Supporting information

Integrated Metabolomics and Network Pharmacology Strategy-Driven Active Traditional Chinese Medicine Ingredients Discovery for the Alleviation of Cisplatin Nephrotoxicity

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1. Methods

1.1. Preliminary experiment scheme

All animal experiments were performed in accordance with the institutional guidelines for the care and use of laboratory animals by the National Research Council of the National Academies and all experimental protocols were approved by the Animal Ethics Committee of China Pharmaceutical University.

Prior to initiation of main experiment, two pilot experiments were conducted to explore the manner, dosage and action duration of kaempferol on renal tissues. All male Sprague-Dawley rats (SPF grade, 140 to160 g body weight, 6 weeks of age) in animal experiments were purchased from Sino–British SIPPR/BK Lab Animal Ltd. (Shanghai, China) and fed with a standard commercial diet while kept in a light- and temperature-controlled condition (12/12 h light/dark, 22–25 °C, 45–55% humidity). After one-week adaptation, rats were randomly divided into differently groups.

In the first pilot study, kaempferol at three different doses i.e. 2, 10 and 50 mg/kg was selected to explore suitable dose by intraperitoneal injection for 11 consecutive days based on previous kaempferol and its analogues research literature[1,2,3]. On 4th day, animals were administered with a single tail vein injection of cisplatin (5 mg/kg dissolved in normal saline). Blood samples were collected through retro-orbital plexus at day1,4,8,9,10 and 11 and serum was separated for the estimation of serum specific renal injury indicators (BUN and Scr). The result showed just a protective trend on rats but no statistically significant differences **(Supplementary Figure S5)**. It was speculated that the number of animal only four per group were influenced by individual differences or the manner, dosage and action duration of kaempferol and even the dosage of cisplatin not the most suitable. In the second pilot study, kaempferol was administered by gavage at 50 and 100 mg/kg for 14 consecutive day. On 7th day, 8 mg/kg cisplatin was selected for animal administration by tail vein a single injection. Blood samples were collected at day1,4,7,8,10,12,13 and 14. From this pilot study, it was observed that serum levels of BUN and Scr were just showing

statistically significant differences between cisplatin, kaempferol at 50 mg/kg administered group of rats **(Supplementary Figure S6B&C&D&E)**. Histopathological findings of kidneys from the rats treated with vehicle revealed the same result **(Supplementary Figure S6A)**. It was speculated that the dosage of kaempferol and cisplatin not the best. Even though, we knew BUN and Scr indicators would begin to recover at 6th days after cisplatin administration according to the two pilot experiments.

1.2. Untargeted metabolomic analysis

1.2.1. Sample Preparation for Metabolomic Analysis.

For kidney, the medulla part and cortex part were separated. Approximately 30 mg of tissue was firstly placed into pre-cooled 2 mL homogenization tubes containing 8 ceramic beads and homogenized in a 10:1 ratio of methanol to tissue for three times (6.5 m/s for 30 s), with 60 s intervals between homogenization steps respectively. After two centrifugations (14000 rpm, 4 ℃, 10 min), the supernatant was obtained and named as kidney medulla or cortex tissue homogenate. For LC-MS analysis, 100μL acetonitrile was added to a 20μL aliquot of kidney tissue homogenate. The solution was mixed thoroughly and centrifuged twice (14000 rpm, 4 ℃, 10 min), and the supernatant was removed for LC-MS analysis. For GC-MS analysis, 100μL methanol was added to a 10μL aliquot of the kidney homogenate. After mixed thoroughly and centrifuged twice like before, 80μL second supernatant was transferred to corresponding brown glass vial and oximated with 25μL O-methoxyamine hydrochloride (10 mg/mL in pyridine) at 1200 rpm at 37 ℃ for 90 min. And then, the mixture was vacuum dried at 50 ℃ for 2 h (Labconco CentriVap®, Kansas, MO, USA). Later, 120 μL MSTFA /ethyl acetate (1:1, v/v) was added to the dried extracts and kept at 1200 rpm at 37 ℃ for 2 h with trimethylsilylation. Finally, the mixture was prepared for GC-MS analysis.

For serum, orbital venous blood was rest before centrifugation for 1.5 h and the supernatant was serum. For LC-MS analysis, 120μL acetonitrile was added to a 20μL aliquot of serum and the following steps are consistent with the kidney tissue homogenate. At last, take the supernatant for

LC-MS analysis. For GC-MS analysis, 100μL methanol was added to a 10μL aliquot of serum and the following steps are consistent with the kidney tissue homogenate. Finally, the mixture was removed for GC-MS analysis.

1.2.2 Liquid Chromatography-Mass Spectrometry (LC-MS) Metabolomic Analysis.

LC-MS analysis was operated on Shimadzu Prominence series ultra-fast liquid chromatography (UFLC) system coupled with ion trap/time-of-flight hybrid mass spectrometry (IT-TOF/MS) (Shimadzu Inc., Japan). Phenomenex Kinetex C18 (100 × 2.1 mm, 2.6μm) (Phenomenex, Torrance, CA, USA) was used for chromatographic separation. The column temperature was kept at 40 ℃. The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution was programmed as follows: linear gradient from 5% B to 95% B for 20min, maintained with 95% B for 3 min, and returned to 5% B for 7 min. The injection volume was 5μL and the flow rate was 0.4 mL/min. Electrospray ionization (ESI) was employed.

1.2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Metabolomic Analysis.

GC-MS analysis was operated on Shimadzu GCMSQP2010 Ultra (Ultra GC-Q/MS; Shimadzu Inc., Japan) in equipment with a fused silica capillary column (Rtx-5MS; 30m×0.25mm, 0.25μm, Restek, USA). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The programmed oven temperature was started at 70 ℃ for 2 min, then increase to 320 ℃ at the rate of 10 ℃/min for 25 min and finally maintained at 320 ℃ for 2 min. The temperatures of the injector and ion source were held at 250and 200 ℃, respectively. Electron impact mode with the energy of 70 eV for the ionization and full scan mode with the mass to charge ratio (m/z) from 45 to 600 for data acquisition were programmed. The injection volume was 1μL, and the split ratio was 20:1 for both kidney and serum. GCMS Solution software (Shimadzu Inc., Japan) was employed for

auto-acquisition of total ion chromatograms (TICs) and fragmentation patterns.

## 1.2.4. Data Preprocessing and Statistical Analysis

Data extraction was performed by Profiling Solution Software (Shimadzu Inc., Kyoto, Japan). After the data pretreatment[4], a matrix containing grouping information, sample names, retention times and normalized peak intensities were obtained. Mass spectrometry total useful signal (MSTUS) method was used for the normalization of signal intensities. OPLS-DA was performed by SIMCA-P software. Features (a feature here was defined as a unique pair of RT and m/z record) were treated as differential if the following conditions were met. First, variable importance in the projection (VIP) value should be greater than 1.0 in OPLS-DA constructed between control and each experimental group. Second, confidence intervals on VIP column plot should be positive. Third, adjusted p value of Wilcoxon Mann-Whitney Test and stricter false discovery rate (FDR) correction based on Benjamini-Hochberg method (MeV, Version 4.6.1, http://www.tm4.org/) should be lower than 0.05. After the feature screening process, those differential features were prepared for metabolite identification.

1.2.5. Metabolite Identification.

For GC-MS analysis, the preliminary identification of metabolites was based on NIST 11 (National Institute of Standards and Technology). Peaks with similarity of more than 80% were assigned for compound names and were further confirmed by comparing with the reference standards available in our lab. In the case of metabolites detected by LC-MS, they were first identified by referring to existing literature and online databases such as HMDB (http://www.hmdb.ca/), METLIN (http:// metlin.scripps.edu/), and Lipid MAPS (http://www.lipidmaps.org/). LC-MS-measured mass signals matched small molecules present in the databases if their exact masses were within 30 ppm (ppm ≤ 30). Then, those metabolites were

further confirmed by comparing with the standards available in our lab.

