ingested was estimated from the water consumption data to be 4.85, 4.91, and 4.89 mg/kg·bw/day, for D, DQ1, and DQ2 groups, respectively. After a 12-week treatment, the serum samples of rats were collected for metabonomics analysis. Ten potential biomarkers were identified for which intensities were significantly increased or reduced as a result of the treatment. These metabolites included isorhamnetin 4′-O-glucuronide, 3-indolepropionic acid, tetracosahexaenoic acid, lysophosphatidylcholine (LysoPC) (20:5), lysoPC (18:3), lysophosphatidylethanolamine (LysoPE) (20:5/0:0), bicyclo-prostaglandin E2, sulpholithocholylglycine, lithocholyltaurine, and glycocholic acid. Results indicated that quercetin exerted a protective effect against cadmium-induced toxicity by regulating lipid and amino acid metabolism, enhancing the antioxidant defense system and protecting liver and kidney function.

KEYWORDS
cadmium, metabonomics, quercetin, rat serum, UPLC–MS

1 | INTRODUCTION

Cadmium is a heavy metal used in electroplating, paint, dyes, and metallurgy.[1] It inevitably enters the environment as a pollutant given its widespread use. Nonprofessionals are mainly exposed to cadmium through food, water, air and smoking.[2] Cadmium is transported throughout the body through blood circulation, and its main target organs are kidney, liver, testis, lung, placenta, bone, and pancreas.[3] Epidemiological data show that cadmium in the environment is associated with numerous diseases, including chronic kidney, heart, neurological system, and male reproductive system diseases.[4–6] Cadmium easily accumulates in the body because it has a long biological half-life (20–40 years) and a low excretion rate.[7] Therefore, cadmium toxicity and its attenuation have attracted widespread attention.

Quercetin is a natural flavonoid that is widely distributed in fruits and vegetables.[8] It has antioxidant, anti-inflammatory, antitumor, and antimutation physiological functions.[9–12] Epidemiologic studies have suggested that increasing quercetin intake can reduce the risk of certain chronic diseases, such as cardiovascular disease, osteoporosis, and diabetes.[13–17] Quercetin has a protective effect against several exogenous toxic compounds, including cadmium,

acrylamide, alcohol, and ferrous sulphate.[18–21] Quercetin can remarkably reduce serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and thiobarbituric acid reactant concentration, drastically increase liver tissue catalase (CAT), superoxide dismutase (SOD) activities, L-glutathione (GSH) level, and reduce the malondialdehyde (MDA) level in cadmium-treated rats.[22,23] However, studies on the antioxidant activity of quercetin and cadmium-induced oxidative stress have mainly focused on the blood or specific organs rather than the global systems of animals. Therefore, the effects of quercetin on cadmium-induced toxicity on the metabolic level should be studied systematically.

Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification."[24] The map constructed by using metabonomics techniques for measuring biological fluids (serum, urine) contains abundant biomarker information, which reflects the biological effects of chemical toxicity on different metabolic pathways. The information provided by metabonomics analysis can reveal the physiological and biochemical functional status of biological systems.[25–28] In our previous work, we subjected rats under long-term exposure to low levels of cadmium to serum metabonomics analysis and identified the potential serum biomarkers of cadmium-induced toxicity.[29] Metabonomics has been widely used in various fields, such as drug discovery, disease diagnosis, and food safety, given its advantages of high speed, resolution, and sensitivity.

Cadmium toxicity is related to oxidative stress[30] and quercetin has strong antioxidant biological effects,[31] which led us to pose a hypothesis that quercetin has a protective effect against cadmium-induced toxicity on the metabolic level. Therefore, the purpose of this study is to systematically investigate and clarify the mechanism of the protective effect exerted by quercetin against cadmium toxicity by using metabonomics techniques and provide a theoretical basis for the chemoprevention of cadmium toxicity.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Cadmium chloride (99.99% purity) and quercetin (98% purity) were obtained from Sigma-Aldrich (India). Leucine enkephalin was purchased from Sigma-Aldrich (St. Louis, MO). Methanol and acetonitrile (high-performance liquid chromatography [HPLC] grade) were supplied by Dikma Science and Technology Co. Ltd (Canada). HPLC-grade formic acid was purchased from Beijing Reagent Company (Beijing, China). Assay kits for serum ALT, AST, triglyceride (TG), and total cholesterol (TCHO) were obtained from Wako Pure Chemical Industries Ltd (Nagoya, Japan). Kits for SOD, CAT, GSH, and MDA purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Kits for phospholipase A2 (PLA2) and cyclooxygenase-2 (COX-2) purchased from Jiangsu Jingmei Biological Technology Co. Ltd (Jiangsu, China). Carboxymethylated cellulose (CMC) and other chemicals were of analytical grade. Deionized water was filtered using a Milli-Q system (Millipore, Billerica).

2.2 | Animal treatment

A total of 60 male Sprague-Dawley rats (180 ± 20 g) were provided by Vital Laboratory Animal Technology Co. Ltd (Beijing, China). The rats were housed individually in metabolic cages under the following controlled circumstances: temperature (22 ± 2℃), humidity (50%-60%), and time-controlled lighting (12 hours of light per day). The animals had free access to distilled water and the standard diet proposed by American Institute of Nutrition (AIN)-93M.

All experimental procedures were carried out in accordance with the international guidelines for care and use of laboratory animals, and the animal experimental protocol was approved by the Medical Ethics Committee of Harbin Medical University (HMUPHIRB 2015007).

The rats were acclimated for 1 week before the experiment. Rats were randomly divided into six groups, including C: the control, Q1: low-dose quercetin (10 mg/kg·bw), Q2: high-dose quercetin (50 mg/kg·bw), D: $CdCl_2$ (4.89 mg/kg·bw), DQ1: low-dose quercetin plus $CdCl_2$, and DQ2: high-dose quercetin plus $CdCl_2$. Each group contained 10 rats. Rats were weighed weekly and water consumption of each rat was recorded daily during the experiment.

The dose of quercetin are designed as low-dose (10 mg/kg.bw) and high-dose (50 mg/kg.bw), which was according to the minimum and maximum intake in some countries population.[32,33] The dose of $CdCl_2$ was selected based on the results of our previous research,[29] which can produce significant toxic effects on rats. $CdCl_2$ was administered to rats by drinking water ad libitum in a concentration of 40 mg/L. The final amount of $CdCl_2$ ingested was estimated from the water consumption data to be 4.85, 4.91, and 4.89 mg/kg.bw/day, for D, DQ1, and DQ2 groups, respectively. Rats were given normal distilled water in groups C, Q1, and Q2. Quercetin (10 mg/kg.bw and 50 mg/kg.bw) were dissolved in 0.5% CMC, the final concentrations of quercetin are 2 mg/mL and 10 mg/mL, respectively. Quercetin was given to rats via gavage in a volume of 5 ml/kg.bw in groups Q1, Q2, DQ1, and DQ2. Rats received 0.5% CMC through the same way in groups C and D. Quercetin and $CdCl_2$ were daily administered to the rats continually for 12 weeks. Water consumption of rat in every week showed no significant difference between the treatment group and the control group ($P > .05$; Table S1).

2.3 | Sample collection

At the end of the experiment, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (3 ml/kg.bw). Blood samples were collected from abdominal aorta and serum samples were obtained by centrifuging the blood at 3000 rpm for 15 minutes. Then, serum biochemical indicators (ALT, AST, TCHO, and TG) were

detected using Hitachi 7100 automated biochemical analyzer (Hitachi Co., Japan). Serum samples were kept at −80°C for metabonomics analysis and PLA2 detection.

