Supporting Information

Metabonomic and transcriptomic analyses of *Tripterygium* glycosides tablet–induced liver injury in rats

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Table S1. Primers designed for qRT-PCR validation of candidate mRNAs.

No.	Gene Symbol	Forward primer	Reverse primer	Product length(bp)	Ta(°C)
1	Ddc	CATGTGGGTGAAGAAGAGAAC	CCTCAGATAAACAGGGTCCATA	64	60
2	Cyp1a2	GCTGTGGACTTCTTTCCG	GCAGAAACAGCACAAAGTTATC	91	60
3	Ido2	GCCAGTTTGCTTGCTAGGAA	GCCCACAGAATGAAGACAC	88	60
4	Aldh1b1	ATCAGAATGTTAGTGAAGGACG	GCAAGCAGTTGTTCCCTTA	87	60
5	Ehhadh	GCCTTACTGGACCATCATTG	CAGCTTCACAGAGCATATCG	93	60
6	Gpt	CAGTTCGAGGACCCATTG	GCACGGATGACCTCAGTAA	91	60
7	Asns	CTGATTGGCTGACCCATTATT	GCTTTGGCAGTTGACTTGTA	94	60
8	Got1	CAAGAACTTCGGGCTCTACA	CTTCTCCATCTGGGAAAGG	97	60

Table S2. Serum levels of six biochemical markers in rats with *Tripterygium glycosides* tablet–induced liver injury over 6 weeks: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and triglyceride (TG). The control group received 0.9% sodium chloride solution. The low- and high-dose groups received 9.5 mg/kg and 18.9 mg/kg Tripterygium glycosides tablets, respectively.

Group	AST （U/L）	ALT （U/L）	TBIL （μmol/L)	GGT （U/L）	ALP （U/L）	TG （mmol/L)
week-1						
control1-1	125	35	0.2	0.9	198.3	0.94
control1-2	101	36	0.3	1	194.2	0.4
control1-3	125	29	0.1	0.8	169.7	0.61
control1-4	97	36	0.2	0.7	204.6	0.57
control1-5	98	39	0.1	0.6	184.2	0.52
control1-6	102	32	0.3	0.5	214.5	0.74
mean	108.00	34.50	0.20	0.75	194.25	0.63
SD	12.14	3.20	0.08	0.17	14.36	0.17
low-dose1-1	133	41	0.2	0.9	174.7	0.93
low-dose1-2	95	42	0	0.6	210.3	0.95
low-dose1-3	109	51	0.1	0.9	205.2	0.83
low-dose1-4	106	49	0	0.6	211.2	0.54
low-dose1-5	94	36	0.1	0.6	197.1	0.89
low-dose1-6	101	47	0	0.9	194	0.75
mean	106.33	44.33	0.07	0.75	198.75	0.82
SD	14.33	5.65	0.08	0.16	13.67	0.15
t-test	0.68	**0.04**	0.08	1.00	0.65	0.12
high-dose1-1	143	43	0.4	0.6	245.5	0.83
high-dose1-2	136	40	0.2	0.8	238.7	0.37
high-dose1-3	162	37	0.2	0.4	215.4	0.44
high-dose1-4	114	45	0.3	0.4	227.9	0.54
high-dose1-5	153	47	0.3	0.7	287.2	0.49
high-dose1-6	135	46	0.5	0.8	184.7	0.52
mean	140.50	43.00	0.32	0.62	233.23	0.53
SD	16.60	3.85	0.12	0.18	34.04	0.16
t-test	**0.00**	**0.00**	0.06	0.29	0.08	**0.03**
week-2						
control2-1	92	51	0.45	0.3	141.8	0.98
control2-2	103	52	0.6	0.2	179.4	0.83
control2-3	127	49	0.38	0.1	127.4	0.85
control2-4	105	43	0.53	0.6	168.6	0.6
control2-5	88	47	0.68	0.1	186.6	0.84
control2-6	131	40	0.6	0.5	127	0.72
mean	107.67	47.00	0.54	0.30	155.13	0.80
SD	17.77	4.69	0.11	0.21	26.45	0.13
low-dose2-1	148	44	0.6	0.5	149.3	0.68
low-dose2-2	121	43	0.38	0.6	106.5	0.37