2. Figures
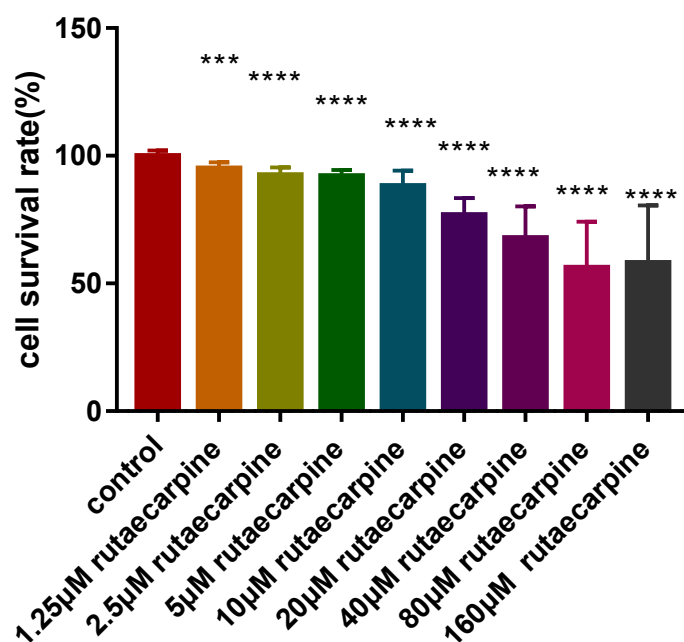
Figure S1 Effect of rutaecarpin on cell viability by CCK8 assay. ∗∗∗p < 0.001, ∗∗∗∗ p < 0.001 compared to the control.
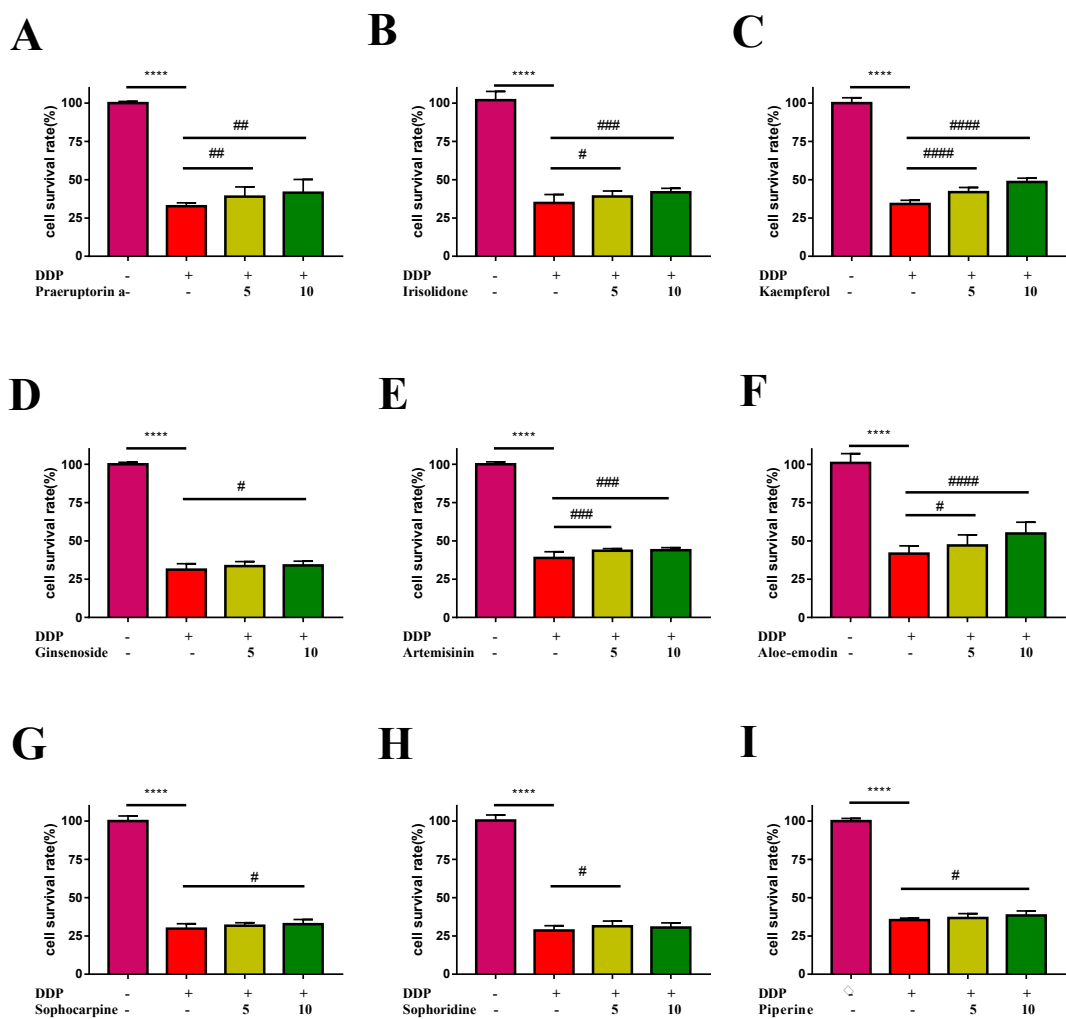
Figure S2 Effect of first discovered monomers on cell viability with or without cisplatin treatment. Data represent the mean ± SEM for 3 independent experiments. ∗p<0.05, ∗∗p<0.01, ∗∗∗p < 0.001, ∗∗∗∗ p < 0.001 compared to the control. # p<0.05, ## p<0.01, ###p < 0.01, #### p < 0.001 compared to cisplatin-treated group. DDP, cisplatin. The concentration units of DDP and kaempferol both are micromole (μM).
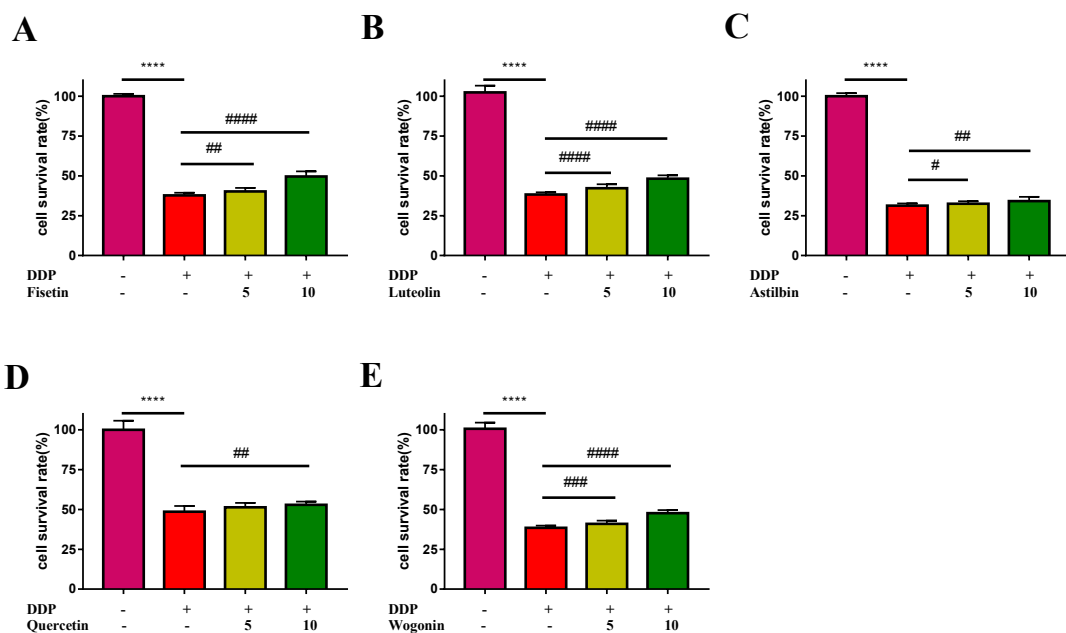
Figure S3 Effect of reported monomers on cell viability with or without cisplatin treatment. Data represent the mean ± SEM for 3 independent experiments. ∗p<0.05, ∗∗p<0.01, ∗∗∗p < 0.001, ∗∗∗∗ p < 0.001 compared to the control. # p<0.05, ## p<0.01, ###p < 0.001, #### p < 0.0001 compared to cisplatin-treated group. DDP, cisplatin. The concentration units of DDP and kaempferol both are micromole (μM).
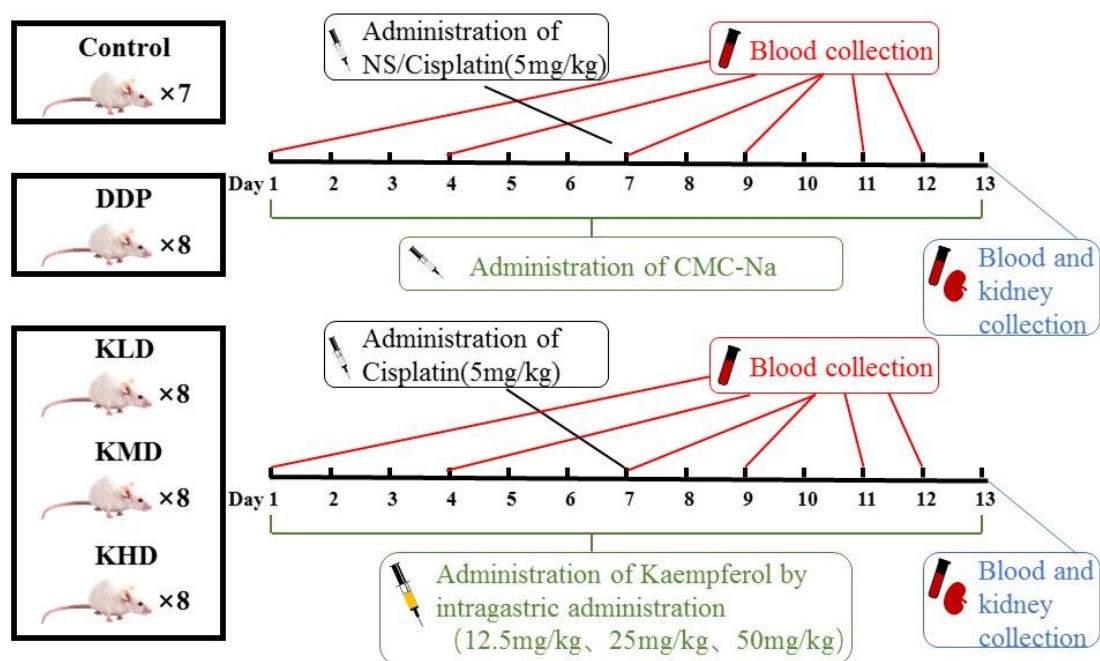
