

Toxicological risks of Renqingchangjue in rats evaluated by ¹H-NMR based serum and urine metabolomics analysis

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Xia Wang and Caidan Rezeng contributed equally to this work.

Figure Captions:

Figure S1. Body weight of rats in four groups during RQCJ administration and withdrawal recovery. Day 0: one day before treatment, days 5–15: the time points of administration, days 18–30: the time points of stop administration. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.

Figure S2. PLS-DA score plot of serum(A, B) and urine (C, D) samples from the four groups at day 15 and day 30. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.

Figure S3. OPLS-DA score plots (A, C, E and G) and color-coded coefficient plots (B, D, F and H) of serum samples from LD, MD and NC group at day 15 and day 30. NC: Normal control group, LD: Low-dose group, MD: middle-dose group.

Figure S4. PCA score plot of urine samples from the four groups at day 0 (A), 3(B), 6 (C), 9 (D), 12 (E), 15 (F), 18 (G), 23 (H), 26 (I) and 30 (J). Day 0: one day before treatment, days 3–15: the time points of administration, days 18–30: the time points of stop administration. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.

Figure S5. OPLS-DA score plots (A, C, E and G) and color-coded coefficient plots (B, D, F and H) of urine samples from LD, MD and NC group at day 15 and day 30. NC: Normal control group, LD: Low-dose group, MD: middle-dose group.

Figure S6. The network of potential biomarkers associated with RQCJ in serum by metscape analysis. The detected metabolites in our study are represented by red hexagons. Hexagons with green lines means that the alteration of the metabolite in HD groups had statistical significance ($P < 0.05$). The size of hexagons indicates the fold change of the corresponding metabolite in HD relative to control. In addition, pink hexagons indicate metabolites participating in the metabolic pathway but not been detected in the study.

Figure S7. The network of potential biomarkers associated with RQCJ in urine by metscape analysis. The detected metabolites in our study are represented by red hexagons. Hexagons with green lines means that the alteration of the metabolite in HD groups had statistical significance ($P < 0.05$). The size of hexagons indicates the fold change of the corresponding metabolite in HD relative to control. In addition, pink hexagons indicate metabolites participating in the metabolic pathway but not been detected in the study.

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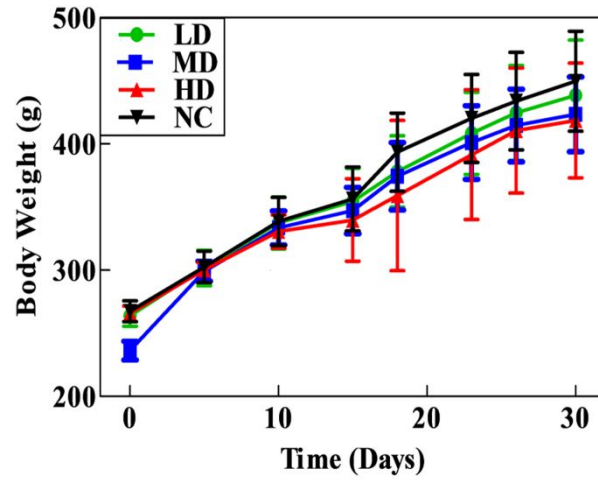