low-dose2-3	178	51	0.53	0.3	146.4	0.43
low-dose2-4	164	57	0.38	0.8	215.5	0.7
low-dose2-5	140	51	0.75	0.7	140.4	1
low-dose2-6	133	53	0.53	0.3	145.7	0.37
mean	147.33	49.83	0.53	0.53	150.63	0.59
SD	20.84	5.38	0.14	0.21	35.51	0.25
t-test	**0.01**	0.50	0.86	0.08	0.82	0.11
high-dose2-1	124	48	0.3	0.1	196	1.19
high-dose2-2	95	52	0.68	0.4	134.7	0.71
high-dose2-3	145	48	0.38	0.5	132.9	0.52
high-dose2-4	133	59	0.68	0.3	263.9	1.01
high-dose2-5	122	45	0.45	0.2	133.1	0.73
high-dose2-6	126	45	0.6	0	199.5	0.48
mean	124.17	49.50	0.52	0.25	176.68	0.77
SD	16.56	5.32	0.16	0.19	53.07	0.28
t-test	0.08	0.43	0.68	0.73	0.44	0.81
			week-3			
control3-1	121	47	1.1	0.6	207.8	0.46
control3-2	96	50	1	0.1	216	0.41
control3-3	93	50	0.5	0.3	204.3	0.71
control3-4	89	46	1	0.7	249.8	0.72
control3-5	99	42	0.8	0.3	174.6	0.6
control3-6	104	49	0.9	0.3	197.4	0.66
mean	100.33	47.33	0.88	0.38	208.32	0.59
SD	11.34	3.08	0.21	0.22	24.71	0.13
low-dose3-1	134	46	0.9	0	197.4	0.34
low-dose3-2	151	31	0.7	0.6	196.1	0.44
low-dose3-3	122	33	0.7	0.1	173.5	0.72
low-dose3-4	139	44	1	0.1	172.5	0.45
low-dose3-5	129	41	0.8	0.4	203.7	0.52
low-dose3-6	144	46	1	0.4	252.9	0.84
mean	136.50	40.17	0.85	0.27	199.35	0.55
SD	10.45	6.62	0.14	0.23	29.29	0.19
t-test	**0.00**	0.09	0.68	0.54	0.66	0.53
high-dose3-1	105	42	1.1	0.2	163.7	0.79
high-dose3-2	126	41	0.9	0.1	178.2	0.66
high-dose3-3	126	46	1.3	0.2	217.1	0.53
high-dose3-4	93	43	1.2	0.2	185.4	0.36
high-dose3-5	137	39	1	0.2	147.9	1.08
high-dose3-6	107	34	1.1	0.4	166.1	0.41
mean	115.67	40.83	1.10	0.22	176.40	0.64
SD	16.56	4.07	0.14	0.10	23.75	0.27
t-test	0.14	**0.02**	0.15	0.14	**0.03**	0.77
			week-4			
control4-1	81	47	1.7	0.4	138.5	0.73
control4-2	64	33	1.9	0.3	100.1	1.19

control4-3	78	48	1.9	0.4	133.6	0.89
control4-4	82	41	1.7	0.1	107.1	0.6
control4-5	80	48	1.7	0.3	122.2	0.75
control4-6	89	47	1.8	0.3	130.8	0.66
mean	79.00	44.00	1.78	0.30	122.05	0.80
SD	8.25	6.00	0.10	0.11	15.40	0.21
low-dose4-1	81	47	1.8	0.1	151.7	0.8
low-dose4-2	99	42	1.9	0.2	122.3	0.73
low-dose4-3	104	40	1.9	0.1	100.1	0.32
low-dose4-4	101	40	1.6	0.2	157.4	0.55
low-dose4-5	95	43	1.6	0.4	130.8	0.62
low-dose4-6	91	49	1.8	0.1	146.7	0.43
mean	95.17	43.50	1.77	0.18	134.83	0.58
SD	8.30	3.73	0.14	0.12	21.51	0.18
t-test	**0.03**	0.84	0.61	0.18	0.30	0.07
high-dose4-1	91	40	1.8	0.2	117.6	0.54
high-dose4-2	87	42	1.5	0.3	116.8	0.62
high-dose4-3	96	43	1.8	0.2	91.3	0.48
high-dose4-4	92	36	1.7	0.5	114.1	0.53
high-dose4-5	98	39	1.6	0.1	110.8	0.38
high-dose4-6	94	46	1.7	0.4	130.9	0.43
mean	93.00	41.00	1.68	0.28	113.58	0.50
SD	3.90	3.46	0.12	0.15	12.89	0.09
t-test	**0.00**	0.31	0.20	0.87	0.37	**0.01**
			week-5			
control5-1	90	53	1.5	0.4	160.3	0.78
control5-2	89	57	1.8	0.2	112.1	0.66
control5-3	88	43	1.8	0.1	136.2	1.12
control5-4	76	40	1.9	0.4	88.4	0.79
control5-5	82	40	1.6	0.3	111.5	0.66
control5-6	106	40	1.8	0.2	127.2	0.91
mean	88.50	45.50	1.73	0.27	122.62	0.82
SD	10.07	7.56	0.15	0.12	24.62	0.17
low-dose5-1	99	40	2	0.5	126	0.63
low-dose5-2	92	41	2.2	0.4	148.7	1.48
low-dose5-3	84	51	1.6	0.3	117.9	0.41
low-dose5-4	79	47	1.5	0.2	96	0.64
low-dose5-5	75	39	1.7	0.3	135	0.46
low-dose5-6	76	59	1.5	0.2	177.7	0.41
mean	84.17	46.17	1.75	0.32	133.55	0.67
SD	9.58	7.81	0.29	0.12	27.90	0.41
t-test	0.48	0.91	0.92	0.46	0.45	0.52
high-dose5-1	97	50	1.6	0.1	99	0.49
high-dose5-2	98	47	1.5	0.3	86.9	0.3
high-dose5-3	96	47	1.8	0.3	106.3	0.29
high-dose5-4	87	43	1.8	0.1	110.1	0.65