Figure S4 Animal experiment scheme. Control, vehicle control group; DDP, cisplatin treated group; KLD, cisplatin with low-dose kaempferol group; KMD, cisplatin with middle-dose kaempferol; KHD, cisplatin with high-dose kaempferol; NS, normal saline.
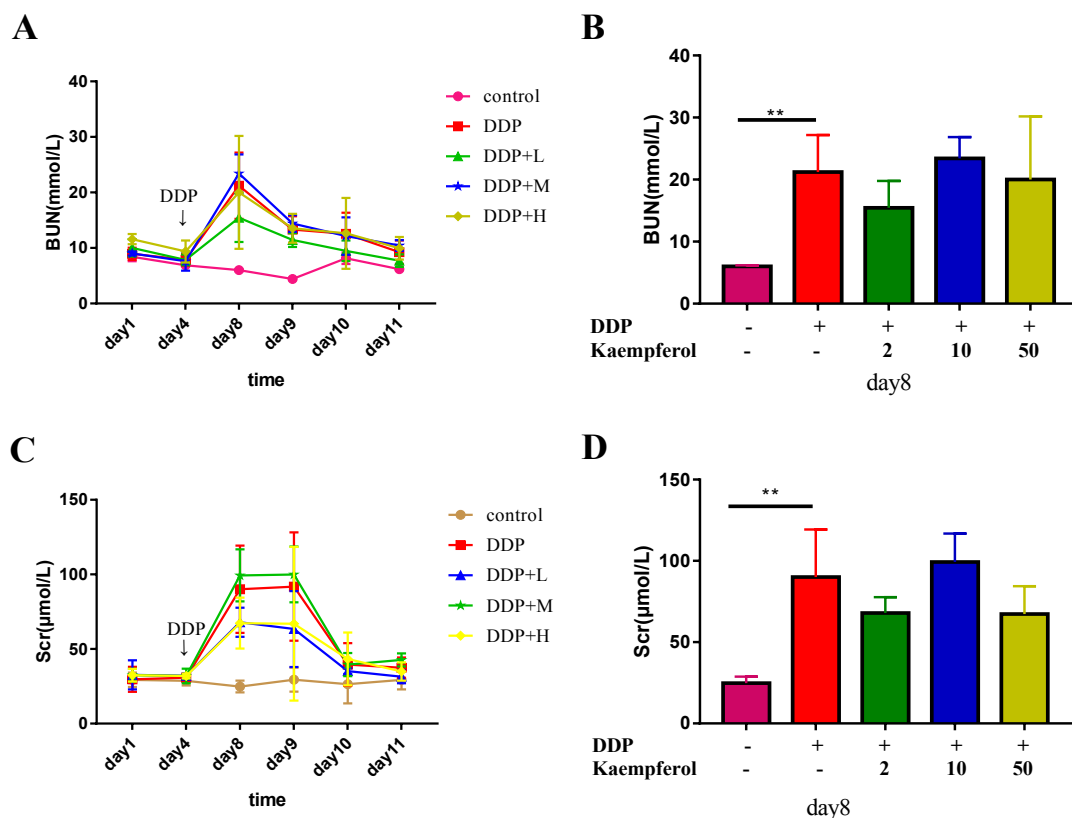
Figure S5 Biochemical assay. (A) BUN trend since cisplatin treat and (B) BUN peak (B); (C) Scr trend since cisplatin treat and (D) Scr peak. Results of Scr and BUN index show that treatment of kaempferol have the alleviating trend for renal function in cisplatin nephropathy, but no significant difference. Data represent the mean ± SEM for 4 rats. *p<0.05, **p<0.01, ***p < 0.001, **** p < 0.001 compared to control. # p<0.05, ## p<0.01, ###p < 0.001, #### p < 0.0001 compared to model group. Control, vehicle control group; DDP, cisplatin treated group; DDP+L, cisplatin with 2 mg/kg kaempferol group; DDP+M, cisplatin with 10 mg/kg kaempferol; DDP+H, cisplatin with 50 mg/kg kaempferol.
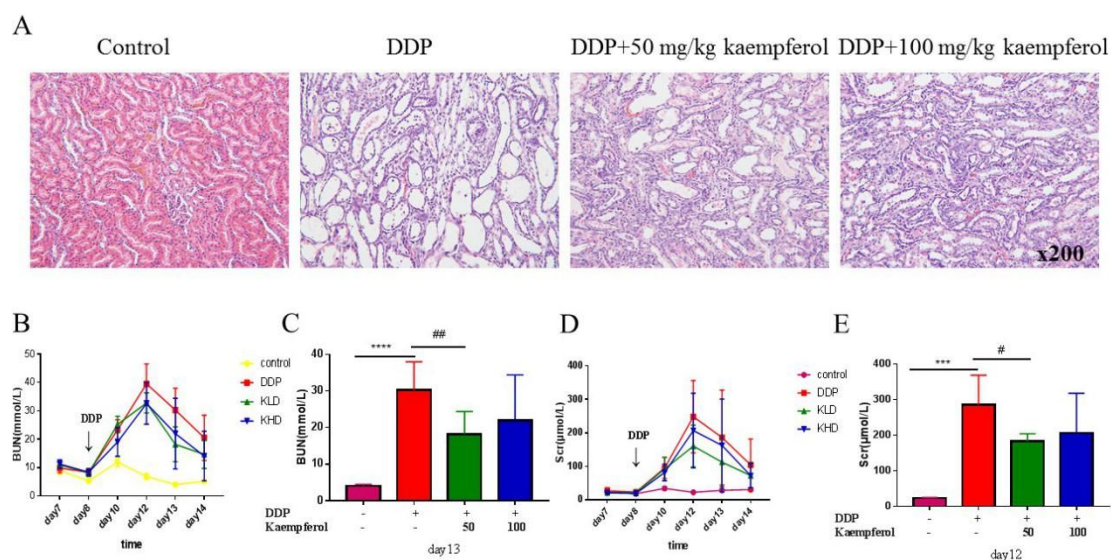