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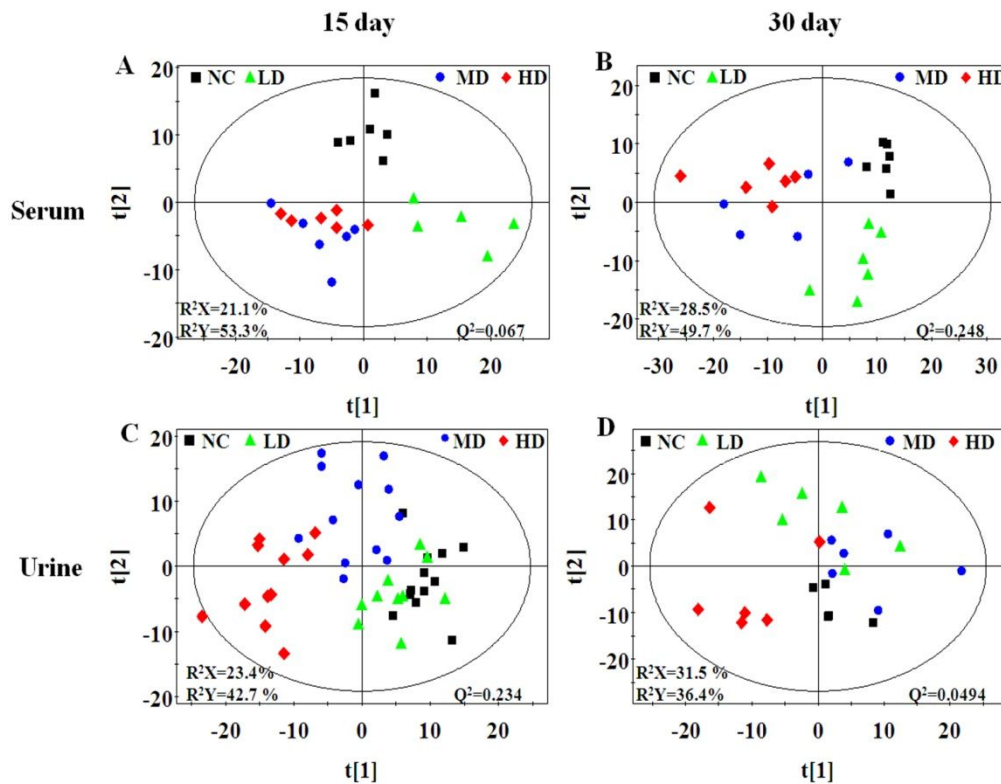


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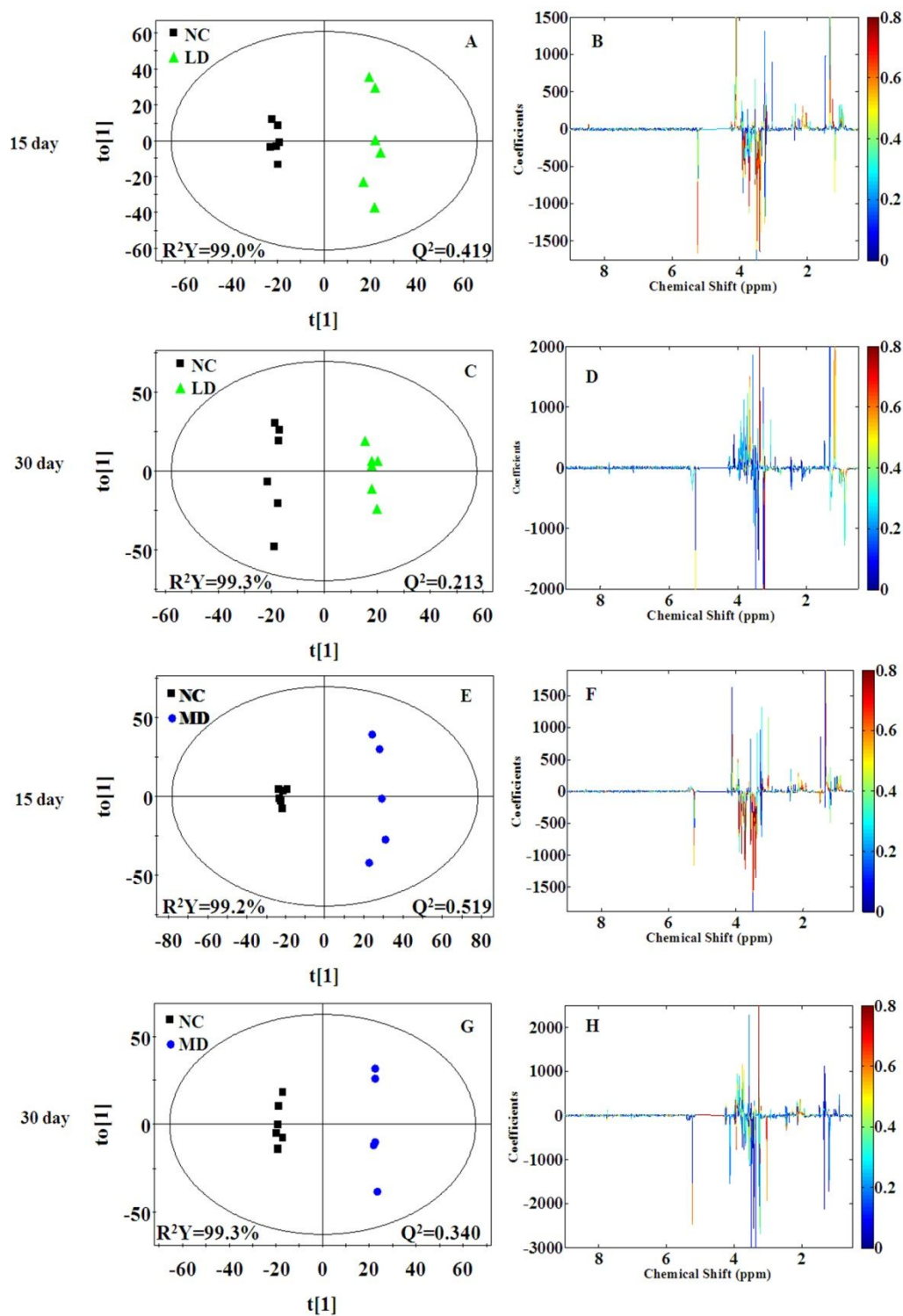