The liver tissue of rats were collected and weighed, a portion of fresh tissue was used for histopathology examination, and the remaining liver tissue was snap-frozen in liquid nitrogen and stored at −80°C for cadmium content, COX-2, antioxidant indicators (SOD, CAT, and GSH) and lipid peroxidation indicator (MDA) detections.

2.4 | Detection of cadmium in the liver

The content of Cd in the liver was detected by atomic absorption spectrophotometry (AAS, ICE 3500; Thermo Fisher Scientific). Briefly, 500 mg samples were placed in conical flask with 10 mL acid ($HClO_4/HNO_3$ = 1/4, V/V), the sample was soaked for 12 hours. Next, samples were digested in digestion furnace (KXL-1010) until the liquid of digestion was colorless and transparent. The cooled samples were transferred to colorimetric tube and diluted to 10 mL with distilled water. Finally, the liquid of digestion were analyzed by AAS with the wavelength of 228.8 nm.

2.5 | Detection of antioxidant indices and lipid peroxidation indicator in liver

The liver tissues were homogenized in physiological saline and centrifuged at 2500 rpm for 10 minutes at 4°C. The supernatants were collected to detect the levels of MDA and GSH, and activities of SOD and CAT by using commercial kits following the manufacturer's instructions.

2.6 | Indicators related with metabolic pathways in serum and liver

One hundred milligrams of liver tissues was homogenized in 900 μL phosphate buffered saline, and centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatants were collected to detect the activity of COX-2 by using commercial kits according to the manufacturer's instructions.

Serum samples were centrifuged at 3000 rpm for 20 minutes at 4°C, the supernatant were collected to detect the activity of PLA2 by using commercial kits following the manufacturer's instructions.

2.7 | Histopathology

The fresh liver tissues were fixed in 10% formalin for 24 hours. The liver samples were dehydrated in a graded alcohol series (75%-100%), and cleared in xylene. The tissues were embedded in paraffin, sectioned at 5-μm thickness, and stained with haematoxylin–eosin. To avoid sampling errors, we prepared five histological sections from 10 different areas of each liver specimen. The sections were viewed and photographed using an Olympus optical microscope (Olympus BX53; Olympus Optical Co. Ltd., Japan).

2.8 | Serum metabonomics

Six hundred microliters of methanol was added to 300 μL serum, centrifuged at 12 000 rpm for 10 minutes at 4°C, and the supernatant was concentrated by vacuum concentrator (Christ RCV2-25 CDplus) for 4 hours, then reconstituted in 600 μL mixture of acetonitrile and water (1:2, v/v). The samples were vortexed for 2 minutes and centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatants were then transferred to autosampler vials for metabonomics analysis. At the same time, 10 μL of each sample was uniformly mixed as a quality control sample (QC). The stability of the system throughout the experiment was evaluated by analyzing QC sample, which was run five times at the start of the experiment to equilibrate the system, and then injected after every 10 sample runs to further monitor system performance.

2.8.1 | Chromatography

Waters' ACQUITY UPLC System (Waters Corp., Milford, MA) was used for chromatographic separation with a UPLC BEH C18 column (100 mm × 2.1 mm, i.d. 1.7 μm, Waters Corp). The volume of each sample entering the column was 2 μL, the autosampler and column temperatures were maintained at 4°C and 35°C, respectively. The flow rate was maintained at 0.45 mL/min. The UPLC mobile phase consisted of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B). Gradient elution was performed as follows: 2 to 20% B for 0 to 1.5 minutes, 20 to 70% B for 1.5 to 6 minutes, 70 to 98% B for 6 to 10 minutes, 98% B for 10 to 12 minutes, 98 to 70% B for 12 to 14 minutes, 70 to 2% B for 14 to 16 minutes.

2.8.2 | Mass spectrometry

Rat serum samples were analyzed in positive and negative modes using a Waters Xevo G2 Q-TOF Mass Spectrometry (Waters, Manchester, UK) with electrospray ionization, which has a full scan mode from m/z 50 to 1200 and 0 to 16 minutes in both positive and negative modes. Test condition: the desolvent gas flow rate was set to 900 L/h and cone gas rate was set to 50 L/h, respectively. The desolvation gas temperature and the source temperature were set to 450°C and 120°C, respectively. The capillary voltage was set to 0.5 kV for the positive and negative modes, the cone voltage was set to 30 V. To ensure accuracy mass acquisition, a solution of leucine enkephalin was used as the mass lock solution at a flow rate of 10 μL/min for monitoring in the positive ion mode ($[M+H]^+$ = 556.2771) and the negative ion mode ($[M-H]^-$ = 554.2615). The lock spray frequency is 10 seconds in the positive ion mode and 15 seconds in the negative ion mode.

2.8.3 | Data processing and metabolite identification

The ultra performance liquid chromatography mass spectrometer data were analyzed using the Progenesis QI Software (version 2.1; Waters Corporation). The metabolites were filtered based on the one-way analysis of variance (ANOVA), $P < .05$ and maximum fold change ≥2. Then, the selected data were imported into EZinfo software (version 2.0; Umetrics AB, Umeå, Sweden) for multivariate statistical analysis. Before multivariate statistical analysis, the data were mean-centered and Pareto-scaled. Principal component analysis (PCA) was performed on QC and other groups to visualize the data and determine the reproducibility and reliability of the approach. Then, the partial least-squares discriminant analysis (PLS-DA) model was established. To avoid the over-fitting of PLS-DA models, a default seven-fold cross-validation and testing with 200 random permutations in positive and negative modes were performed using SIMCA-P software (version 12.0; Umetrics AB). The metabolites with the variable importance in projection values above 1.0 were selected into Progenesis QI software for metabolite identification.

Preliminary identification of metabolites was performed by the Human Metabolome Database (HMDB; the mass tolerance was set at 10 ppm or 5 mDa). Finally, metabolites were confirmed by comparison of retention time, m/z, and fragmentation pattern with standard substances. Potential pathways for biomarkers are explained by the databases, including HMDB (http://www.hmdb.ca/) and METLIN (http://metlin.scripps.edu).

2.9 | Statistical analysis

Statistical analysis was performed using SPSS (version 21.0; Beijing Stats Data Mining Co. Ltd., China). All data results are expressed as mean ± standard deviation (SD). To examine the difference between control and experimental groups, ANOVA was selected when the homogeneity test of variance was satisfied, and the Kruskal–Wallis nonparametric test was selected on the contrary. $P < .05$ indicates that the difference is statistically significant. Receiver operating characteristic (ROC) curve analysis was plotted by SPSS, the area under the corresponding curve (AUC) was calculated to assess the predictive power (sensitivity and specificity) of the biomarker.

3 | RESULTS

3.1 | Viscera coefficient

The body weights of rats did not significantly differ between groups ($P > .05$) at each time point. As shown in Figure S1, the rat liver viscera coefficient was calculated (liver weight/body weight × 100) after the experiment. No significant difference was observed among all treatment groups and the control group ($P > .05$).

3.2 | Cadmium content of the liver

Cadmium content of the liver is shown in Figure S2. The cadmium contents of groups C, Q1, and Q2 were below the limit of quantitative detection (0.003 mg/kg). The cadmium content of the cadmium treatment groups significantly increased compared with that of group C ($P < .01$). The cadmium content of group DQ2 significantly decreased compared with that of group D ($P < .01$).