Figure S6 Renal protective and anti-apoptotic effects of kaempferol in DDP-induced acute kidney injury by pathological changes and biochemical assay. (A) Representative images of H&E staining (x200). (B) BUN trend since cisplatin treat and (C) BUN peak; (D) Scr trend since cisplatin treat and (E) Scr peak. Results of Scr and BUN index Indicator show that treatment of kaempferol restored renal function in cisplatin nephropathy with 50 mg/kg kaempferol. (E) MDA and (F) SOD for oxidative stress assay. Results of MDA and SOD indicate kaempferol anti-oxidation caused by cisplatin. Data represent the mean ± SEM for 8-9 rats. $**p < 0.01$, $****p < 0.0001$ compared to control. $\#p < 0.05$, $\#\#p < 0.01$ compared to model group. Control, vehicle control group; DDP, cisplatin treated group; KLD, cisplatin with 50 mg/kg kaempferol group; KHD, cisplatin with 100 mg/kg kaempferol.
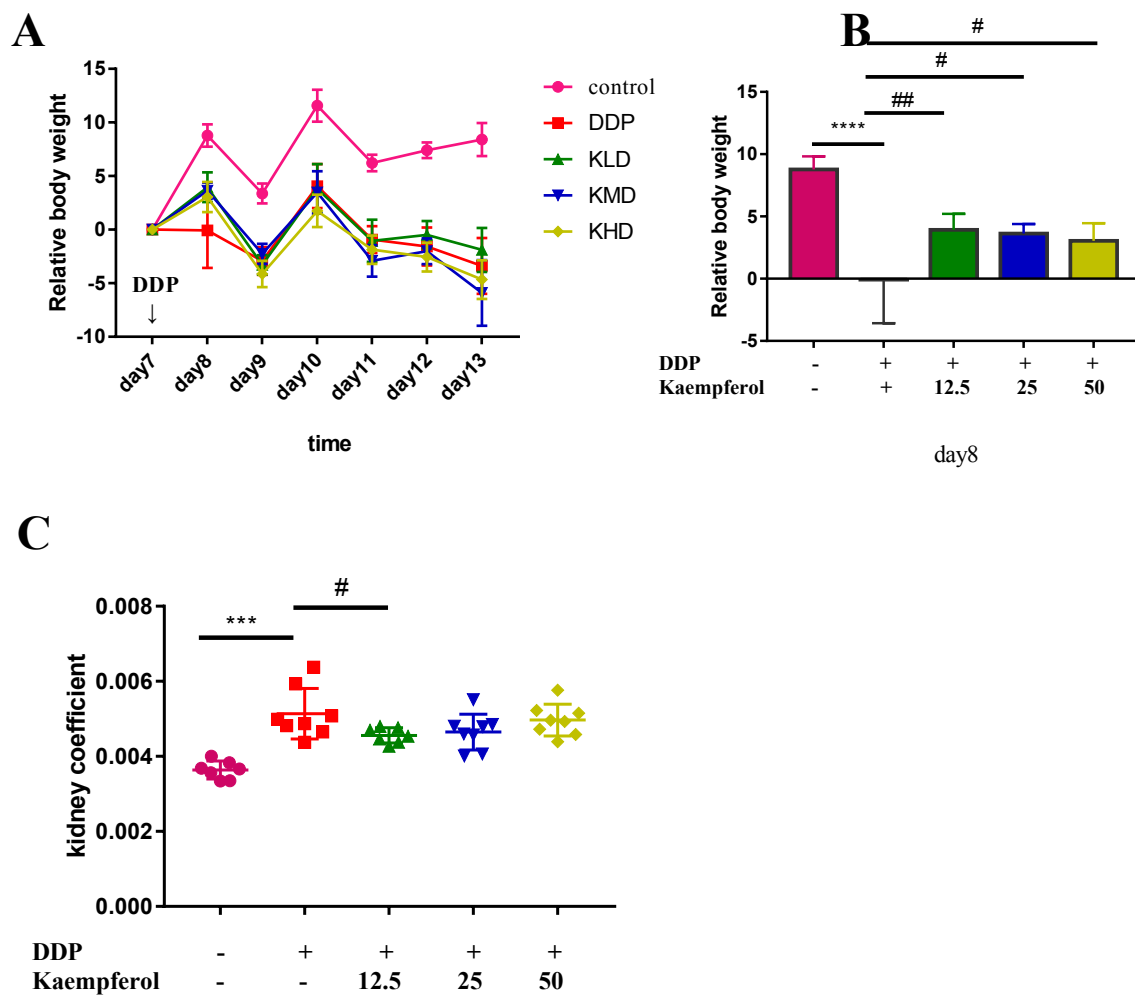
Figure S7 General condition. (A) relative body weight trend (B) body weight loss in the second day after cisplatin administration; (C) kidney coefficient. Results of relative body weight and kidney coefficient show that treatment of kaempferol restored renal function in cisplatin