high-dose5-5	93	36	1.5	0.2	94.6	0.4
high-dose5-6	81	31	1.7	0.2	76.4	0.43
mean	92.00	42.33	1.65	0.20	95.55	0.43
SD	6.69	7.37	0.14	0.09	12.50	0.13
t-test	0.57	0.24	0.19	0.47	0.07	**0.01**
week-6						
control6-1	144	45	0.7	0.2	130.1	0.7
control6-2	105	56	1.7	0.1	156.7	0.49
control6-3	/	/	/	/	/	/
control6-4	101	59	1.3	0.1	122.9	0.44
control6-5	99	46	1	0.2	101.2	0.42
control6-6	96	48	0.9	0.1	105	0.64
mean	109.00	50.80	1.12	0.14	123.18	0.54
SD	19.84	6.30	0.39	0.05	22.28	0.12
low-dose6-1	100	47	1	0.1	112.5	0.45
low-dose6-2	97	48	0.9	0.4	131	0.48
low-dose6-3	90	40	0.8	0.4	98.7	0.96
low-dose6-4	120	53	1.4	0.2	171	0.48
low-dose6-5	97	41	0.7	0.1	115.6	0.49
low-dose6-6	101	38	0.8	0.1	119.6	0.52
mean	100.83	44.50	0.93	0.22	124.73	0.56
SD	10.15	5.75	0.25	0.15	24.97	0.20
t-test	0.60	0.06	0.44	0.62	0.64	0.41
high-dose6-1	73	37	0.8	0.3	87.7	0.45
high-dose6-2	105	50	0.6	0.3	97.1	0.57
high-dose6-3	103	39	1.1	0.3	99.4	0.56
high-dose6-4	92	38	1.1	0.1	90	0.35
high-dose6-5	104	37	0.7	0.3	76.4	0.58
high-dose6-6	109	52	0.8	0.1	88.7	0.6
mean	97.67	42.17	0.85	0.23	89.88	0.52
SD	13.35	6.91	0.21	0.10	8.13	0.10
t-test	0.46	0.12	0.20	0.10	**0.01**	0.71

Table S3. Summary of potential biomarkers from control group and high dose group by LC-ESI/MS analysis in positive and negative ion mode.

	Positive ion mode					Negative ion mode			
No.	*m/z*	Rt (min)	Fold change[a]	P value	No.	*m/z*	Rt (min)	Fold change[a]	P value
1	123.0441	3.04	0.86	2.78E-02	1	215.0322	1.17	0.67	5.09E-
2	130.0862	1.60	1.55	2.98E-03	2	153.0180	6.32	2.03	6.54E-
3	141.0656	1.64	1.41	4.32E-02	3	163.0389	6.81	2.40	3.93E-
4	143.0814	1.30	4.07	1.67E-05	4	165.0546	6.96	2.59	5.97E-
5	148.0602	1.16	1.26	1.59E-02	5	179.0551	1.26	0.66	5.97E-
6	154.0584	1.24	0.86	1.97E-02	6	187.0061	6.64	1.46	4.16E-
7	165.0543	3.04	0.85	2.32E-02	7	199.0063	7.42	2.04	2.39E-
8	165.0908	10.28	0.63	1.55E-03	8	221.0663	2.72	0.56	5.15E-
9	172.0399	2.01	0.79	1.37E-02	9	245.0125	5.80	3.99	5.36E-
10	173.0418	1.25	0.80	1.47E-02	10	269.0545	10.78	11.24	3.12E-
11	177.1020	3.93	0.81	3.26E-02	11	333.2077	9.44	2.03	3.98E-
12	203.0522	1.14	0.67	7.04E-06	12	343.1919	12.78	11.69	7.27E-
13	219.0261	1.14	0.72	1.99E-04	13	357.1020	8.16	0.72	1.11E-
14	226.0445	3.04	0.88	4.70E-02	14	367.1054	1.24	0.63	1.83E-
15	248.1488	2.85	1.52	1.23E-03	15	403.2494	10.70	3.97	2.41E-
16	261.0106	1.15	0.69	9.95E-06	16	409.1333	8.41	4.93	2.29E-
17	265.1112	1.21	0.79	2.65E-03	17	433.1146	8.01	1.87	4.52E-
18	267.0582	1.75	0.86	3.71E-02	18	435.2758	11.50	3.23	3.49E-
19	343.1897	8.89	3.87	9.69E-14	19	449.2553	10.70	5.26	1.63E-
20	345.2054	12.80	13.52	4.36E-24	20	455.3174	14.29	3.44	2.84E-
21	383.1151	1.15	0.55	2.02E-05	21	460.2714	9.32	2.44	9.15E-
22	520.3389	13.58	0.86	3.89E-02	22	462.2870	8.87	1.85	3.38E-
23	523.3581	15.29	0.83	3.42E-02	23	471.2795	10.78	3.45	4.00E-
24	546.3542	14.99	0.81	3.48E-02	24	530.2743	8.87	1.85	3.20E-
25	548.3702	16.71	0.72	3.63E-03	25	539.2247	10.70	2.68	3.39E-
26	548.3702	17.69	0.71	2.00E-03					
27	569.3419	13.66	0.72	2.18E-03					
28	570.3521	17.69	0.71	2.00E-03					
29	572.3702	17.03	0.72	2.33E-03					
30	590.3202	13.66	0.73	3.02E-03					