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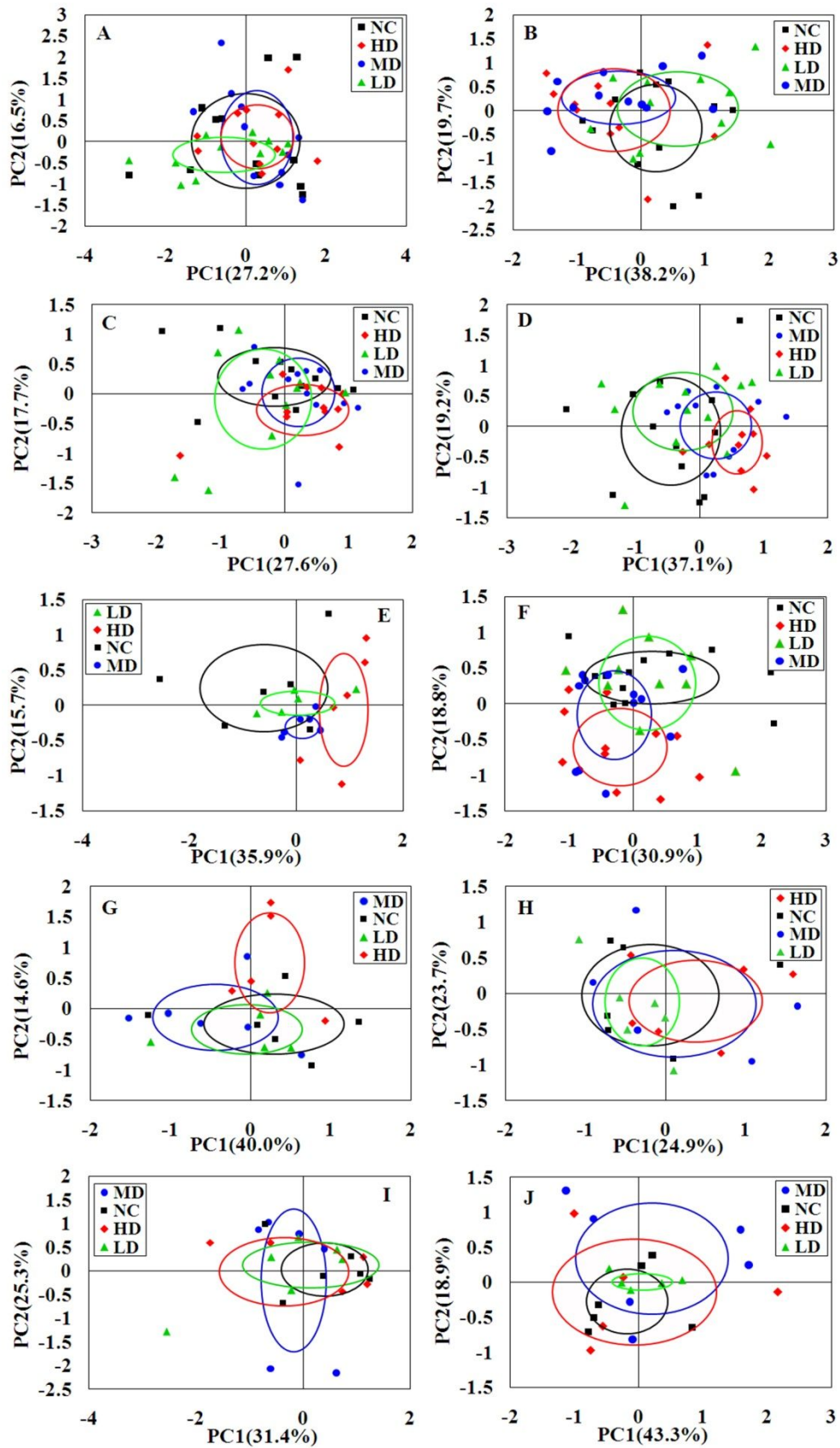