nephropathy. Results of MDA and SOD indicate kaempferol anti-oxidation caused by cisplatin. Data represent the mean ± SEM for 7–8 rats. **p < 0.01, ****p < 0.0001 compared to control. #p < 0.05, ##p < 0.01 compared to model group. Control, vehicle control group; DDP, cisplatin treated group; KLD, cisplatin with 12.5 mg/kg kaempferol group; KMD, cisplatin with 25 mg/kg kaempferol; KHD, cisplatin with 50 mg/kg kaempferol.
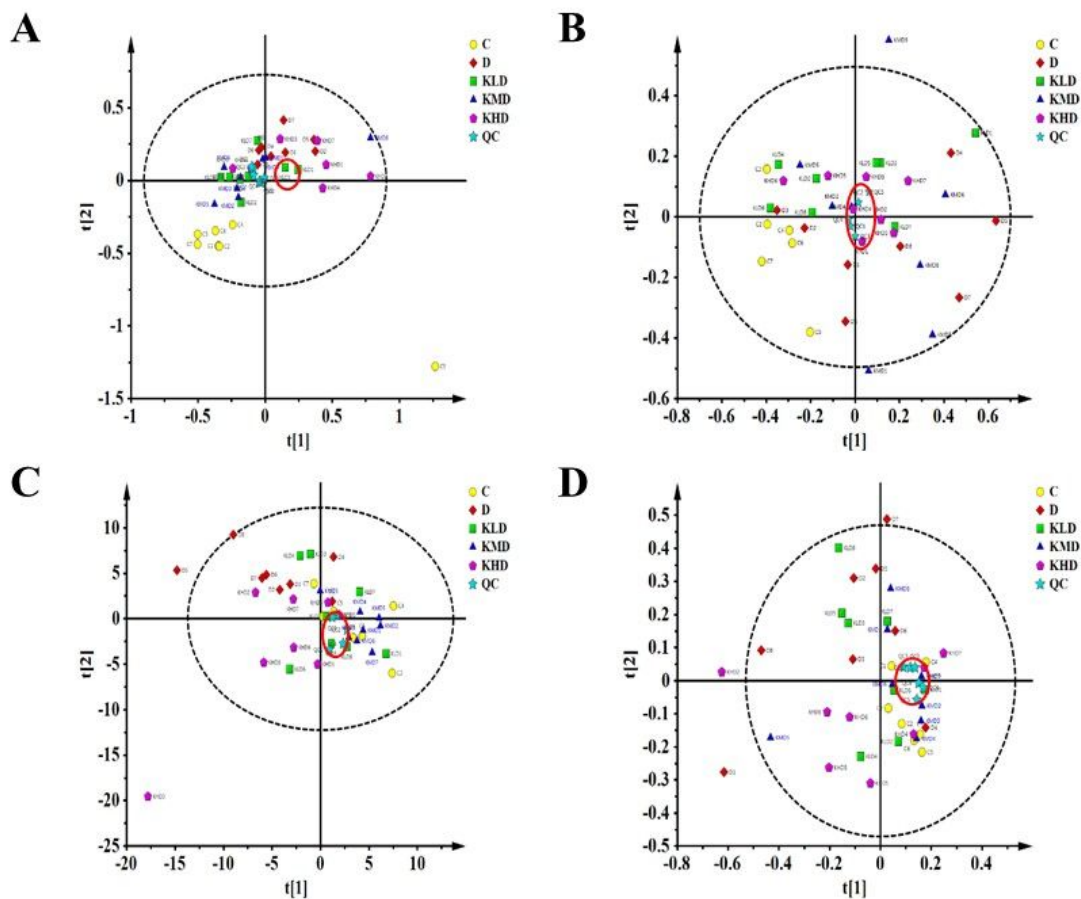
Figure S8 PCA plots detected by GC-MS. (A) serum collected at the day 12. (B) serum collected at day 13. (C) medulla collected at day 13. (D) cortex collected at day 13. C, vehicle control group; D, cisplatin treated group; KLD, cisplatin with 12.5 mg/kg kaempferol group; KMD, cisplatin with 25 mg/kg kaempferol; KHD, cisplatin with 50 mg/kg kaempferol; QC, quality control. Red ellipse, QC samples.
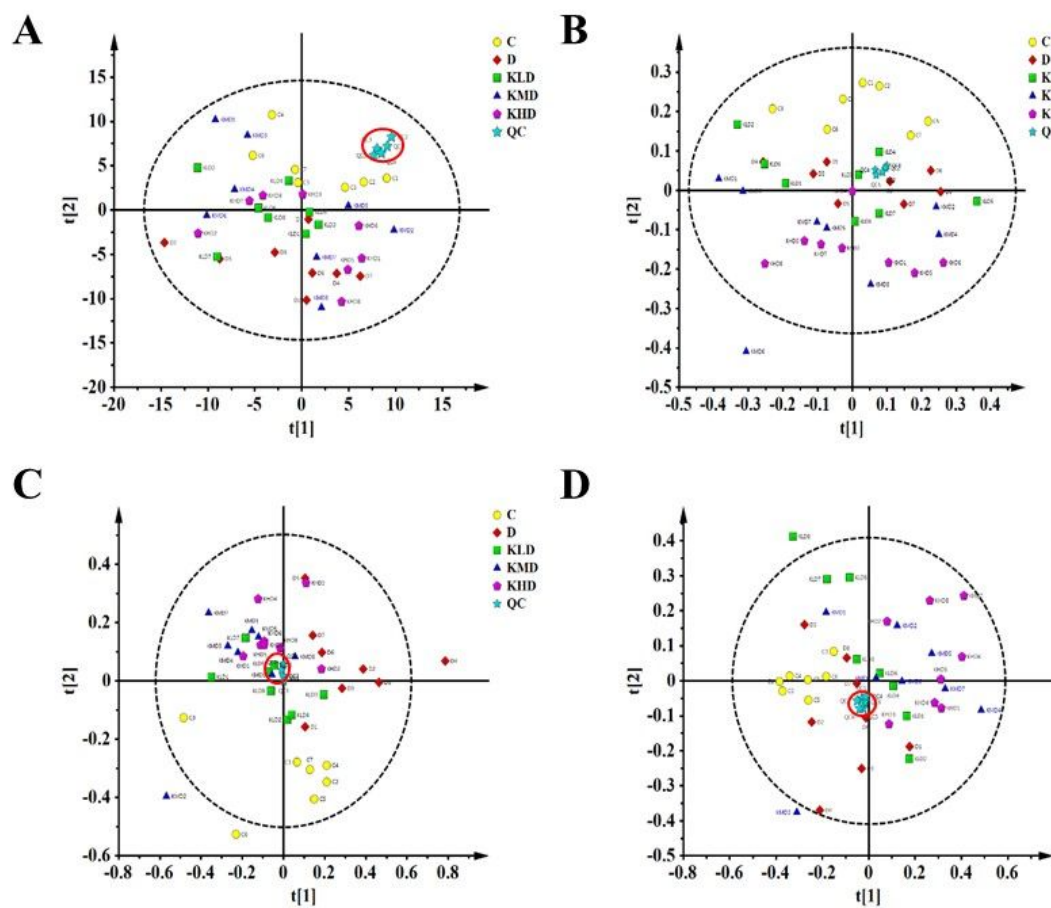
Figure S9 PCA plots detected by LC-MS ESI (+). (A) serum collected at the day 12. (B) serum collected at day 13. (C) medulla collected at day 13. (D) cortex collected at day 13. C, vehicle control group; D, cisplatin treated group; KLD, cisplatin with 12.5 mg/kg kaempferol group; KMD, cisplatin with 25 mg/kg kaempferol; KHD, cisplatin with 50 mg/kg kaempferol; QC, quality control. Red ellipse, QC samples.
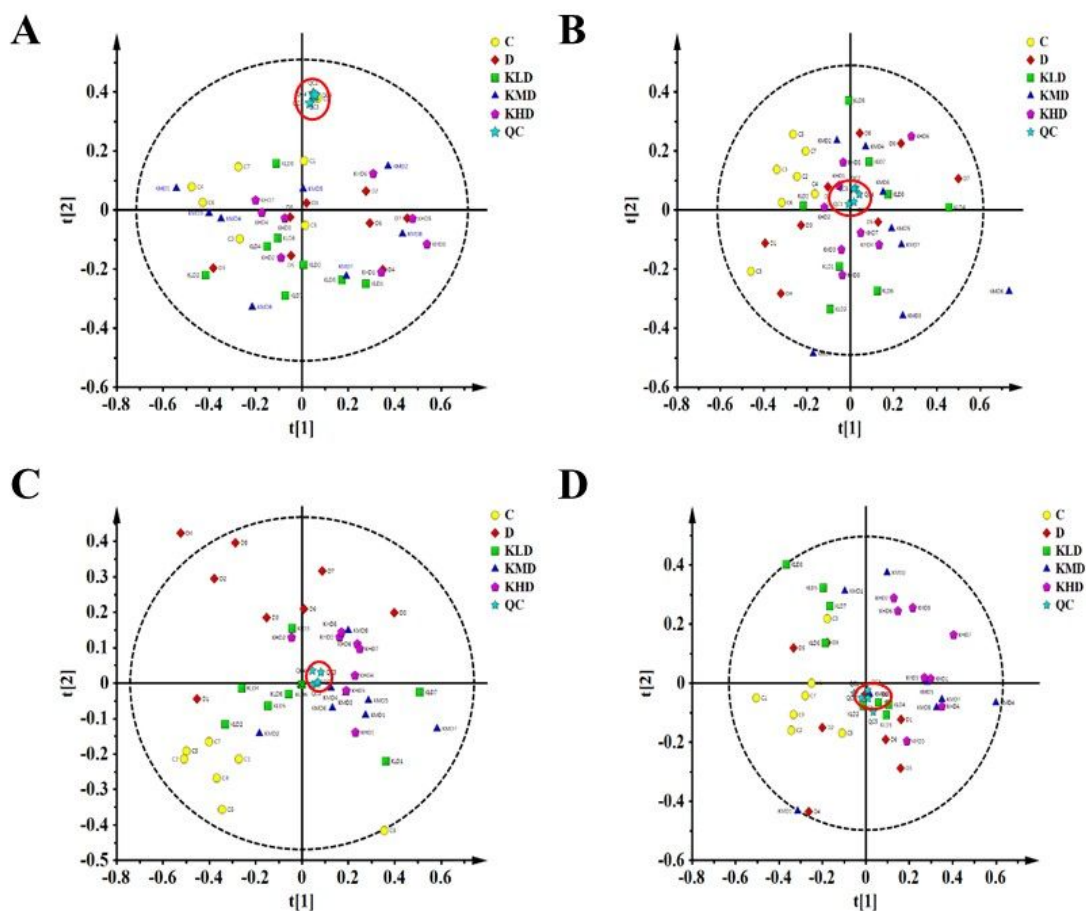
Figure S10 PCA plots detected by LC-MS ESI (-). (A) serum collected at the day 12. (B) serum collected at day 13. (C) medulla collected at day 13. (D) cortex collected at day 13. C, vehicle control group; D, cisplatin treated group; KLD, cisplatin with 12.5 mg/kg kaempferol group; KMD, cisplatin with 25 mg/kg kaempferol; KHD, cisplatin with 50 mg/kg kaempferol; QC, quality control. Red ellipse, QC samples.