a. Fold change was calculated by the ratio of mean value of control group to high dose group.

Figure S1. The PCA score plots of QC samples of serum deriving from (a) LC-(+)ESI/MS and (b) LC-(-)ESI/MS data.
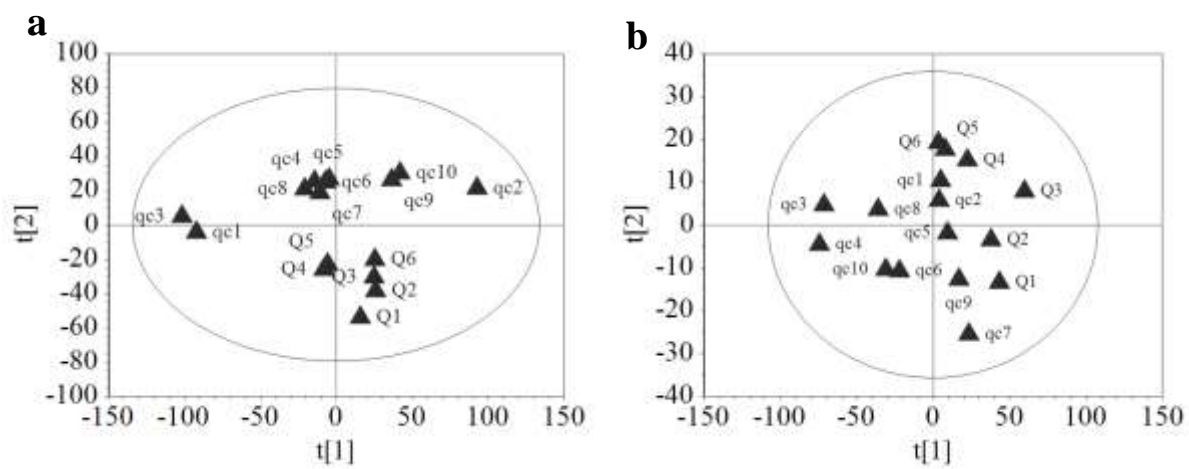
Figure S2. Line plots of QC samples of serum generated by PCA using (a) first component and (b) second component deriving from LC-(+)ESI/MS; Line plots of QC samples of serum generated by PCA using (c) first component and (d) second component deriving from LC-(-)ESI/MS. X-axis: run order; Y-axis: standard deviation.
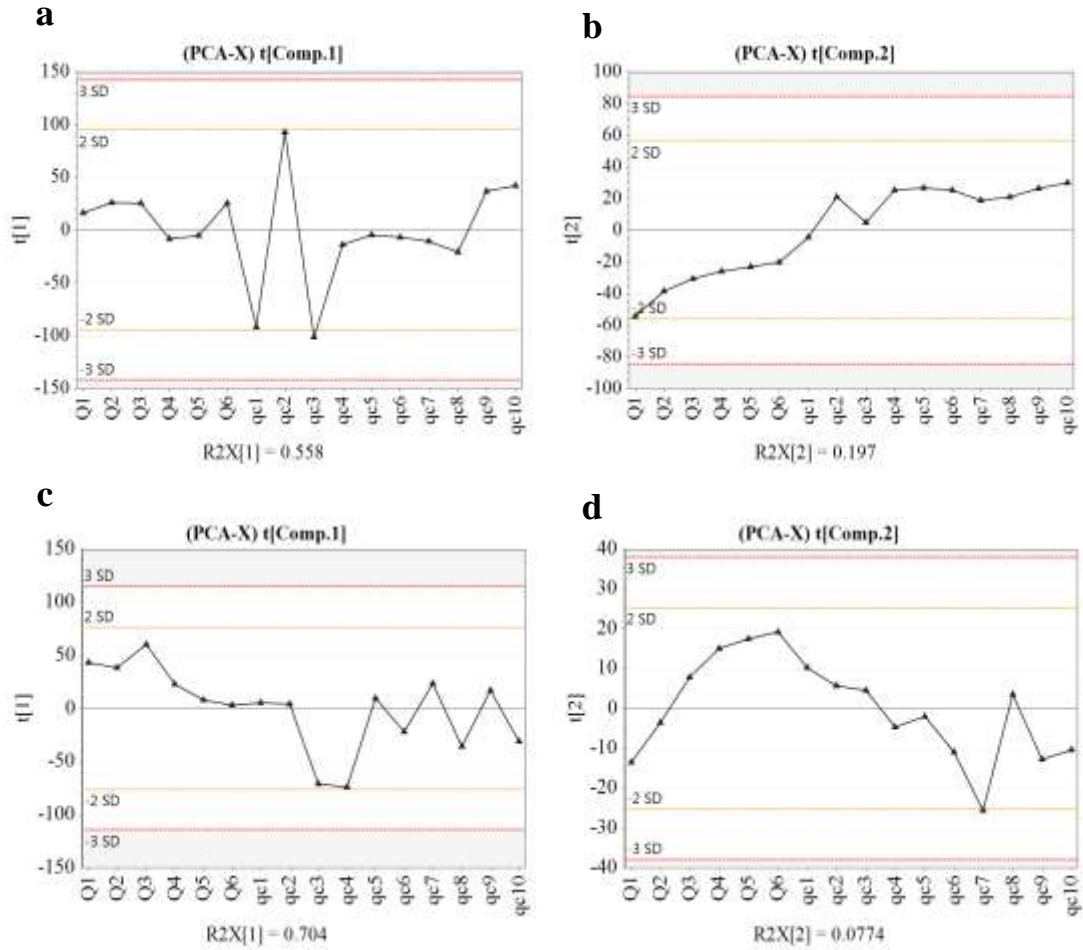
a