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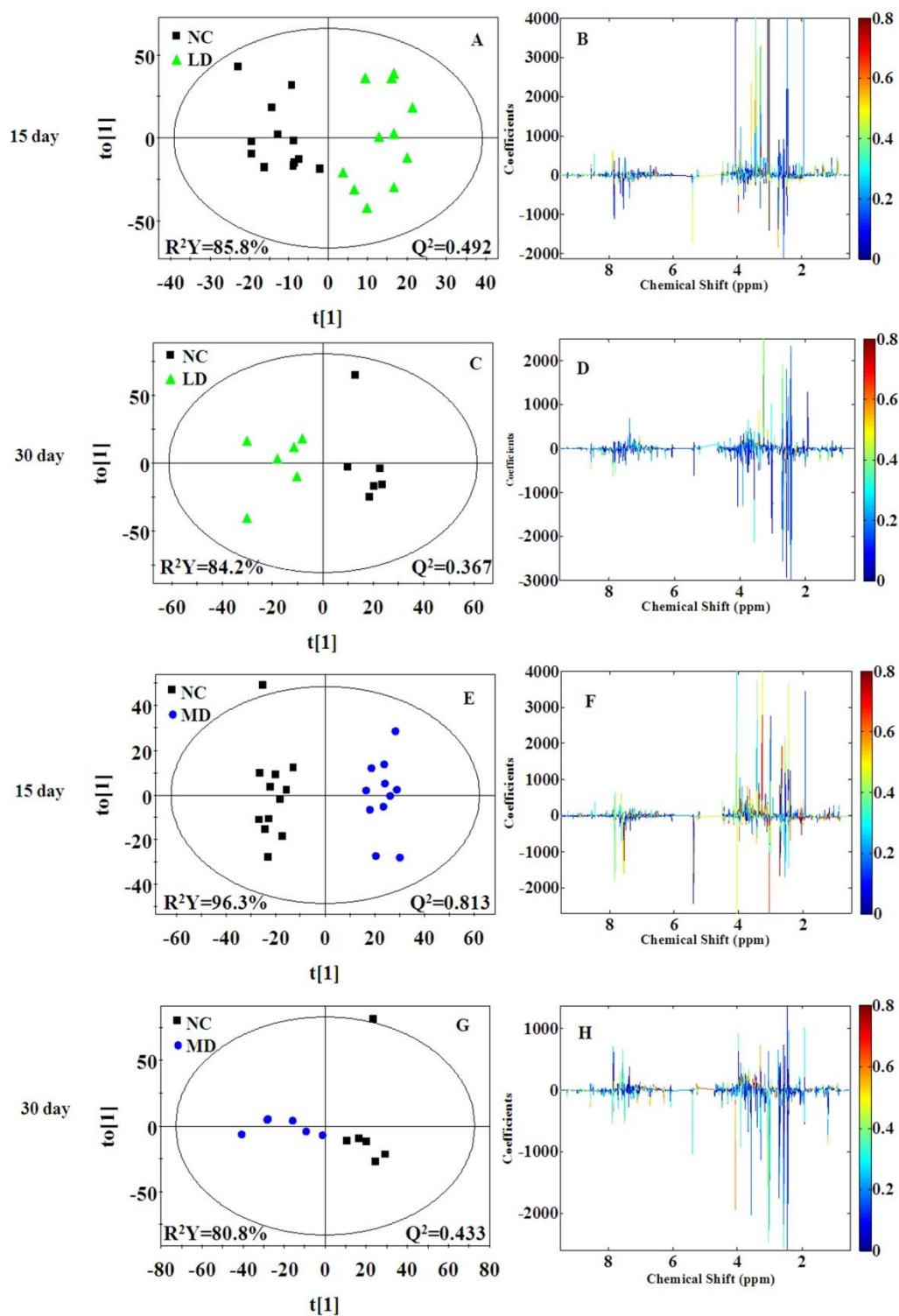