3. Tables

Table S1 Collection of differential metabolites involved in different metabolism pathways during cisplatin-induced renal injury from literature and our previous research.

Amino acids metabolism	Lipid metabolism	Energy metabolism	Other metabolism pathways
Asparagine	LPC (14:0)	Glucose	Ascorbic acid
Glycine	LPC (15:0)	Malic acid	Elaidic acid
Ornithine	LPC (16:1)	Fumaric acid	Ascorbate 2-sulfate
Tryptophan	LPC (20:1)	Pyruvic acid	Acetate
Glutamine	LPC (20:2)	Citrate	Acetoacetate
Alanine	LPC (20:3)	cis-Aconitate	2-oxoglutarate
Glutamic acid	LPE (20:2)	Succinic acid	Dimethylamine
Isoleucine	LPE (20:5)	fucose	Allantoin
Leucine	LPE (22:6)	mannose	Hippurate
Lysine	Phosphate		1-Methylnicotinamide
Phenylalanine	Stearic acid		2-Oxoglutarate
Proline	Acetylcarnitine		Trimethylamine

Serine	Cholic acid	Pipecolate
Threonine	DG (37:1)	3-Indoxyl sulfate
Tyrosine	LPC (18:1)	Guanidoacetate
Valine	LPC (20:4)	choline dehydrogenase
Pyroglutamic acid	LPC (20:5)	betaine
Methionine	PA (22:4)	Glutathione
3-Methylhistidine	PE (38:5)	3-indoxyl sulfate
Arginine	Carnitine	3-ydroxyphenylacetate
3-ethylcrotonylglycine	DG (31:0)	agmatine
histidine	DG (33:0)	spermidine
cysteine	DG (38:2)	sorbitol (glucitol)
glycylproline	FFA C22:6	glucosamine
citrulline	Indoleacrylic acid	1,5-anhydroglucitol
gamma-glutamylphenylalanine	Linoleyl carnitine	monoethanolamine
cysteine	LPE (18:2)	riboflavin (Vitamin B2)
glycylproline	LPE (22:4)	2'-deoxyinosine

		5-methyltetrahydrofolate
citrulline	PI (20:4)	(5MeTHF)
gamma-glutamylphenylalanine	Cholesterol	nicotinamide adenine dinucleotide
	Ethanolamine	phosphate (NADP +)
	LPE (20:4)	Methylamine
	LPE (20:4)	Trigonelline（N-methylnicotinate）
	Linoleic acid	fumarate
	LPC (18:0)	homocysteine
	Ceramide(d18:1/16:0)	asymmetric dimethylarginine
	Cholesterol sulfate	putrescine
	FFA C22:4	cadaverine
	FFA C22:5	Creatinine
	Glycocholic acid	Creatine
	LPC (18:2)	Cytidine

LPC (18:3)   Urea

LPC (22:5)   Uric acid

LPE (16:0)   Uracil

LPE (18:1)   Uridine

Palmitoylcarnitine   Inosine

Phosphoric acid   Hypoxanthine

Sphingosine   Xanthine

Stearoylcarnitine   Xanthurenic acid

3-HBT   Adenine

TMAO   Adenosine

myo-inositol

3-hydroxybutyrate (BHBA)

3-hydroxy-3-methylglutarate

glycerol

Glycerphosphocholine

LPE (18:0)

Table S2 Kidney damage targets collection from disease-related database.

Source	Targets	Source	Targets	Source	Targets
PGKB database	MTHFR	GAD database	CYBA	TTD database	TP53
	FOXP3		AGTR1		
	CCDC22		APOE		
	XRCC1		COMT		
	SLC22A2		TNF-α		
	ERCC1		IL10		
	LARP1B				
	SLC19A1				
	ACE				
	G6PD				
	ABCB1				
	ABCG2				
	CYP2D6				
	CYP3A4				

CYP3A5

DPYD

EPO

ABCC2

Table S3 Herb ingredients collection and their ADME parameters from TCMSP database

No.	Molecule name	Targets	OB	DL
1	quercetin	ABCG2、CYP3A4、TNF-α	46.43	0.28
2	resveratrol	ABCG2、ABCB1、TNF-α	19.07	0.11
3	ginkgolide a	ABCB1、CYP3A4、ABCC2	13.82	0.74
4	(-)-epicatechin	ABCG2、COMT、TNF-α	28.93	0.24
5	(-)-epigallocatechin-3-gallate	COMT、TNF-α	55.09	0.77
6	piperine	ABCB1、TNF-α	42.52	0.23