3.3 | Biochemical indices

Serum biochemical indicators, including ALT, AST, TG, and TCHO, were measured after the experiment (Table 1). The biochemical parameters of the Q1 and Q2 groups did not significantly differ

TABLE 1 Biochemical indices measured in the rat serum

Groups	ALT, U/L	AST, U/L	TG, mmol/L	TCHO, mmol/L
C	44.40 ± 5.44	108.50 ± 21.98	0.76 ± 0.30	1.85 ± 0.33
Q1	43.70 ± 3.77	103.40 ± 16.48	0.69 ± 0.32	1.78 ± 0.28
Q2	42.60 ± 3.27	102.60 ± 9.67	0.64 ± 0.26	1.64 ± 0.35
D	66.20 ± 3.85*	155.40 ± 16.06*	1.73 ± 0.39*	2.77 ± 0.53*
DQ1	65.50 ± 5.81*	146.70 ± 18.05*	1.56 ± 0.31*	2.69 ± 0.25*
DQ2	50.30 ± 5.40**#	130.60 ± 13.27**#	1.19 ± 0.30**#	2.25 ± 0.20**#

Note: C: control group, Q1: treated with low-dose quercetin, Q2: treated with high-dose quercetin, D: treated with $CdCl_2$, DQ1: treated with low-dose quercetin and $CdCl_2$, DQ2: treated with high-dose quercetin and $CdCl_2$. Values are mean ± SD (n = 10).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TCHO, total cholesterol; TG, triglyceride.

*Significantly different from the control group at $P < .01$ (one-way ANOVA).

**Significantly different from the control group at $P < .05$ (one-way ANOVA).

#Significantly different from the D group at $P < .01$ (one-way ANOVA).

TABLE 2 Enzymatic antioxidant activities and GSH, MDA levels in the rat liver

Groups	SOD (U/mgprot)	GSH (mgGSH/gprot)	CAT (U/mgprot)	MDA (nmol/mgprot)
C	364.19 ± 30.45	8.41 ± 1.92	33.40 ± 2.56	0.81 ± 0.29
Q1	367.03 ± 41.97	8.51 ± 2.02	34.67 ± 5.03	0.75 ± 0.36
Q2	378.66 ± 40.69	9.19 ± 1.88	35.55 ± 3.83	0.69 ± 0.16
D	265.60 ± 21.23*	3.80 ± 0.83*	20.22 ± 4.88*	1.53 ± 0.26*
DQ1	273.53. ± 40.00*	4.34. ± 1.33*	21.51. ± 5.44*	1.44 ± 0.23*
DQ2	317.20 ± 17.80**#	6.13 ± 1.27**#	27.66 ± 4.84**#	1.10 ± 0.26**#

Note: C: control group, Q1: treated with low-dose quercetin, Q2: treated with high-dose quercetin, D: treated with $CdCl_2$, DQ1: treated with low-dose quercetin and $CdCl_2$, DQ2: treated with high-dose quercetin and $CdCl_2$. Values are mean ± SD (n = 10).
Abbreviations: CAT, catalase; GSH, L-glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.
*Significantly different from the control group at $P < .01$ (one-way ANOVA).
**Significantly different from the control group at $P < .05$ (one-way ANOVA).
#Significantly different from the D group at $P < .01$ (one-way ANOVA).

from those of the control group ($P > .05$) and were significantly higher in group D compared with those in group C ($P < .01$). The indicators in group DQ2 were significantly lower than those in group D ($P < .01$) and were significantly different from those in group C ($P < .05$).

3.4 | Antioxidant indices and lipid peroxidation indicator

Antioxidant enzyme activities (SOD, CAT) and GSH, MDA levels in the rat liver were measured at the end of the experiment (Table 2). The indicators of groups Q1 and Q2 were not significantly different from those of group C ($P > .05$). SOD, CAT activities, and GSH level significantly decreased ($P < .01$) and MDA level significantly increased ($P < .01$) in group D relative to those in group C. SOD, CAT activities, and GSH level significantly increased ($P < .01$), MDA level significantly decreased ($P < .01$) in group DQ2 relative to those in group D. The levels of these indices in group DQ2 were statistically significantly different from those in group C ($P < .05$).

3.5 | Histopathology

No clear histopathological changes were found in liver tissues from groups C, Q1, and Q2. Histopathological analysis revealed steatosis and hydropic degeneration, inflammatory cell infiltration, and small necrotic foci in groups D, DQ1, and DQ2. Histopathological changes in group DQ2 were less severe than those in groups D and DQ1, and only a small amount of concentrated cells were observed (Figure 1).

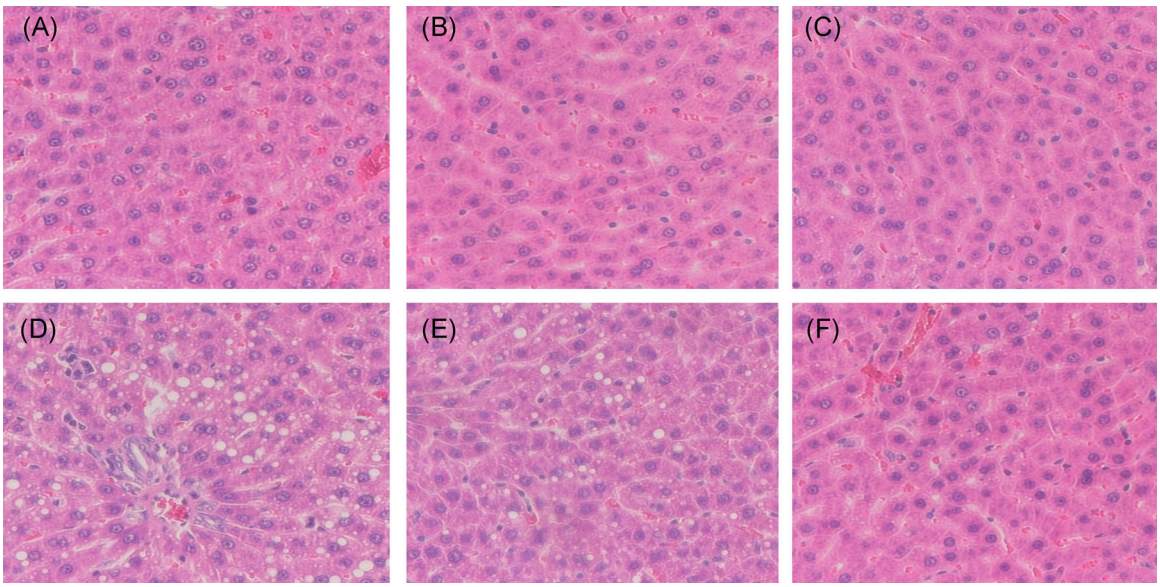

FIGURE 1 The photomicrographs of liver (×200). A, Control group. B, Treated with low-dose quercetin. C, Treated with high-dose quercetin. D, Treated with $CdCl_2$. E, Treated with low-dose quercetin and $CdCl_2$. F, Treated with high-dose quercetin and $CdCl_2$

