

Supplementary files

Tissue metabolomics study to reveal the toxicity of a Traditional Tibetan medicines ‘Renqing Changjue’ in rat

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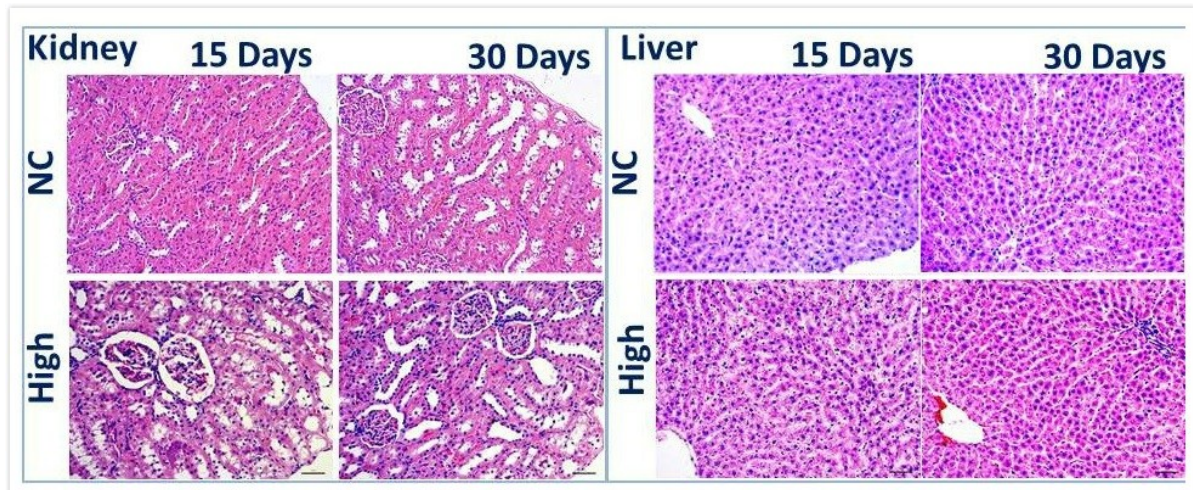


Fig. S1. Histopathological examination of control and HD group liver and kidney tissues by H&E staining (10 \times).