7	daidzein	CYP3A4、TNF-α	19.44	0.19
8	rutaecarpine	CYP3A4、TNF-α	40.3	0.6
9	artemisinin	ABCB1、CYP3A4	49.88	0.31
10	ginsenoside rf	CYP3A4、TNF-α	17.74	0.24
11	kaempferol	CYP3A4、TNF-α	41.88	0.24
12	alpha-humulene	TNF-α	22.98	0.06
13	dl-praeruptorin a	TNF-α	46.46	0.53
14	atractylenolide iii	TNF-α	68.11	0.17
15	bilobetin	TNF-α	7.27	0.63
16	emodin	TNF-α	24.4	0.24
17	tanshinone iia	CYP3A4	49.89	0.4
18	eugenol	ABCC2	56.24	0.04
19	fisetin	TNF-α	52.6	0.24

20	bergaptol	CYP3A4	24.22	0.12
21	glycyrrhizin	TNF-α	9.06	0.11
22	puerarin	TNF-α	24.03	0.69
23	hyperforin	CYP3A4	44.03	0.6
24	sophocarpine	TNF-α	64.26	0.25
25	sophoridine	TNF-α	60.07	0.25
26	wogonin	TNF-α	30.68	0.23
27	zingerone	TNF-α	25.23	0.05
28	genipin	ABCC2	26.06	0.1
29	caffeic acid	TNF-α	25.76	0.05
30	matrine	TNF-α	63.77	0.25
31	capsaicin	ABCB1	10.31	0.2
32	triptolide	TNF-α	51.29	0.68
33	rutin	TNF-α	3.2	0.68
34	aloe-emodin	TNF-α	83.38	0.24
35	astilbin	TNF-α	36.46	0.74
36	luteolin	TNF-α	36.16	0.25

37	citral	TNF-α	22.52	0.02
38	limonin	CYP3A4	21.3	0.57
39	solamargine	TNF-α	31.36	0.06
40	apigenin	TNF-α	23.06	0.21
41	genistein	TNF-α	17.93	0.21
42	ginsenoside rh2	TNF-α	36.32	0.56
43	corilagin	TNF-α	3.01	0.44
44	aucubin	TNF-α	4.17	0.33
45	paeonol	TNF-α	28.79	0.04
46	paeoniflorin	TNF-α	53.87	0.79
47	morin	ABCB1	46.23	0.27
48	diosgenin	ABCC2	80.88	0.81
49	demethoxycurcumin	ABCB1	4.37	0.33
50	coumestrol	CYP3A4	32.49	0.34
51	coumarin	CYP3A4	29.17	0.04
52	ursolic acid	TNF-α	16.77	0.75

53	yakuchinone b	TNF-α	9.13	0.26
54	yakuchinone a	TNF-α	8.2	0.25
55	myricetin	TNF-α	13.75	0.31
56	isovitexin	TNF-α	31.29	0.72
57	cryptotanshinone	TNF-α	52.34	0.4
58	naringin	TNF-α	6.92	0.78
59	irisolidone	TNF-α	37.78	0.3
60	quercitrin	CYP3A4	4.04	0.74

Table S4 Improved metabolites detected by GC-MS. Sday12 means that serum collected at the peak day of BUN and Scr; Sday13 means that serum collected at terminal point day of animal experiment. M means medulla. C means cortex. ↑, up-regulated metabolites; ↓, down-regulated metabolites. DDP means cisplatin treated group; K&DDP means kaempferol combined with cisplatin treated groups

No.	Metabolite	VIP	Similarity	Ion m/z	Ion RT	DDP	K&DDP	Sample
1	3-Hydroxybutyric acid	11.437	88	191.058	8.03	↑	Remission	Sday12
2	Butanoic acid	1.0857	89	148.102	8.032	↑	Remission	Sday12
3	Urea	1.4807	90	147.104	9.31	↑	Remission	Sday12
4	Propanoic acid	2.7952	94	75.0457	6.352	↑	Remission	Sday13
5	Valine	2.4618	94	144.162	8.936	↓	Remission	Sday13
6	Urea	9.4354	90	147.103	9.284	↑	No difference	Sday13
7	Serine	1.0653	91	57.0521	9.574	↓	Remission trend	Sday13
8	Threonine	2.5312	84	130.104	10.14	↓	No difference	Sday13

9	Valine	1.1604	96	220.082	8.927	↓	Remission	M
10	Urea	1.6777	90	100.021	9.238	↑	Remission	M
11	Arabitol	1.3975	90	129.097	15.76	↓	Remission	M
12	Tyrosine	2.1248	91	217.08	18.22	↑	No difference	M
13	Serine	2.1889	91	73.05	11.13	↓	Remission	C
14	Valine	2.077	95	218.098	8.927	↓	Remission	C
15	Urea	2.0264	90	171.1	9.222	↑	Remission	C
16	Leucine	1.4063	94	232.12	9.792	↓	Remission	C
17	Pyrimidine	2.3142	80	245.047	10.82	↑	Remission	C
18	Threonine	2.0395	86	291.161	11.52	↓	Remission	C
19	Aspartic acid	3.6831	86	232.099	13.31	↓	Remission	C
20	Phenylalanine	1.0033	93	219.088	14.7	↓	Remission	C
21	Asparagine	1.5384	90	132.119	15.22	↑	Aggravation	C
22	Alanine	8.6395	93	116.106	7.067	↓	Remission trend	C

23	Norvaline	1.0272	87	117.057	8.966	↓	No difference	C
24	Isoleucine	1.7697	89	219.099	10.14	↓	No difference	C
25	Butanedioic acid	1.3835	80	149.097	12.89	↓	Remission trend	C
26	Proline	3.5611	86	156.128	13.36	↓	No difference	C

Table S5 Improved metabolites detected by LC-MS ESI (+). Sday12 means that serum collected at the peak day of BUN and Scr; Sday13 means that serum collected at terminal point day of animal experiment. M means medulla. C means cortex. ↑, up-regulated metabolites; ↓, down-regulated metabolites. DDP means cisplatin treated group; K&DDP means kaempferol combined with cisplatin treated groups

No.	Metabolite	VIP	Ion m/z	Ion RT	DDP	K&DDP	Sample
1	Acetylcarnitine	1.96895	204.1212	0.65	↓	Aggravation	Sday12
2	Propionylcarnitine	1.14903	218.1354	0.666	↓	Remission trend	Sday12
3	Phenylalanine	1.39198	166.0857	1.171	↑	Remission	Sday12
4	Tryptophan	1.48657	206.0951	1.31	↓	Remission	Sday12
5	Indoleacrylic acid	2.00748	188.0699	2.068	↓	Remission	Sday12
6	Glycocholic acid	1.18438	466.3124	8.826	↓	Remission	Sday12
7	LPC (14:0)	1.35656	468.3091	11.711	↓	Remission	Sday12
8	Sphingosine	1.07221	300.2876	12.133	↓	Remission	Sday12
9	LPC (15:0)	1.14361	482.3216	12.469	↑	Remission	Sday12
10	LPC (18:2)	1.39893	521.3392	12.47	↓	Remission	Sday12
11	LPC (22:6)	1.38973	568.3343	12.761	↓	Remission	Sday12
12	LPC (18:3)	1.38077	508.3358	12.924	↓	Remission	Sday12