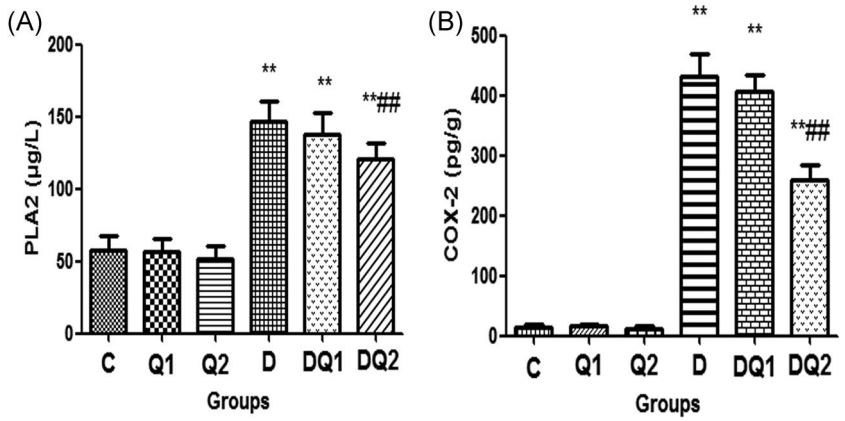

FIGURE 2 Activities of phospholipase A2 (PLA2) in serum (A) and cyclooxygenase-2 (COX-2) in liver (B) of rats in each group. C, Control group, Q1: Treated with low-dose quercetin, Q2: Treated with high-dose quercetin. D, Treated with $CdCl_2$, DQ1: Treated with low-dose quercetin and $CdCl_2$, DQ2: Treated with high-dose quercetin and $CdCl_2$. Values are mean ± SD (n = 10). *Significantly different from the control group at $P < .05$ (one-way ANOVA). **Significantly different from the control group at $P < .01$ (one-way ANOVA). #Significantly different from the D group at $P < .05$ (one-way ANOVA). ##Significantly different from the D group at $P < .01$ (one-way ANOVA)

3.6 | Indicators related to metabolic pathways

The activities of PLA2 in the serum and those of COX-2 in the liver are illustrated in Figure 2. The activities of PLA2 and COX-2 significantly increased in group D relative to those in group C ($P < .01$). The activities of PLA2 and COX-2 significantly decreased ($P < .01$) in group DQ2 relative to those in group D and were still significantly different from those in group C ($P < .01$).

3.7 | Metabolic profiling through UPLC–MS analysis

Serum samples were analyzed through UPLC–MS in positive and negative modes. The PCA score plots obtained in positive and negative modes are shown in Figure S3. QC samples were tightly clustered. Six ions (m/z 174.9388, 272.1381, 393.1441, 490.2297, 509.2221, and 675.5060 in the positive mode) were selected from the chromatographic peaks to validate the reliability of the method. The relative standard deviations of peak intensity, retention time, and m/z were 0.23 to 1.33%, 0.16 to 0.71%, and 0.13 to 0.24%, respectively. These findings show that the run was stable. Subsequently, the PLS-DA score plots of both modes were used to compare differences among samples from the control and treatment groups. As shown in Figure 3, groups D, DQ1, and DQ2 were separated from group C, but groups C, Q1, and Q2 did not present remarkable separation. Overlap between groups D and DQ1 can still be observed, but groups D and DQ2 are clearly separated. Subsequently, PLS-DA verification models in the positive and negative modes were established to prevent model overfitting (Figure S4). The parameters of R2X, R2Y, and Q2 in the positive mode were 0.749, 0.947, and 0.914 (four components). The parameters of R2X, R2Y, and Q2 in the negative mode were 0.810, 0.955, and 0.911 (four components). The point of the blue (Q2) and green line (R2Y) on the left was lower than that on the right side. This finding shows that these models were at a low risk of overfitting. In addition, the results of CV-ANOVA indicate that the PLS-DA models were highly significant (Tables S2 and S3).

Twenty-seven metabolites, including nine in the positive mode and eighteen in the negative mode, were initially identified by using Progenesis QI. Ten biomarkers were finally identified (three in positive mode and seven in negative mode) by using above metabolite identification methods. Information on these biomarkers is shown in Table 3 and Table S4. The intensity values of metabolites identified in the positive and negative modes are shown in Tables S5 and S6. Tetracosahexaenoic acid (THA), 3-indolepropionic acid, and lysoPC (20:5) were identified in the positive mode. Glycocholic acid, lithocholyltaurine, sulpholithocholylglycine, bicyclo-prostaglandin (PG) E2, lysoPC (18:3), lysoPE (20:5/0:0), and isorhamnetin 4′-O-glucuronide were identified in the negative mode. In groups Q1, Q2, DQ1, and DQ2, there was a positive association suggested between the intensities of isorhamnetin 4′-O-glucuronide and the dose of quercetin. The intensities of 3-indolepropionic acid and THA significantly decreased ($P < .01$) and those of the seven other metabolites significantly increased ($P < .01$) in group D relative to those in group C. The intensities of 3-indolepropionic acid and THA increased ($P < .01$ or $P < .05$) and those of the seven other metabolites significantly decreased in group DQ2 ($P < .01$ or $P < .05$) compared with those in group D. Except for those of isorhamnetin 4′-O-glucuronide, the intensities of nine metabolites in group DQ2 were significantly different from those in group C ($P < .05$ or $P < .01$). The intensities of the above metabolites did not significantly differ between groups D and DQ1 ($P > .05$) or among groups C, Q1, and Q2 ($P > .05$).

The ROC curve was used to evaluate the predictive ability of biomarkers. The ROC curve with 1-specificity was the abscissa, and

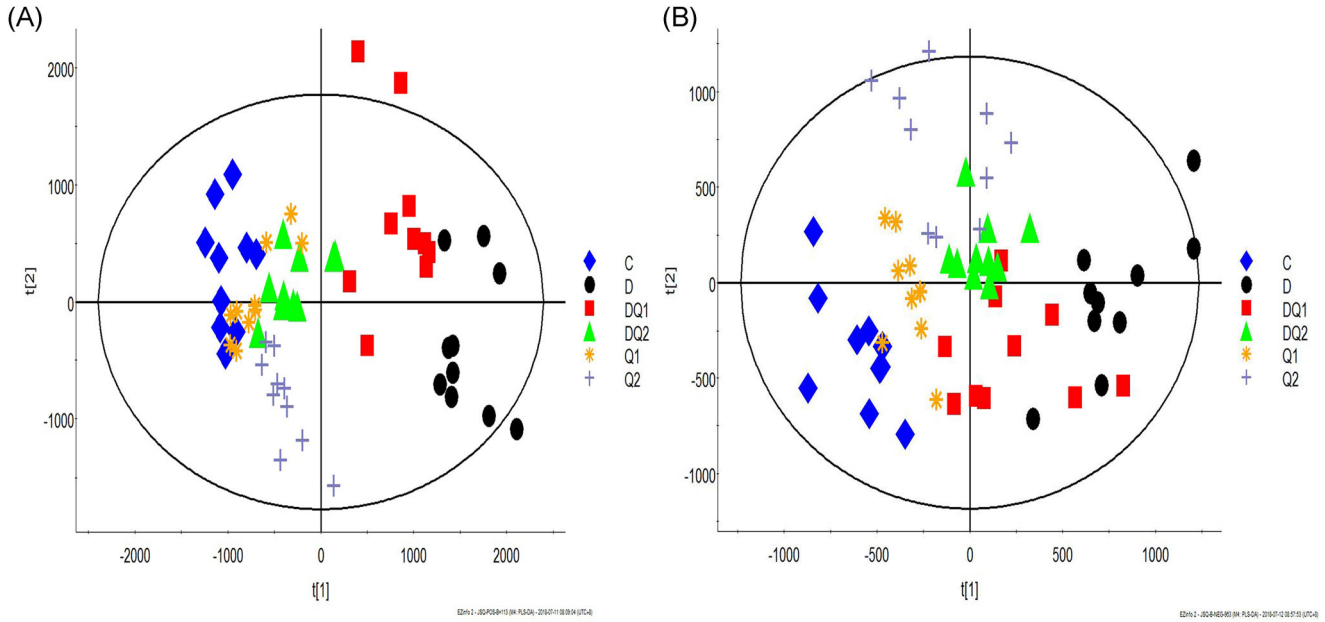

FIGURE 3 Partial least-squares discriminant analysis score plots resulting from ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry spectra of the rat serum in positive (A) and negative modes (B). Blue diamonds, control group (C); stars, treated with low-dose quercetin (Q1); crosses, treated with high-dose quercetin (Q2); black circles, treated with $CdCl_2$(D); red squares, treated with low-dose quercetin and $CdCl_2$ (DQ1); green triangles, treated with high-dose quercetin and $CdCl_2$ (DQ2). N = 10

the sensitivity was the ordinate. The AUC of all metabolites were above 0.9 in the 95% confidence interval. This result indicates that the biomarkers have at least good predictive abilities (Figure 4).