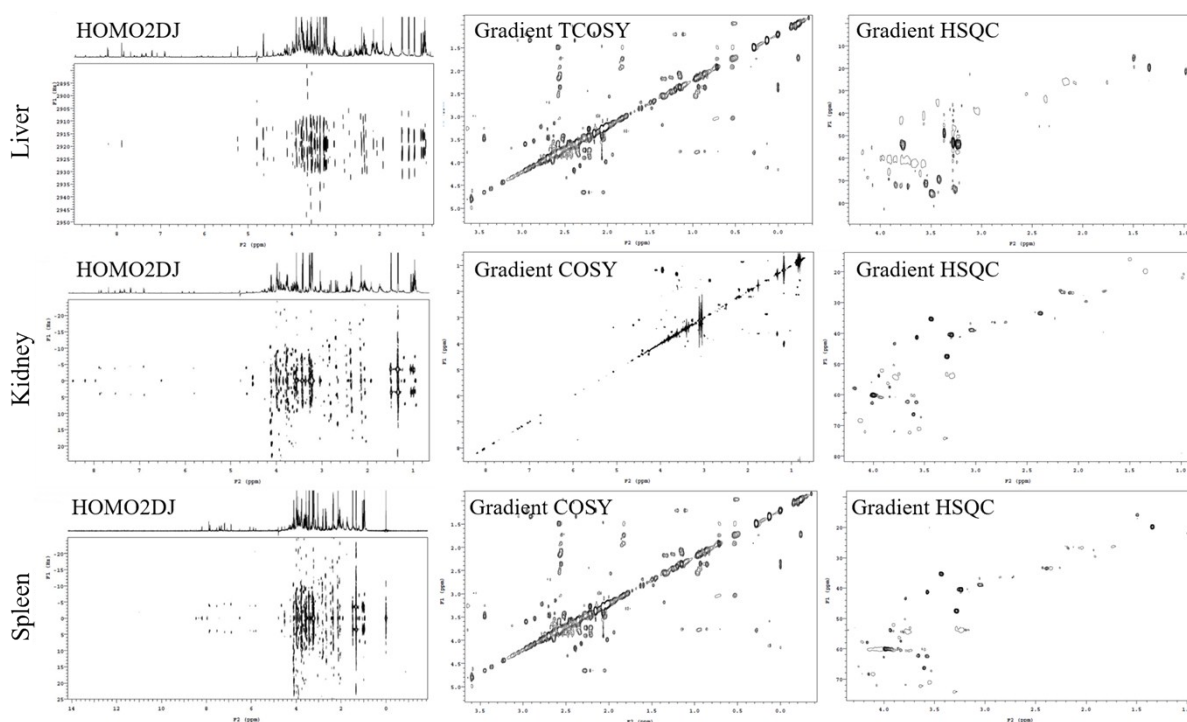


Fig. S2. Standard two-dimensional (2D) NMR spectra of liver, kidney and spleen

Parameters: For COSY and HSQC, 64 transients per increment and 128 increments were collected in the indirect dimension. A SINE function with 2048 and 1024 digital points was used for processing. Exponential multiplications of 0.20 and 0.30 Hz in the direct and indirect dimension were used, respectively. HSQC was processed using the SINE function. Exponential multiplications of 0.20 and 0.30 Hz in the direct and indirect dimension were used, respectively. In addition, a Gaussian multiplication of 0.1 Hz in the indirect dimension was applied for the HSQC. Two dimensional J-resolved spectra were also recorded to aid the assignments of the metabolites. The pulse sequence is of the form -RD-90-t1-180-t1-ACQ. The RD was 2 s, and the number of scans was 1 for all experiments.