b

c

d

Figure S3. The retention time deviation profiles deriving from (a) LC-(+)ESI/MS and (b) LC-(-)ESI/MS analyses. A positive deviation indicates that the samples were eluting after the median retention time, and a negative deviation indicates that the samples was eluting before the median retention time.
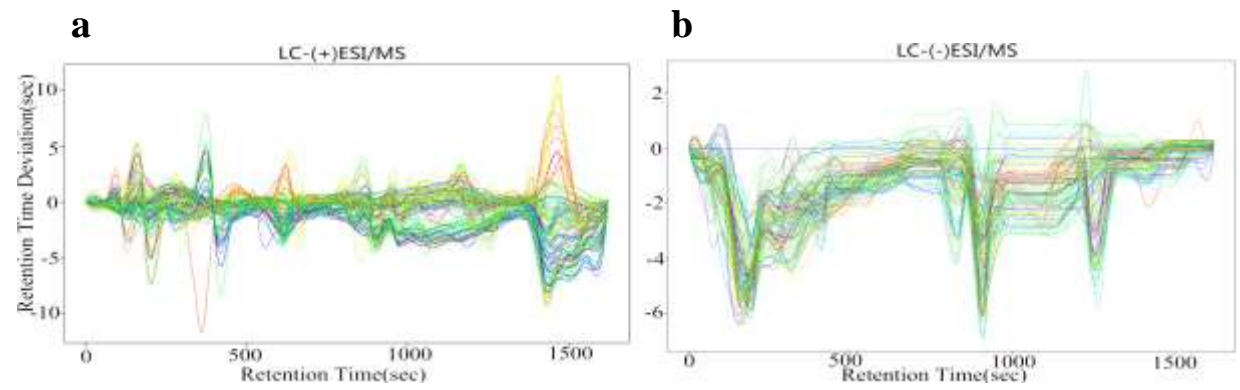
Figure S4. The TIC of LC-MS/MS from QC samples of serum in (a) positive and (b) negative ion mode.