4 | DISCUSSION

The PLA-DA score plots (Figure 3) obtained in this study show a significant separation between groups D and C. This separation indicates that cadmium exposure caused toxicity on the metabolic level. The significant separation of group D from group DQ2 indicates that high doses of quercetin affected the toxicity induced by cadmium. Ten potential biomarkers were identified. The effect of these biomarkers and the protective effect of quercetin on cadmium-induced toxicity are mainly exerted through the four following metabolic pathways (Figure 5).

4.1 | The first pathway involves the effect of quercetin on cadmium-induced oxidative stress

Free radicals are metabolites that are normally present in living organisms. The excess or imbalance of free radicals may cause aging, inflammation, cancer, and cardio-cerebrovascular diseases. LysoPC

TABLE 3 Serum biomarkers identified after treatment in positive and negative mode

RT, min	Measured mass, Da	Calculated mass, Da	Error, Da	Elemental composition	Scan mode	Metabolites
3.65	190.0877	190.0868	0.0009	C11H11NO2	+	3-Indolepropionic acid[ab]
7.50	357.2805	357.2793	0.0012	C24H36O2	+	Tetracosahexaenoic acid[ab]
6.57	542.3251	542.3246	0.0005	C28H48NO7P	+	LysoPC (20:5)[ab]
4.24	464.3025	464.3012	0.0013	C26H43NO6	−	Glycocholic acid[b]
4.63	482.2943	482.2940	0.0003	C26H45NO5S	−	Lithocholyltaurine[ab]
4.71	512.2689	512.2682	0.0007	C26H43NO7S	−	Sulfolithocholylglycine[ab]
4.42	333.2070	333.2066	0.0004	C20H30O4	−	Bicyclo-PGE2[ab]
6.28	498.2623	498.2621	0.0002	C25H42NO7P	−	LysoPE (20:5/0:0)[ab]
7.75	516.3094	516.3090	0.0004	C26H48NO7P	−	LysoPC (18:3)[ab]
4.09	491.0826	491.0825	0.0001	C22H20O13	−	Isorhamnetin 4′-O-glucuronide[b]

[a]The ions were identified by comparison to the standards.
[b]The ions were identified by comparison to the metabolites of the Human Metabolome Database (HMDB).

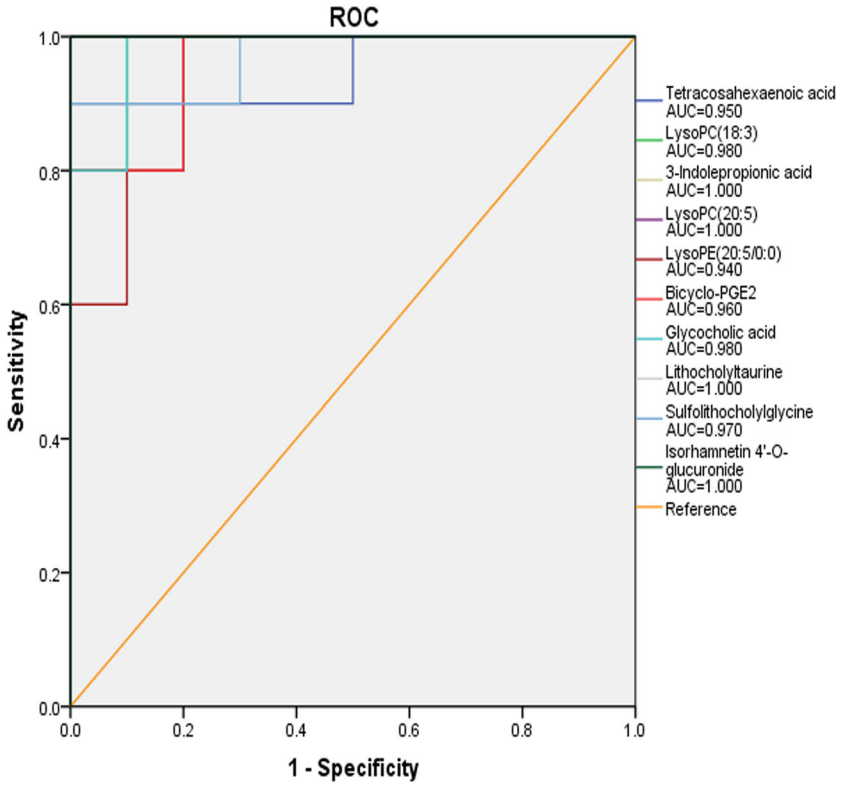

FIGURE 4 Receiver operator characteristic curve analysis of the 10 metabolites

is one of the metabolites of phosphatidylcholine (PC). It is a proinflammatory factor that can increase the production of reactive oxygen species (ROS).[34] 3-Indolepropionic acid is a product of tryptophan deamination and is a free radical scavenger that can effectively prevent oxidative damage. Elevated 3-indolepropionic acid levels can prevent lipid peroxidation.[35] THA is an ω-3 polyunsaturated fatty acid and an intermediate of the metabolism of eicosapentaenoic acid to docosahexaenoic acid (DHA). DHA is an

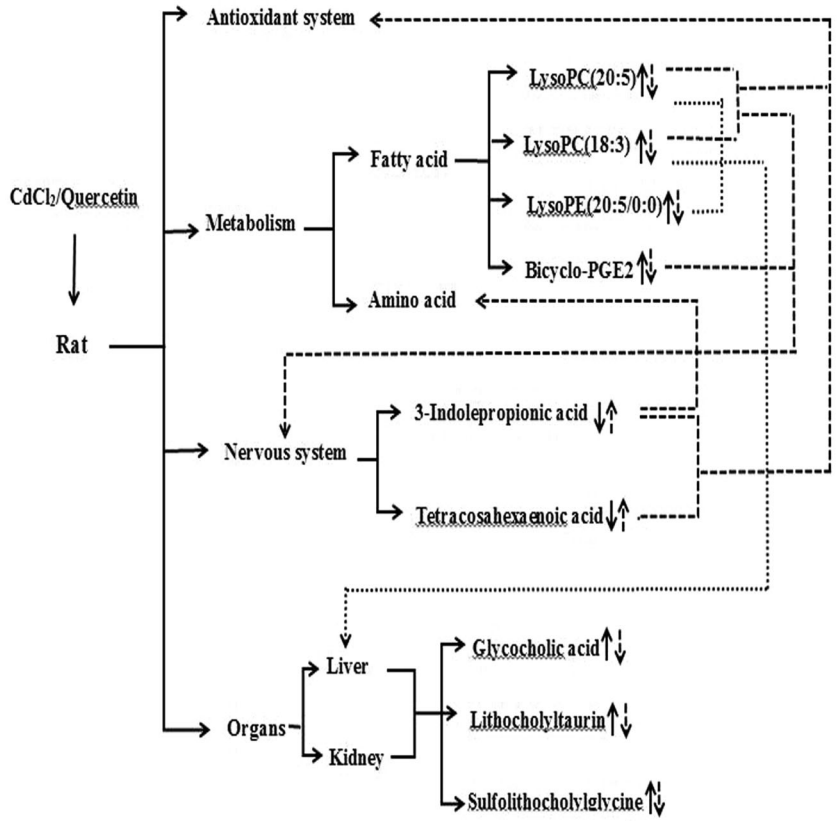

FIGURE 5 Metabolic pathways in response to $CdCl_2$ and/or quercetin treatment

essential fatty acid that can effectively reduce ROS levels in the body. In this study, the intensities of LysoPC significantly increased and those of 3-indolepropionic acid and THA significantly decreased in group D relative to those in group C. These results indicate that cadmium exposure intensifies the oxidative damage experienced by the body. Quercetin has a powerful antioxidant effect and can resist oxidative damage by scavenging ROS.[36] The intensities of above biomarkers in group DQ2 were decreased or increased than those in group D. These results indicate that quercetin has a protective effect against cadmium-induced oxidative stress.