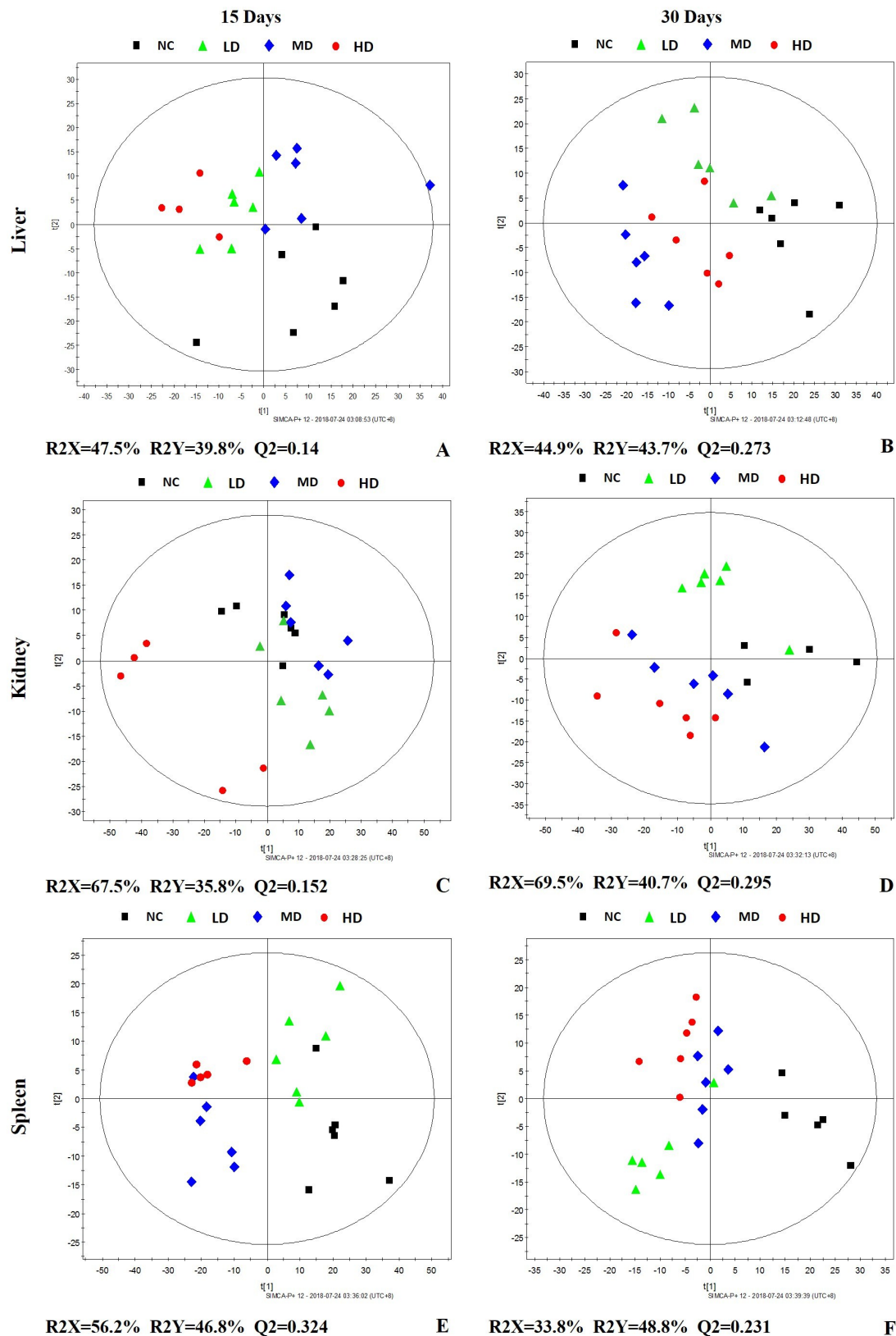


Fig. S3. Representative PLS-DA score plots (t_1 vs. t_2) derived from the ^1H NMR data of liver extract (A and B), kidney extract (C and D), and spleen extract (E and F) from control and dosed groups at day 15 and day 30