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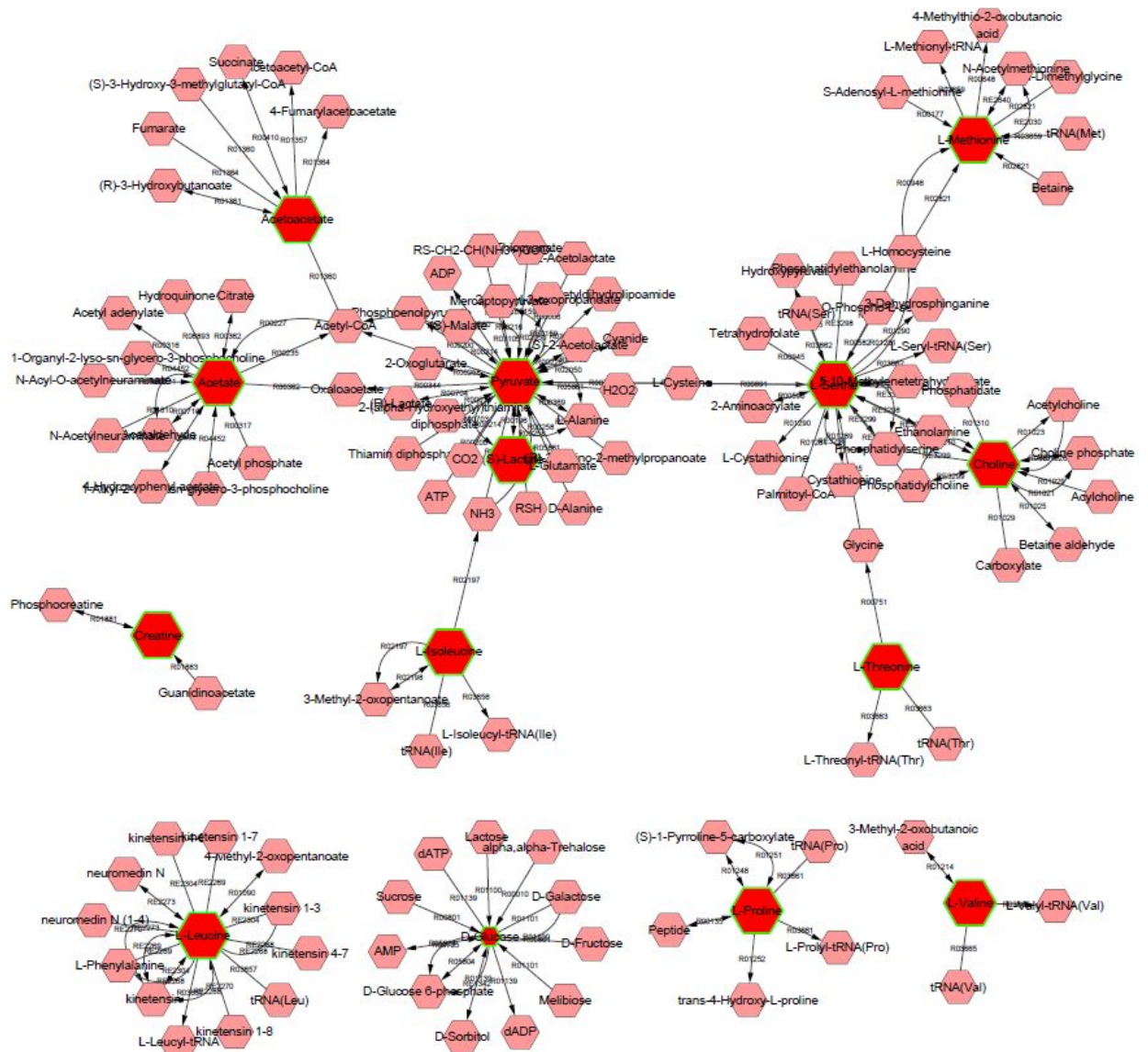