SOD, CAT, and GSH are important antioxidants that can scavenge free radicals. MDA can be used to understand the extent of membrane lipid peroxidation. In our study, the changes in the activities of SOD, CAT, and the levels of GSH, MDA indicate that quercetin had a certain protective effect against cadmium-induced oxidative damage. The results further supported our metabonomics results.

4.2 | The second pathway involves the effect of quercetin on cadmium-induced abnormal lipid metabolism

Lipid metabolism is one of the important physiological functions of the liver. Glycerol phospholipid is the most abundant phospholipid in the body and a component of bile and membrane surfactants. PC and phosphatidylethanolamine (PE) are the most abundant glycerol phospholipids and play a vital role in protein recognition by cell membranes and signal transduction. PLA2 hydrolyzes PC and PE to produce LysoPC and LysoPE. LysoPE levels are low under normal conditions. LysoPE will be released into the blood when the cell membrane is hydrolyzed and destroyed.[37,38] PLA2 is a rate-limiting enzyme in the production of biologically active substances, such as arachidonic acid (ARA), PGs, and platelet-activating factor. Therefore, PLA2 plays an important role in regulating lipid metabolism. The activation of PLA2 causes potentially cytotoxic lysophospholipids (LysoPC and LysoPE) to be released into the blood and then induces lipid metabolism disorders. We speculated that the significant increases in LysoPC and LysoPE intensities in group D observed in this study may be related to enhanced PLA2 activity.[39] Studies have shown that quercetin can inhibit PLA2 activity.[40,41] In this study, the changes in the activity of PLA2 and the intensities of LysoPC, LysoPE indicate that cadmium affected lipid metabolism and high doses of quercetin exert a protective effect against cadmium-induced lipid metabolism disorders.

ARA is a polyunsaturated fatty acid. PG is one of the main metabolites of ARA. ARA is decomposed into its free form by PLA2 and is released into cellular fluid under physiological and pathological stimulation. Free ARA reacts with COX. Finally, PGE2 is produced through a series of metabolic processes. COX-2 is a rate-limiting enzyme that catalyzes the conversion of ARA to PG. It is only expressed when cells are stimulated by proinflammatory factors and by brain damage. PGE2 levels in the blood or tissue are low under normal conditions and drastically increase when the body is affected by external toxicants. High PGE2 levels are associated with pathological and physiological processes, such as inflammation and tumorigenesis. Bicyclo-PGE2 is the final product of PGE2 metabolism. Cadmium exposure can promote PGE2 synthesis, which increases bicyclo-PGE2 levels. In this study, the increase in bicyclo-PGE2 intensity in group D relative to that in group C may be related to the cadmium-induced upregulation of PLA2 and COX-2. The enhancement in PLA2 and COX-2 activities in group D confirms this hypothesis (Figure 2). Quercetin can effectively inhibit the transcriptional activity of the COX-2 promoter.[42] PLA2, COX-2 activities, and bicyclo-PGE2 intensity significantly decreased in group DQ2 relative to those in group D. These suggest that quercetin prevents cadmium-induced lipid metabolism disorders. The results for serum lipid indices (TCHO and TG; Table 1) further support the above metabonomics results.

4.3 | The third pathway involves the effect of quercetin on cadmium-induced amino acid metabolism and nervous system disorders

Tryptophan is an essential amino acid and is a precursor of angiotensin, tryptamine, nicotinic acid, coenzyme I, and coenzyme II. It has numerous physiological activities, such as antistress, antioxidation, and immune response. 3-Indolepropionic acid is produced through tryptophan deamination and is found in human plasma and cerebrospinal fluid.[43] 3-Indolepropionic acid is a neuroprotective agent that can protect primary neurons and neuroblastoma cells from oxidative damage. 3-Indolepropionic acid was identified for which intensity was increased or reduced as a result of the treatment indicates that quercetin exerts a protective effect against cadmium-induced nervous system damage by regulating the metabolism of certain amino acids.

DHA is an essential fatty acid and is an important component of the nervous system cell membrane. It has numerous physiological functions, such as anticancer and anti-inflammatory effects, and acts against cardiovascular diseases. The ability of the liver to synthesize DHA is the key to maintaining the level of THA in the brain. ω-3 Polyunsaturated fatty acids have protective effects against central nervous system diseases, and high doses of DHA can reduce the risk of dementia among the elderly.[44]

Pericytes play pivotal roles in the central nervous system under physiological and pathophysiological conditions. They can prevent neuronal damage stemming from a compromised blood–brain barrier. LysoPC leads to pericyte loss in vivo.[45] LysoPC also can induce neurotoxicity through oligodendrocyte demyelination and can promote neurotoxic protein aggregation.[46–50] LysoPC levels are drastically elevated in patients with brain injury.[51]

COX-2 is widely involved in brain trauma, ischemia-induced neuronal damage, inflammatory responses, and neurodegenerative diseases. The role of COX-2 in neuropathology is related to changes

in neuronal synapses.[52] The abnormal activation of COX-2 promotes the progression of Alzheimer's disease.[53,54]

In this study, THA intensity significantly decreased, LysoPC intensities and COX-2 activity increased in group D compared with those in group C. These results indicate that cadmium induced nervous system damage. Quercetin exerts a multiplicity of neuroprotective actions within the brain by suppressing neuroinflammation and protecting neurons against neurotoxin-induced injury.[55] The restorative changes in the intensity of the above metabolites and the activity of COX-2 in group DQ2 relative to those in group D indicate that quercetin prevented cadmium-induced nervous system damage.

4.4 | The fourth pathway involves the effect of quercetin on cadmium-induced liver and kidney damage

The liver is an important organ for maintaining cholesterol balance. Bile acids, which are synthesized by cholesterol of liver and excreted into bile for promoting the lipid absorption in intestine. Most of intestinal bile acids are reabsorbed in the ileum and recirculated through the portal vein to the liver, this formed bile acid enterohepatic circulation.[56] Enterohepatic circulation is the main pathway for the elimination of steroids from the body and plays an important role in regulating bile acid homeostasis. Enterohepatic circulation disorders develop when the liver is damaged and bile acid in the intestine cannot return to the liver. The latter effect drastically increases the level of bile acid. Bile acids can directly activate signaling pathways in hepatocytes that stimulate the production of proinflammatory mediators, including PGE2; inflammation contributes to liver injury during cholestasis.[57] Glycocholic acid, lithocholyltaurine, and sulpholithocholylglycine are combined bile acids. In this study, the significantly increased intensities of above bile acids in group D relative to those in group C indicate that cadmium caused liver dysfunction. Quercetin can inhibit the production of proinflammatory factors, such as COX-2.[58,59] The intensities of the above bile acids and the activity of COX-2 decreased in group DQ2 in comparison with those in group D. These results indicate that quercetin prevented cadmium-induced liver damage. The results for serum biochemical indicators (ALT, AST; Table 1) and histopathological changes (Figure 1) further support the above metabonomics results.