13	LPC (20:3)	1.70276	546.3469	13.055	↑	Remission trend	Sday12
14	LPC (18:1)	1.86086	522.3522	13.377	↓	No difference	Sday12
15	LPE (18:1)	2.08529	480.3426	13.672	↓	Remission trend	Sday12
16	LPC (20:2)	1.83328	548.3671	14.075	↑	No difference	Sday12
17	LPC (20:1)	1.46303	550.384	15.089	↓	No difference	Sday12
18	LPE (22:0)	1.61471	538.3842	15.601	↓	No difference	Sday12
19	Valine	2.19164	118.0859	0.599	↓	Remission	Sday13
20	Carnitine	2.30563	162.1116	0.645	↓	No difference	Sday13
21	Acetylcarnitine	3.51462	204.1219	0.655	↓	No difference	Sday13
22	Propionylcarnitine	1.69713	218.1369	0.669	↓	Remission trend	Sday13
23	Tryptophan	2.74518	205.0953	1.993	↓	Remission	Sday13
24	Indoleacrylic acid	1.81717	188.0703	2.012	↓	Remission	Sday13
25	LPC (16:1)	4.10658	494.3223	12.087	↓	No difference	Sday13
26	LPC (18:2)	1.16989	521.3409	12.379	↓	Remission	Sday13

27	LPC (20:3)	2.54433	546.353	13.208	↓	Remission	Sday13
28	LPE (18:1)	1.18343	480.3414	13.592	↑	Remission	Sday13
29	LPC (18:0)	1.0682	524.3103	13.719	↓	Aggravation	Sday13
30	LPC (20:2)	1.70769	548.3688	14.004	↓	No difference	Sday13
31	Glycerphosphocholine	1.12609	258.8975	0.534	↑	Remission	M
32	Carnitine	1.28956	162.1105	0.58	↓	Aggravation	M
33	Acetylcarnitine	1.51863	204.1202	0.613	↓	No difference	M
34	Phenylalanine	2.313	166.0857	1.168	↓	Remission	M
35	Tryptophan	1.78831	205.0949	2.013	↓	Remission	M
36	Indoleacrylic acid	1.1116	188.0696	2.06	↓	Remission	M
37	Sphingosine	2.40023	300.2874	12.103	↑	Remission trend	M
38	LPC (20:4)	2.12954	544.337	12.523	↑	Remission	M
39	LPC (18:1)	1.23775	522.3524	13.358	↑	Remission	M
40	PE(P-16:0e/0:0)	1.76501	438.2959	13.546	↑	Remission	M
41	LPC (15:0)	1.34531	482.3564	13.576	↑	Remission	M

42	LPE (18:1)	1.09215	480.3402	13.646	↑	Remission trend	M
43	LPE (18:0)	1.64008	482.3216	14.644	↑	No difference	M
44	Glycocholic acid	2.14555	466.327	15.118	↑	Remission	M
45	Acetylcarnitine	3.36135	204.121	0.619	↓	Remission	C
46	Phenylalanine	1.8194	166.0848	0.678	↑	Remission	C
47	Glycocholic acid	1.45893	466.3141	8.773	↑	Aggravation	C
48	Sphingosine	2.52457	300.2886	12.053	↑	Remission trend	C

Table S6 Improved metabolites detected by LC-MS ESI (-). Sday12 means that serum collected at the peak day of BUN and Scr; Sday13 means that serum collected at terminal point day of animal experiment. M means medulla. C means cortex. DDP, cisplatin administration; K&DDP, kaempferol combined with cisplatin administration. ↑, up-regulated metabolites; ↓, down-regulated metabolites.

No.	Metabolite	VIP	Ion m/z	Ion RT	DDP	K&DDP	Sample
1	Taurocholic acid	2.15529	514.2785	8.022	↑	Remission	Sday12
2	LPC (16:1)	2.99995	538.3082	12.173	↓	No difference	Sday12
3	LPE (20:2)	1.0808	504.3038	12.462	↓	Remission	Sday12
4	LPC (15:0)	1.75004	526.3086	12.463	↓	Remission	Sday12
5	LPC (18:2)	2.84596	564.3229	12.464	↓	Remission	Sday12
6	LPC (22:6)	1.7154	612.3216	12.755	↓	Remission	Sday12
7	LPC (16:0)	2.56364	540.3235	12.93	↓	No difference	Sday12
8	LPC (20:3)	2.1452	590.3378	13.283	↓	Remission	Sday12
9	LPC (18:1)	3.2421	566.338	13.627	↓	Remission	Sday12
10	LPC (22:5)	2.21522	568.3541	14.473	↓	No difference	Sday12
11	FFA C22:6	1.65863	327.2296	16.893	↓	No difference	Sday12
12	Glycocholic Acid	3.57049	464.2984	8.728	↑	Remission	Sday13

13	Taurocholic acid	3.56295	514.2806	7.982	↑	No difference	Sday13
14	LPC (16:1)	4.27169	538.3106	12.08	↓	Remission trend	Sday13
15	LPC (18:2)	2.85274	564.3255	12.37	↓	Remission	Sday13
16	LPE (20:5)	2.78801	544.2654	12.423	↓	Aggravation	Sday13
17	LPC (20:2)	1.36597	592.3566	13.997	↓	No difference	Sday13
18	LPC (20:1)	1.27425	594.3723	15.026	↓	Remission trend	Sday13
19	12-Oxo-20-trihydroxy-leukotriene B4	1.78075	381.1719	18.423	↑	No difference	Sday13
20	Xanthine	2.10127	218.1023	0.666	↓	Remission	M
21	Xanthurenic acid	1.37152	250.0368	0.653	↓	Remission	M
22	Ascorbate 2-sulfate	3.2644	254.98	0.635	↑	No difference	M
23	Glycocholic acid	2.25082	464.2971	8.798	↑	Remission	M
24	LPE (20:5)	1.24043	544.2635	12.416	↓	No difference	M
25	PI (20:4)	2.8105	619.2806	12.432	↓	Remission trend	M

26	LPC (20:4)	1.48924	588.3234	12.517	↑	Remission	M
27	LPE (22:4)	1.71906	528.3054	13.564	↑	Remission	M
28	Cholesterol sulfate	2.57553	465.2993	22.286	↓	No difference	M
29	Xanthine	1.51887	368.073	0.636	↓	Remission	C
30	Ascorbate-2-sulfate	1.9067	306.0552	0.641	↑	Remission	C
31	Glycocholic acid	2.57762	464.2979	8.766	↓	Remission	C
32	LPE (20:5)	1.38509	544.2652	12.404	↓	Aggravation	C
33	LPC (22:5)	1.02522	568.3556	14.437	↓	Aggravation	C
34	LPE (20:4)	2.81304	500.2743	12.41	↓	No difference	C
35	PI (20:4)	1.92445	619.2828	12.435	↓	No difference	C

4. Reference

(1) Suchal, K.,Malik, S.,Khan, S. I.,Malhotra, R. K.,Goyal, S. N.,Bhatia, J.,Ojha, S.Arya, D. S., (2017) Molecular Pathways Involved in the Amelioration of Myocardial Injury in Diabetic Rats by Kaempferol. *International journal of molecular sciences* 18 (5).

(2) Sanchez-Gonzalez, P. D.,Lopez-Hernandez, F. J.,Perez-Barriocanal, F.,Morales, A. I.Lopez-Novoa, J. M., (2011) Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 26 (11), 3484-95.

(3) Sahu, B. D.,Kalvala, A. K.,Koneru, M.,Mahesh Kumar, J.,Kuncha, M.,Rachamalla, S. S.Sistla, R., (2014) Ameliorative effect of fisetin on cisplatin-induced nephrotoxicity in rats via modulation of NF-kappaB activation and antioxidant defence. *PloS one* 9 (9), e105070.

(4) Zhang, P.,Chen, J. Q.,Huang, W. Q.,Li, W.,Huang, Y.,Zhang, Z. J.Xu, F. G., (2017) Renal Medulla is More Sensitive to Cisplatin than Cortex Revealed by Untargeted Mass Spectrometry-Based Metabolomics in Rats. *Scientific reports* 7, 44804.