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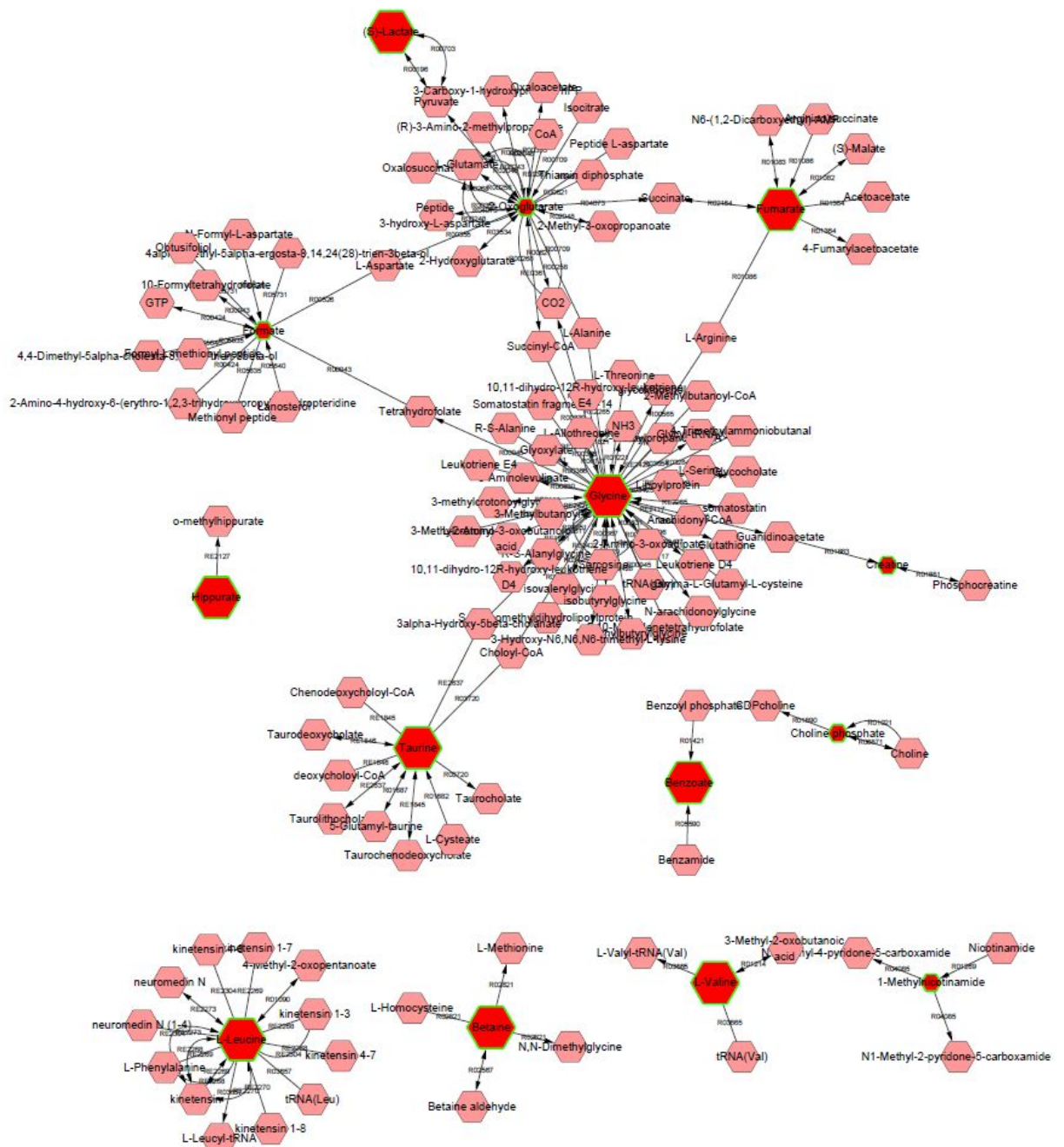