Kidney is an important organ of the urinary system, and is deemed to play a certain role in bile acid excretion.[60] Bile acid is filtered through the glomerulus. The serum levels of bile acid increase when the filtration of bile acid through glomeruli is reduced.[60] Renal dysfunction can cause the metabolic disorders of bile acids. Many studies have shown the increase of serum bile acid level and alteration of bile acid homeostasis in both clinical and animal model studies on chronic renal failure.[61] Bile acid is an essential pathogenetic factor in cholemic nephropathy[62] and the mechanism may be related with oxidative stress, inflammation, and membrane alterations.[63] Bile acid cause oxidative damage to tubular cell membranes by stimulating the production of ROS from mitochondria.[64] Oxidative stress can promote the formation of vasoactive mediators. These mediators can affect renal function by causing renal vasoconstriction.[65,66] In our study, the intensities of glycocholic acid, lithocholyltaurine, and sulpholithocholylglycine significantly increased in group D compared with those in group C, suggesting kidney damage in the cadmium treatment group. The pathogenetic mechanism of kidney damage caused by bile acids alteration should be addressed in the further study. When high-dose quercetin was given to rats with $CdCl_2$, the intensities of above bile acids in group DQ2 obviously decreased relative to those in group D, which indicate quercetin exerts a protective effect against cadmium-induced kidney damage. As a potent antioxidant, quercetin may attenuate cadmium-induced kidney damage by renewing mitochondrial function, elevation of the enzymic antioxidants levels and attenuating lipid peroxidation.[67]

5 | CONCLUSIONS

This study applied metabonomics technology to explore the protective effect of quercetin against the toxicity induced in rats by chronic exposure to low levels of cadmium. The results of this study demonstrate that quercetin exerts a protective effect against cadmium-induced toxicity by regulating lipid and amino acid metabolism, enhancing antioxidant defense systems and protecting liver and kidney function.

ACKNOWLEDGMENT

The authors specially thank the Laboratory of Nutrition and Food Hygiene in Harbin Medical University. It is the key laboratory of Heilongjiang Province and Heilongjiang Higher Education Institutions.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTION

Siqi Jia designed the study plan, supervised all parts of this project, and did the final edition of the manuscript. Siqi Jia, Yajing Liu, and Yanli Liu carried out the experimental work. Xia Zhang and Tong Guan analyzed data and manuscript modification. Xiujuan Zhao contributed to conception or design, and is accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