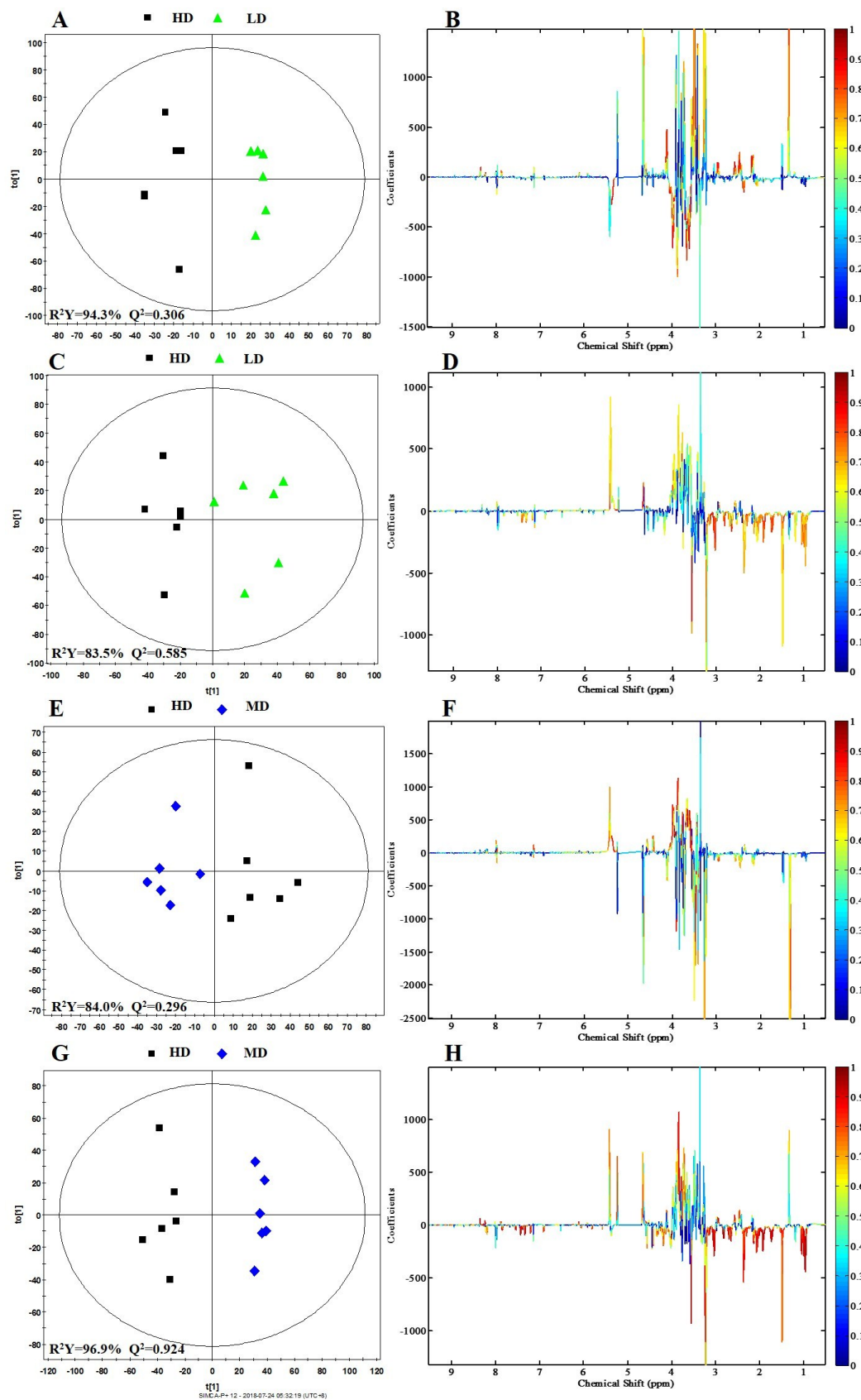


Fig. S4. OPLS-DA scores plots (A, C, E and G) and coefficient loading plots (B, D, F and H) derived from ^1H NMR spectra of liver from LD/ NC and MD/ NC group at day 15 and day 30.