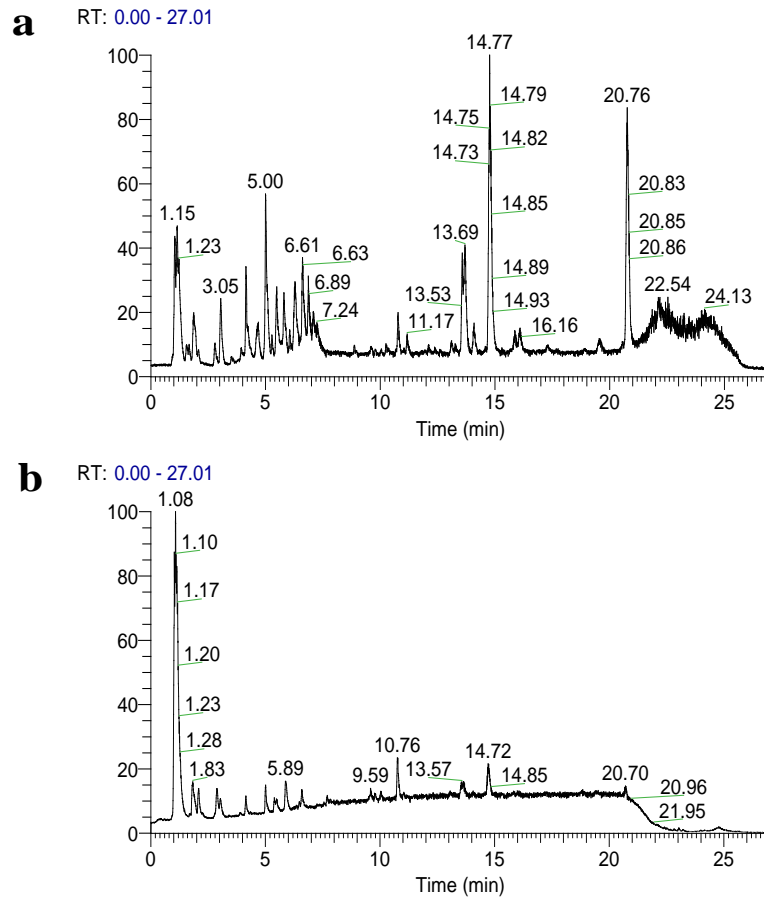
Figure S5. The OPLS-DA score plots of control group samples and five weeks administration of high-dose group samples deriving from (a) LC-(+)ESI/MS and (b) LC-(-)ESI/MS data. (●: control; ■: treated for 1 week; ▲: treated for 2 weeks; ▼: treated for 3 weeks; ◆: treated for 4 weeks; +: treated for 5 weeks)
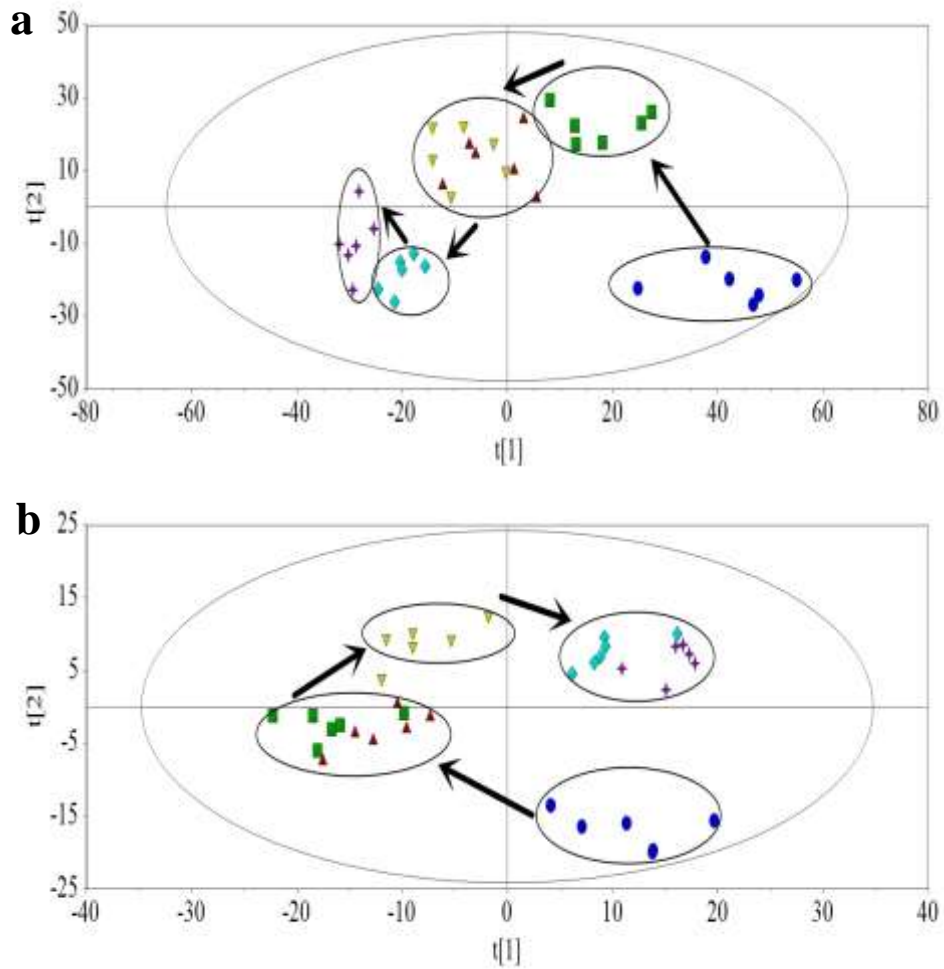