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Table S1. Summary of serum clinical biochemical parameters at day 15 and day 30.

Time	Biochemical parameters	NC	LD	MD	HD
Day 15	AST (IU/L)	152.90±52.30	136.80±14.01	190.60±56.91*	224.28±35.01*
	ALT (IU/L)	54.85±10.70	52.06±2.82*	56.67±10.00*	61.33±8.33*
	ALP (IU/L)	347.80±15.28	323.60±29.61	327.30±28.50	293.20±73.10*
	BUN (mmol/L)	9.43±1.54	6.88±1.18*	7.93±0.42*	7.17±1.28*
Day 30	AST (IU/L)	193.60±43.01	177.40±41.40	196.30±48.67	203.00±58.20
	ALT (IU/L)	57.18±9.16	57.22±3.98	48.68±4.13	60.13±10.30
	ALP (IU/L)	268.30±84.03	252.70±47.80	259.70±33.01*	265.60±34.10
	BUN (mmol/L)	9.39±0.44	9.02±1.40	7.52±0.95	8.93±0.87

Data were expressed as mean±SD (n=12). *P<0.05 each dosed group vs. control group. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.

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

Pathway Name	Total	Expected	Hits	Raw p	-Log(p)	Holm adjust	FDR	Impact
Aminoacyl-tRNA biosynthesis	67	0.66904	7	1.10E-06	13.723	8.88E-05	8.02E-05	0.13793
Valine, leucine and isoleucine biosynthesis	11	0.10984	4	1.98E-06	13.133	0.000158	8.02E-05	0.99999
Glycine, serine and threonine metabolism	32	0.31954	5	7.77E-06	11.765	0.000614	0.00021	0.2428
Pyruvate metabolism	22	0.21969	3	0.001093	6.8189	0.084153	0.017705	0.24337
Glycolysis or Gluconeogenesis	26	0.25963	3	0.001802	6.3189	0.13694	0.024325	0.12753
Cysteine and methionine metabolism	28	0.2796	3	0.002244	6.0997	0.16827	0.025961	0.1388
Butanoate metabolism	20	0.19971	2	0.015882	4.1425	1	0.16081	0.10145
Synthesis and degradation of ketone bodies	5	0.049929	1	0.04901	3.0157	1	0.44109	0.6
Arginine and proline metabolism	44	0.43937	2	0.068977	2.674	1	0.50792	0.07598
Methane metabolism	9	0.089872	1	0.086602	2.4464	1	0.58456	0.4
Citrate cycle (TCA cycle)	20	0.19971	1	0.18299	1.6983	1	1	0.07184
Starch and sucrose metabolism	23	0.22967	1	0.20758	1.5722	1	1	0.03778
Galactose metabolism	26	0.25963	1	0.23149	1.4632	1	1	0.03644
Glycerophospholipid metabolism	30	0.29957	1	0.26233	1.3382	1	1	0.02315

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 urine.