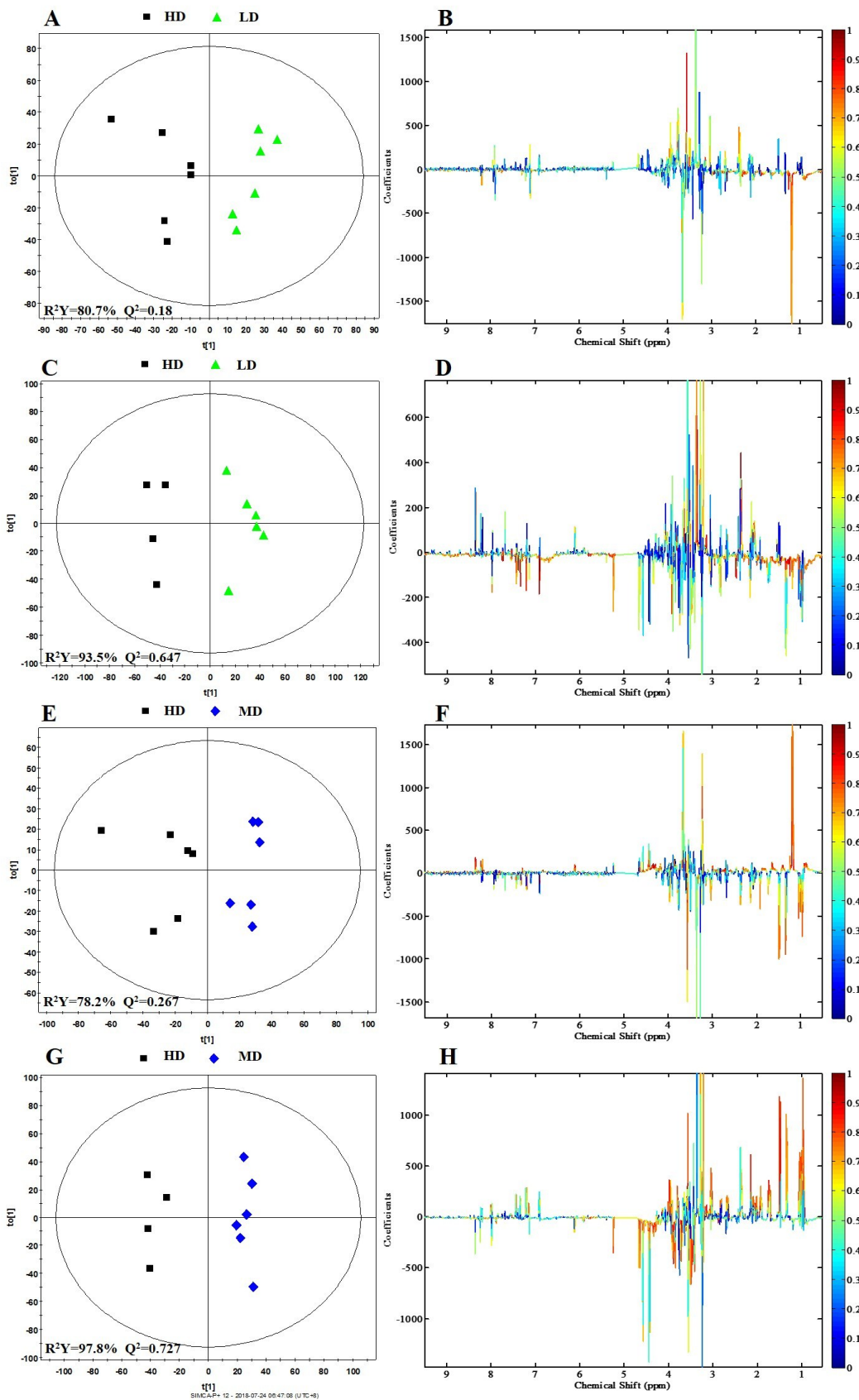


Fig. S5. OPLS-DA scores plots (A, C, E and G) and coefficient loading plots (B, D, F and H) derived from 1H NMR spectra of kidney from LD/ NC and MD/ NC group at day 15 and day 30.