Figure S6. The ROC curve of 13 potential biomarkers for the diagnosis of DILI. (a)The ROC curve of 4 metabolites up-regulated in DILI model. (b)The ROC curve of 9 metabolites down-regulated in DILI model.
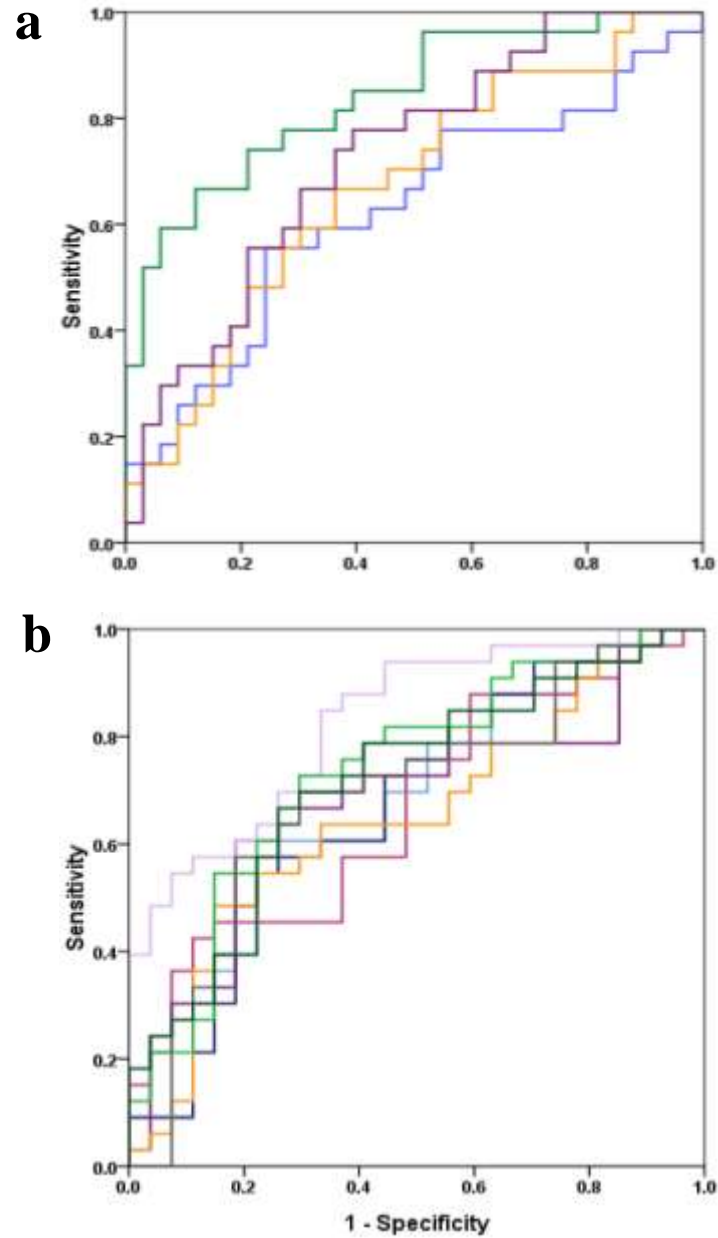
Figure S7. PCA score plot of pre-dose group (BK0) and high-dose group at week 1, week 2
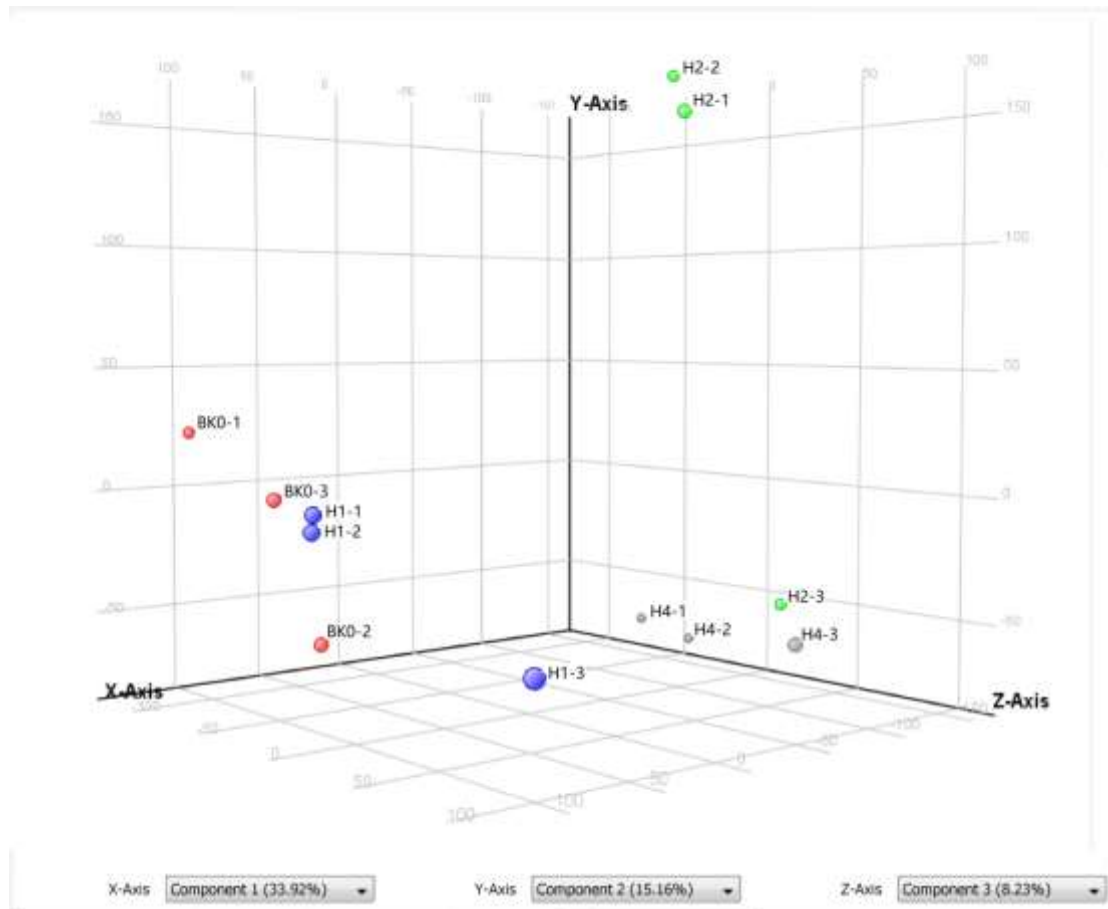
and week 4 (H1, H2, H4).