Pathway Name	Total	Expected	Hits	Raw p	-Log(p)	Holm adjust	FDR	Impact
Valine, leucine and isoleucine biosynthesis	11	0.11769	2	0.005561	5.192	0.44488	0.22522	0.66666
Citrate cycle (TCA cycle)	20	0.21398	2	0.01817	4.008	1	0.36794	0.09487
Alanine, aspartate and glutamate metabolism	24	0.25678	2	0.02575	3.6593	1	0.41715	0.06645
Glycine, serine and threonine metabolism	32	0.34237	2	0.044048	3.1225	1	0.46104	0.29197
Taurine and hypotaurine metabolism	8	0.085592	1	0.082653	2.4931	1	0.52549	0.42857
Primary bile acid biosynthesis	46	0.49215	2	0.084338	2.4729	1	0.52549	0.05952
Nicotinate and nicotinamide metabolism	13	0.13909	1	0.13103	2.0324	1	0.70754	0.125
Glyoxylate and dicarboxylate metabolism	16	0.17118	1	0.1589	1.8395	1	0.75709	0.11111
Glutathione metabolism	26	0.27817	1	0.24589	1.4029	1	0.98012	0.00573
Glycerophospholipid metabolism	30	0.32097	1	0.27827	1.2792	1	1	0.04444

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. Contents of As and Hg in serum sample of control and dosed-group. (ng/g)

Time	Element	NC	LD	MD	HD
Day 15	As	2283±949	5456±1052*	20754±5919*	64081±5472**
	Hg	0.232±0.12	0.898±0.23*	4.745±1.31*	5.396±2.51**
Day 30	As	2429±407.73	11428±2561*	20262±2666*	50831±6961*
	Hg	0.550±0.26	1.034±0.40*	1.155±0.33*	1.940±0.96*

Data were expressed as mean±SD (n=12). *P<0.05, **P<0.01 each dosed group vs. control group. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.

Table S5. Contents of As and Hg in urine sample of control and dosed-group. (ng/g)

Time	Element	NC	LD	MD	HD
Day 0	As	11.81±8.77	16.95±5.53	12.18±4.32	13.09±53.23
	Hg	not detected	not detected	not detected	not detected
Day 3	As	8.25±5.18	133.50±42.70*	334.30±48.90*	707.70±67.50**
	Hg	not detected	not detected	not detected	not detected
Day 6	As	9.13±4.44	172.80±64.70*	336.10±131.00*	965.5±202.30**
	Hg	0.499±0.59	not detected	not detected	0.85±0.12
Day 9	As	7.83±4.00	142.20±26.30*	286.30±77.60*	1168±330**
	Hg	0.725±0.23	not detected	5.380±1.78*	8.880±1.60*
Day 12	As	14.64±7.53	209.20±19.60*	471.70±100*	1243±305**
	Hg	not detected	2.308±0.97*	6.783±1.35*	15.98±3.66*
Day 15	As	12.30±4.55	186.10±88.50*	395.30±131.50*	1258±206**
	Hg	not detected	19.40±1.89*	25.56±3.05*	63.75±18.20**
Day 18	As	10.23±4.72	60.84±26.1*	108.20±52.0*	211.50±43.90**
	Hg	1.407±0.99	5.415±0.78*	4.004±0.99*	43.14±9.10*
Day 23	As	7.942±4.12	36.60±8.66*	107.2±43.0*	157.20±54.30*
	Hg	not detected	2.476±1.17*	5.537±1.37*	13.02±0.97*
Day 26	As	9.671±4.78	32.461±10.56*	64.54±25.90*	125.5±33.10*
	Hg	not detected	1.397±3.76*	not detected	6.029±0.73*
Day 30	As	10.65±3.57	12.84±12.50	71.07±26.50*	124.8±47.50*
	Hg	not detected	not detected	not detected	2.197±0.59*

Data were expressed as mean±SD (n=12). *P<0.05, **P<0.01 each dosed group vs. control group. NC: Normal control group, LD: Low-dose group, MD: middle-dose group, HD: high-dose group.