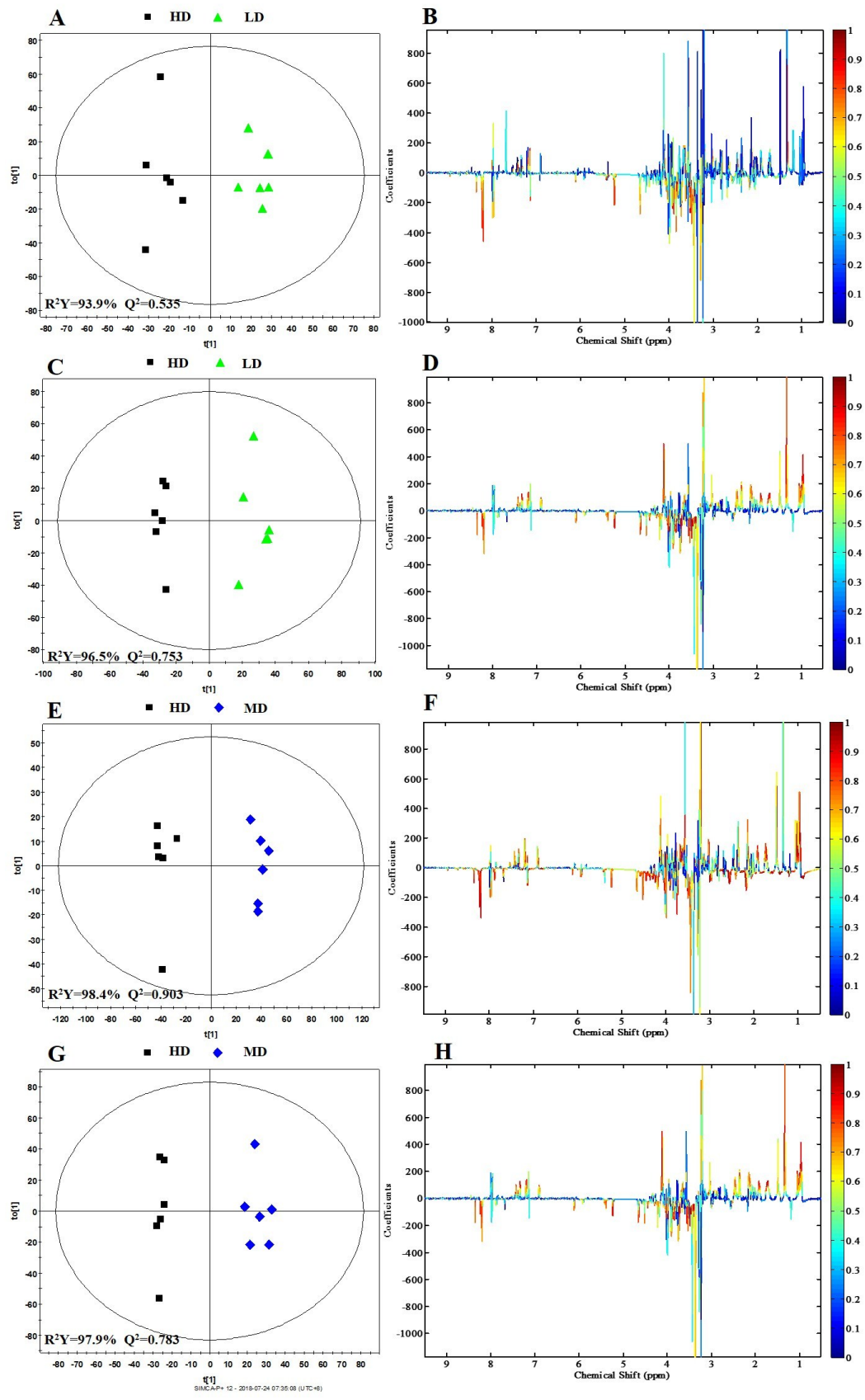


Fig. S6. OPLS-DA scores plots (A, C, E and G) and coefficient loading plots (B, D, F and H) derived from 1H NMR spectra of spleen from LD/ NC and MD/ NC group at day 15 and day 30.

Table S1. The level of serum biochemical indices of RQCJ successive administration ($\bar{x} \pm s$).

	Biochemical indices	HD group	MD group	LD group	NC group
Day 15	AST (IU/L)	224.28±35.0	190.6±56.91	136.8±14.1	152.9±52.3
	ALT (IU/L)	61.33±8.33	56.67±10.0	52.06±2.82*	54.85±10.7
	ALP (IU/L)	327.3±73.1*	293.2±28.5*	323.6±29.6	347.8±15.28
	BUN (mmol/L)	7.717±1.28*	7.933±0.42*	6.880±1.18	9.432±1.54
	CHE (IU/L)	28.45±9.12	26.35±6.11	19.34±1.38*	28.67±15.28
Day 30	AST (IU/L)	203.0±58.2	196.3±48.67	177.4±41.4	193.6±43.0
	ALT (IU/L)	60.13±10.3	48.62±4.13*	57.22±3.98	57.18±9.16
	ALP(IU/L)	265.6±34.1	259.7±33.0	252.7±47.8	268.3±84.03
	BUN (mmol/L)	8.935±0.87	7.517±0.95	9.020±1.40	9.395±0.44
	CHE (IU/L)	28.47±4.06	31.15±5.68	32.86±4.86	33.67±4.08

Note:* compared with control group, $p < 0.05$

Table S2. Results from Pathway Analysis with MetPA from liver.

Pathway Name	Total	Hits	Raw p	=-LOG(p)	Holm P	FDR	Impact
D-Glutamine and D-glutamate metabolism	5	2	0.000363	7.9214	0.029033	0.013544	1
Phenylalanine, tyrosine and tryptophan biosynthesis	4	1	0.025458	3.6707	1	0.34369	0.5
Alanine, aspartate and glutamate metabolism	24	2	0.0094	4.6671	0.72378	0.15228	0.40928
Phenylalanine metabolism	9	1	0.05647	2.874	1	0.50823	0.40741
Histidine metabolism	15	2	0.003685	5.6034	0.28745	0.074627	0.24194
Nicotinate and nicotinamide metabolism	13	1	0.080645	2.5177	1	0.65322	0.2381
Glyoxylate and dicarboxylate metabolism	16	1	0.098413	2.3186	1	0.72441	0.11111

Total is the total number of compounds in the pathway; the hits is the actually matched number from the user uploaded data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis.