Figure S8. Top 40 GO terms (a) and top 40 pathways of KEGG analysis (b) for the differences

in co-expressed lncRNA genes between DILI group and control group.
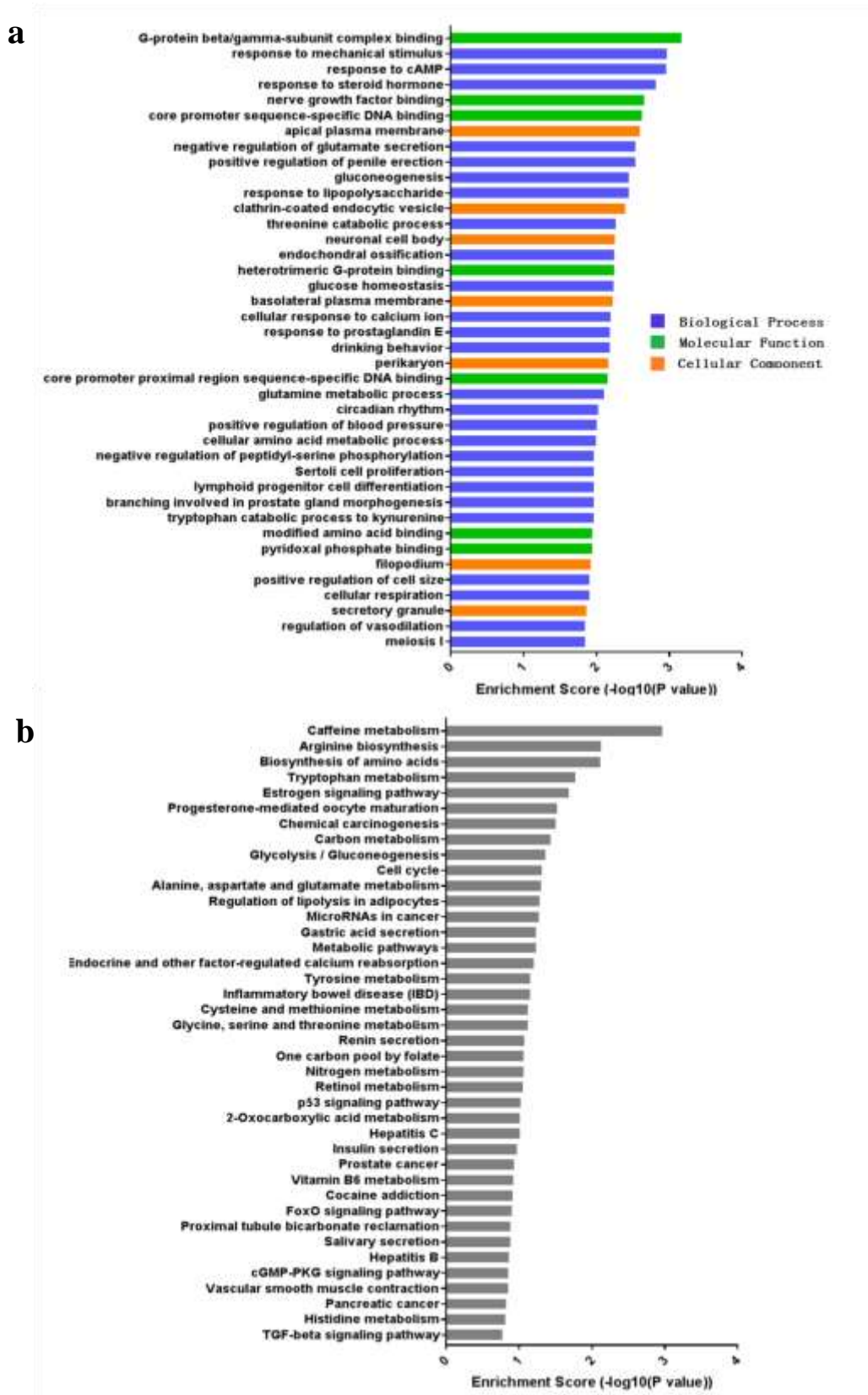

Figure S9. Validating microarray results of mRNAs identified involved in tryptophan

metabolism and alanine, aspartate and glutamate metabolism using qRT-PCR.