ORCID

Xiujuan Zhao http://orcid.org/0000-0001-6953-4148

REFERENCES

[1] M. O. Alese, M. A. Agbaje, O. O. Alese, *J. Trace Elem. Med. Biol.* **2018**, *50*, 276.
[2] A. Ashok, N. K. Rai, S. Tripathi, S. Bandyopadhyay, *Toxicol. Sci.* **2015**, *143*, 64.
[3] H. Wang, R. Zhang, Y. Song, T. Li, M. Ge, *Biol. Trace Elem. Res.* **2019**, *187*, 281.
[4] M. P. Waalkes, *Mutat. Res.* **2003**, *533*, 107.
[5] G. M. Buck Louis, R. Sundaram, E. F. Schisterman, A. M. Sweeney, C. D. Lynch, R. E. Gore-Langton, Z. Chen, S. Kim, K. L. Caldwell, D. B. Barr, *Chemosphere* **2012**, *87*, 1201.
[6] E. García-Esquinas, M. Pollan, M. Tellez-Plaza, K. A. Francesconi, W. Goessler, E. Guallar, J. G. Umans, J. Yeh, L. G. Best, A. Navas-Acien, *Environ. Health Perspect.* **2014**, *122*, 363.
[7] D. Tucovic, A. Popov Aleksandrov, I. Mirkov, M. Ninkov, J. Kulas, L. Zolotarevski, V. Vukojevic, J. Mutic, N. Tatalovic, M. Kataranovski, *Ecotoxicol. Environ. Saf.* **2018**, *164*, 12.
[8] M. Zhuang, H. Qiu, P. Li, L. Hu, Y. Wang, L. Rao, *Drug Des. Devel. Ther.* **2018**, *12*, 955.
[9] O. L. Woodman, E. Chan, *Clin. Exp. Pharmacol. Physiol.* **2004**, *31*, 786.
[10] S. Chirumbolo, *Inflamm. Allergy. Drug. Targets.* **2010**, *9*, 263.
[11] R. Sinha, S. Srivastava, A. Joshi, U. J. Joshi, G. Govil, *Eur. J. Med. Chem.* **2014**, *79*, 102.
[12] C. Burak, S. Wolffram, B. Zur, P. Langguth, R. Fimmers, B. Alteheld, P. Stehle, S. Egert, *Nutrition* **2019**, *58*, 47.
[13] K. Griffiths, B. B. Aggarwal, R. B. Singh, H. S. Buttar, D. Wilson, F. De Meester, *Diseases* **2016**, *4*, 28.
[14] L. Z. Xing, H. J. Ni, Y. L. Wang, *Biomed. Pharmacother.* **2017**, *89*, 1136.
[15] H. H. Donmez, N. Donmez, I. Kisadere, I. Undag, *Biotech. Histochem.* **2019**, *94*, 1.
[16] H. E. Eitah, Y. A. Maklad, N. F. Abdelkader, A. A. Gamal El Din, M. A. Badawi, S. A. Kenawy, *Toxicol. Appl. Pharmacol.* **2019**, *365*, 30.
[17] G. J. Shi, Y. Li, Q. H. Cao, H. X. Wu, X. Y. Tang, X. H. Gao, J. Q. Yu, Z. Chen, Y. Yang, *Biomed. Pharmacother.* **2019**, *109*, 1085.
[18] S. M. Prabu, K. Shagirtha, J. Renugadevi, *J. Food Sci.* **2010**, *75*, T132.
[19] L. Bo, Y. Liu, S. Jia, Y. Liu, M. Zhang, S. Li, X. Zhao, C. Sun, *Food Funct.* **2018**, *9*, 5965.
[20] L. Zhao, N. Zhang, D. Yang, M. Yang, X. Guo, J. He, W. Wu, B. Ji, Q. Cheng, F. Zhou, *Nutrients* **2018**, *10*, 1754.
[21] F. Gholampour, N. Saki, *Gen. Physiol. Biophys.* **2019**, *38*, 27.
[22] R. Li, C. Yuan, C. Dong, S. Shuang, M. M. Choi, *Naunyn Schmiedebergs Arch. Pharmacol.* **2011**, *383*, 437.
[23] S. Milton Prabu, M. Muthumani, K. Shagirtha, *Eur. Rev. Med. Pharmacol. Sci.* **2013**, *17*, 582.
[24] J. K. Nicholson, J. C. Lindon, E. Holmes, *Xenobiotica* **1999**, *29*, 1181.
[25] Z. Liu, H. Li, M. Soleimani, K. Girijashanker, J. M. Reed, L. He, T. P. Dalton, D. W. Nebert, *Biochem. Biophys. Res. Commun.* **2008**, *365*, 814.
[26] S. Primrose, J. Draper, R. Elsom, V. Kirkpatrick, J. C. Mathers, C. Seal, M. Beckmann, S. Haldar, J. H. Beattie, J. K. Lodge, M. Jenab, H. Keun, A. Scalbert, *Br. J. Nutr.* **2011**, *105*, 1277.
[27] V. De Preter, *Methods Mol. Biol.* **2015**, *1277*, 245.
[28] L. Zhao, T. Hartung, *Methods Mol. Biol.* **2015**, *1277*, 209.
[29] L. Hu, L. Bo, M. Zhang, S. Li, X. Zhao, C. Sun, *Xenobiotica* **2018**, *48*, 1079.
[30] F. Bakti, C. Sasse, T. Heinekamp, I. Pocsi, G. H. Braus, *Front. Microbiol.* **2018**, *9*, 744.
[31] A. V. Anand David, R. Arulmoli, S. Parasuraman, *Pharmacogn. Rev.* **2016**, *10*, 84.
[32] S. Ranka, J. M. Gee, L. Biro, G. Brett, S. Saha, P. Kroon, J. Skinner, A. R. Hart, A. Cassidy, M. Rhodes, I. T. Johnson, *Eur. J. Clin. Nutr.* **2008**, *62*, 1131.
[33] Y. Zhang, Y. Li, C. Cao, J. Cao, W. Chen, Y. Zhang, C. Wang, J. Wang, X. Zhang, X. Zhao, *Nutr. Cancer* **2010**, *62*, 1120.
[34] E. A. Kim, J. Ae Kim, M. H. Park, S. C. Jung, S. H. Suh, M. G. Pang, Y. J. Kim, *J. Matern. Fetal. Neonatal. Med.* **2009**, *22*, 325.
[35] W. Qi, R. J. Reiter, D. X. Tan, L. C. Manchester, A. W. Siu, J. J. Garcia, *J. Pineal Res.* **2000**, *29*, 54.
[36] D. Xu, M. J. Hu, Y. Q. Wang, Y. L. Cui, *Molecules* **2019**, *24*, 1123.
[37] M. Gauster, G. Rechberger, A. Sovic, G. Hörl, E. Steyrer, W. Sattler, S. Frank, *J. Lipid Res.* **2005**, *46*, 1517.
[38] E. Gonzalez, S. van Liempd, J. Conde-Vancells, V. Gutierrez-de Juan, M. Perez-Cormenzana, M. Perez-Cormenzana, R. Mayo, A. Berisa, C. Alonso, C. A. Marquez, J. Barr, S. C. Lu, J. M. Mato, J. M. Falcon-Perez, *Metabolomics* **2012**, *8*, 997.
[39] C. J. Packard, *Curr. Opin. Cardiol.* **2009**, *24*, 358.
[40] M. Lindahl, C. Tagesson, *Inflammation* **1993**, *17*, 573.
[41] M. Lindahl, C. Tagesson, *Inflammation* **1997**, *21*, 347.
[42] S. Kamaraj, R. Vinodhkumar, P. Anandakumar, S. Jagan, G. Ramakrishnan, T. Devaki, *Biol. Pharm. Bull.* **2007**, *30*, 2268.
[43] S. N. Young, G. M. Anderson, S. Gauthier, W. C. Purdy, *J. Neurochem.* **1980**, *34*, 1087.
[44] M. Ruiz-Roso, V. Echeverry-Alzate, B. Ruiz-Roso, J. Quintela, S. Ballesteros, V. Lahera, N. de las Heras, J. López-Moreno, B. Martín-Fernández, *Nutrients* **2018**, *11*, 11.
[45] R. Muramatsu, M. Kuroda, K. Matoba, H. Lin, C. Takahashi, Y. Koyama, T. Yamashita, *J. Biol. Chem.* **2015**, *290*, 11515.
[46] N. Takahara, A. Kashiwagi, Y. Nishio, N. Harada, H. Kojima, H. Maegawa, H. Hidaka, R. Kikkawa, *Diabetologia* **1997**, *40*, 662.
[47] A. M. Sheikh, M. Michikawa, S. U. Kim, A. Nagai, *Neuroscience* **2015**, *292*, 159.
[48] J. Qiao, F. Huang, R. P. Naikawadi, K. S. Kim, T. Said, H. Lum, *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **2006**, *291*, L91.
[49] Z. Ou, Y. Sun, L. Lin, N. You, X. Liu, H. Li, Y. Ma, L. Cao, Y. Han, M. Liu, Y. Deng, L. Yao, Q. R. Lu, Y. Chen, *J. Neurosci.* **2016**, *36*, 10560.
[50] I. Kaya, D. Brinet, W. Michno, M. Başkurt, H. Zetterberg, K. Blenow, J. Hanrieder, *ACS Chem. Neurosci.* **2017**, *8*, 2778.
[51] R. Tzekov, C. Dawson, M. Orlando, B. Mouzon, J. Reed, J. Evans, G. Crynen, M. Mullan, F. Crawford, *PLoS One* **2016**, *11*, e0153608.
[52] C. Chen, J. C. Magee, N. G. Bazan, *J. Neurophysiol.* **2002**, *87*, 2851.
[53] I. Bezprozvanny, M. P. Mattson, *Trends Neurosci.* **2008**, *31*, 454.
[54] V. Chhor, T. Le Charpentier, S. Lebon, M. V. Oré, I. L. Celador, J. Josserand, V. Degos, E. Jacotot, H. Hagberg, K. Sävman, C. Mallard, P. Gressens, B. Fleiss, *Brain Behav. Immun.* **2013**, *32*, 70.
[55] D. Vauzour, K. Vafeiadou, A. Rodriguez-Mateos, C. Rendeiro, J. P. Spencer, *Genes Nutr.* **2008**, *3*, 115.
[56] C. H. Lin, R. Kohli, *Clin. Transl. Gastroenterol.* **2018**, *9*, 164.
[57] K. Allen, H. Jaeschke, B. L. Copple, *Am. J. Pathol.* **2011**, *178*, 175.
[58] V. García-Mediavilla, I. Crespo, P. S. Collado, A. Esteller, S. Sánchez-Campos, M. J. Tuñón, J. González-Gallego, *Eur. J. Pharmacol.* **2007**, *557*, 221.
[59] C. C. Lee, S. R. Shen, Y. J. Lai, S. C. Wu, *Food Funct.* **2013**, *4*, 794.
[60] L. Chu, K. Zhang, Y. Zhang, X. Jin, H. Jiang, *Int. Urol. Nephrol.* **2015**, *47*, 345.
[61] F. Jimenez, M. J. Monte, M. Y. El-Mir, M. J. Pascual, J. J. Marin, *Dig. Dis. Sci.* **2002**, *47*, 2398.

[62] P. Fickert, E. Krones, M. J. Pollheimer, A. Thueringer, T. Moustafa, D. Silbert, E. Halilbasic, M. Yang, H. Jaeschke, G. Stokman, R. G. Wells, K. Eller, A. R. Rosenkranz, G. Eggertsen, C. A. Wagner, C. Langner, H. Denk, M. Trauner, *Hepatology* **2013**, *58*, 2056.
[63] S. Erlinger, *Clin. Res. Hepatol. Gastroenterol.* **2014**, *38*, 392.
[64] E. Krones, M. Wagner, K. Eller, A. R. Rosenkranz, M. Trauner, P. Fickert, *Dig. Dis.* **2015**, *33*, 367.
[65] A. Bomzon, S. Holt, K. Moore, *Semin. Nephrol.* **1997**, *17*, 549.
[66] M. P. Panozzo, D. Basso, L. Balint, M. Zaninotto, P. Bonvicini, M. Plebani, *Eur. Surg. Res.* **1995**, *27*, 332.
[67] L. Wang, S. Lin, Y. He, G. Llu, Z. Wang, *Biomed. Environ. Sci.* **2013**, *26*, 258.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Jia S, Guan T, Zhang X, Liu Y, Liu Y, Zhao X. Serum metabonomics analysis of quercetin against the toxicity induced by cadmium in rats. *J Biochem Mol Toxicol*. 2020;e22448. https://doi.org/10.1002/jbt.22448