Table S3. Results from Pathway Analysis with MetPA from kidney.

Pathway Name	Total	Hits	Raw p	=-LOG(p)	Holm P	FDR	Impact
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	0.000472	7.6595	0.037252	0.012732	1
D-Glutamine and D-glutamate metabolism	5	1	0.045574	3.0884	1	0.24968	1
Valine, leucine and isoleucine biosynthesis	11	2	0.004167	5.4806	0.3125	0.048214	0.66666
Alanine, aspartate and glutamate metabolism	24	4	4.27E-05	10.061	0.003419	0.001731	0.45253
Taurine and hypotaurine metabolism	8	1	0.071991	2.6312	1	0.32396	0.42857
Phenylalanine metabolism	9	2	0.002756	5.894	0.21221	0.037206	0.40741
Glycine, serine and threonine metabolism	32	3	0.002647	5.9342	0.20648	0.037206	0.29197
Tyrosine metabolism	42	1	0.32775	1.1155	1	1	0.14045
Arginine and proline metabolism	44	3	0.006624	5.0171	0.49018	0.067068	0.10545

Total is the total number of compounds in the pathway; the hits is the actually matched number from the user uploaded data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis.

Table S4. Results from Pathway Analysis with MetPA from spleen.

Pathway Name	Total	Hits	Raw p	=-LOG(p)	Holm P	FDR	Impact
Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	0.000723	7.2316	0.057147	0.019531	1
D-Glutamine and D-glutamate metabolism	5	1	0.055852	2.8851	1	0.41127	1
Valine, leucine and isoleucine biosynthesis	11	2	0.006328	5.0628	0.48094	0.08543	0.66666
Alanine, aspartate and glutamate metabolism	24	4	0.000105	9.1608	0.008406	0.004256	0.45569
Taurine and hypotaurine metabolism	8	1	0.087945	2.431	1	0.49822	0.42857
Phenylalanine metabolism	9	2	0.004198	5.4732	0.32322	0.068003	0.40741
Tryptophan metabolism	41	1	0.37965	0.96849	1	1	0.15684
Tyrosine metabolism	42	2	0.080649	2.5176	1	0.49822	0.14045
Glyoxylate and dicarboxylate metabolism	16	1	0.1686	1.7802	1	0.75869	0.11111
Arginine and proline metabolism	44	4	0.001168	6.7524	0.091114	0.023655	0.10545

Total is the total number of compounds in the pathway; the hits is the actually matched number from the user uploaded data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis.

Table S5. Concentrations of As and Hg in control and dosed-group tissues sample.

sample	time points	Element	Control group	Low dosed group	Medium dosed group	High dosed group
Liver	15 day	As	0.593±0.05	1.816±0.21*	3.696±0.50*	9.963±1.53*
		Hg	0.658±0.19	1.749±0.21*	4.039±1.09*	9.891±1.52*
	30 day	As	0.970±0.14	1.923±0.29	4.099±0.41*	8.938±1.48
		Hg	0.332±0.05	0.451±0.11	0.632±0.27*	0.839±0.56*
Kidney	15 day	As	1.208±0.28	2.942±0.14*	6.862±0.62*	19.75±5.67*
		Hg	0.159±0.03	2.173±0.38*	3.909±1.28*	8.840±1.79*
	30 day	As	1.397±0.35	2.982±0.58*	6.711±0.54*	16.04±2.60*
		Hg	0.105±0.04	2.553±0.71*	4.463±0.88*	6.266±0.95*
Spleen	15 day	As	5.037±1.21	13.90±3.75*	28.46±4.04*	58.13±16.17*
		Hg	Non-Detected	Non-Detected	Non-Detected	Non-Detected
	30 day	As	5.307±1.38	13.52±2.83*	33.29±8.06*	77.59±15.36*
		Hg	Non-Detected	Non-Detected	Non-Detected	Non-Detected

Data were presented as mean±SD of n=8 animals per groups. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test.

* P<0.05 versus control group. * *P<0.01 versus control group.