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Protection of baicalin on lipopolysaccharide induced liver and kidney injury based on ^{1}H NMR metabolomic profiling

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Table S1 The sequences of primers used for real-time PCR assays

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	CGACTTCAACAGCAACTC	GTAGCCGTATTCATTGTCAT
TNF-α	GACAGTGACCTGGACTGTGG	GAGACAGAGGCAACCTGACC
IL-1β	TGCCACCTTTTGACAGTGATG	ATGTGCTGCTGCGAGATTTG
IL-10	TAAGGCTGGCCACACTTGAG	TGAGCTGCTGCAGGAATGAT
IL-6	CAGAAGGAGTGGCTAAGGACC	AACGCACTAGGTTTGCCGA
PK	CCGAGATACGCACTGGAGTC	GTGGTAGTCCACCCACACTG
CS	TGGTCCCAGGATACGGTCAT	TTGTACAGCTGAGCCACCAG

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; TNF-α: Tumor Necrosis Factor-α; IL-1β: interleukin-1β; IL-10: interleukin-10; IL-6: interleukin-6; PK: pyruvate kinase； CS: citrate synthase.

Table S2 ^{1}H NMR assignment of metabolites in serum of NC, LPS and Bai mice

No.	Metabolite	Assignments	Chemical shift (ppm)
1	LDL/VLDL	CH_2	0.89 (m), 1.20-1.30 (m)
2	3-hydroxybutyrate	γCH_3, βCH	1.26 (d), 4.21 (m)
3	lactate	CH_3, CH	1.33 (d), 4.12 (q)
4	alanine	βCH_3, αCH	1.49 (d), 3.78 (q)
5	acetate	CH_3	1.92 (s)
6	N-acetylglucosamine	CH_3	2.04 (d)
7	N-acetylglycoprotein	CH_3	2.09 (s)
8	O-acetylglycoprotein	$OHCHCH_3$	2.19 (s)
9	2-OG	CH_2	2.45 (t), 3.02 (t)
10	pyruate	βCH_3	2.46 (s)
11	citrate	$1/2CH_2$, $1/2CH_2$	2.55 (d), 2.66 (d)
12	NADPH	CH_2	2.80 (dt)
13	creatinine	$N\text{-}CH_3$, $N\text{-}CH_2\text{-}CO$	3.05 (s), 4.05 (s)
14	taurine	$NH_2\text{-}CH_2$, $SO_3\text{-}CH_2$	3.25 (t), 3.43 (t)
15	betaine	$N(CH_3)_3$, CH_2	3.25 (s), 3.89(s)
16	TMAO	CH_3	3.27 (s)
17	acetoacetate	CH_3	2.30 (s), 3.42 (s)
18	glucose	CH, CH_2	3.20-3.90

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets, dt, doublet of triplets.

Table S3 ^{1}H NMR assignment of metabolites in liver of NC, LPS and Bai mice

No.	Metabolite	Assignments	Chemical shift (ppm)
1	LDL/VLDL	CH_2	0.89 (m),1.20-1.30 (m)
2	3-hydroxybutyrate	γCH_3, βCH	1.20 (d), 4.13 (m)
3	isoleucine	δCH_3, γCH_3, αCH	0.93 (t), 0.99 (d), 2.65 (d)
4	leucine	δCH_3, δCH_3, γCH, αCH	0.97 (dd), 1.70 (m), 3.73 (t)
5	valine	γCH_3, γCH_3	0.99 (d),1.05 (d), 2.26 (m), 3.60 (d)
6	lactate	CH_3, CH	1.33 (d), 4.12 (q)
7	alanine	βCH_3, αCH	1.49 (d), 3.78 (q)
8	acetate	CH_3	1.92 (s)
9	acetoacetate	CH_3	2.31 (s)
10	NADPH	CH_2	2.80 (dt)
11	creatine	CH_2, CH_3	3.04 (s), 3.93 (s)
12	creatinine	N-CH_3, N-CH_2-CO	3.05 (s), 4.05 (s)
13	choline	$N(CH_3)_3$, N-CH_2	3.19 (s),4.1 (d)
14	phosphocholine	N-CH_2	3.21 (s)
15	betaine	$N(CH_3)_3$, CH_2	3.25 (s), 3.88 (s)
16	taurine	NH_2-CH_2, SO_3-CH_2	3.27 (t),3.43 (t)
17	TMAO	CH_3	3.27 (s)
18	glucose	CH, CH_2	3.20-3.90
19	uridine	H_5, H_6, H_1'	5.9 (d), 7.9 (d)
20	tyrosine	H_3/H_5, C_5H/C_6H	3.04 (m), 6.90 (d),7.20 (d)
21	phenylalanine	CH=CH, CH_2, CH-NH_2	3.13 (m), 3.28 (m), 4.00 (m), 7.33 (m) 7.38(m) 7.43(m)
22	adenosine	CH-OH, N=CH-N	8.25 (s), 8.34 (s)
23	inosine	O-CH-N, N-CH=N CH=N	6.10 (d), 8.23 (s), 8.34 (s)

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets, dt, doublet of triplets.

Table S4 ^{1}H NMR assignment of metabolites in kidney of NC, LPS and Bai mice

No.	Metabolite	Assignments	Chemical shift (ppm)
1	LDL/VLDL	CH_2	0.89 (m),1.20-1.30 (m)
2	3-HB	γCH_3, βCH	1.19 (d)
3	lactate	CH_3, CH	1.32 (d), 4.1 (q)
4	alanine	βCH_3, αCH	1.47 (d), 3.77 (q)
5	acetoacetate	CH_3	2.35 (s)
6	2-OG	CH_2	2.45 (t), 3.02 (t)
7	sarcosine	CH_2	2.71 (s)
8	NADPH	CH	2.80 (dt)
9	creatine	CH_2, CH_3	3.02 (s), 3.92 (s)
10	creatinine	$N-CH_3$, $N-CH_2-CO$	3.02 (s), 3.92 (s)
11	choline	$N(CH_3)_3$, $N-CH_2$	3.19 (s), 4.1 (d)
12	phosphocholine	$N-CH_2$	3.21 (s)
13	TMAO	CH_3	3.27 (s)
14	taurine	NH_2-CH_2, SO_3-CH_2	3.25 (t), 3.41 (t)
15	myo-inositol	CH	3.52 (dd), 3.61 (t), 4.1 (s)
16	betaine	$N(CH_3)_3$, CH_2	3.25 (s), 3.88 (s)
17	inosine	O-CH-N, N-CH=N	4.27 (s), 4.42 (s), 6.09 (d), 8.22 (s)
18	lactose	CH	3.65 (m), 3.94 (m), 5.22 (d)
19	sucrose	CH, CH_2	3.67 (s), 3.82 (m), 4.21d, 5.39 (d)
20	maltose	CH	3.58 (m), 3.72 (m), 3.84 (m), 3.92 (m), 5.40 (d)
21	anserine	CH_3, CH_2, CH, N=CH-N	2.68 (m), 3.03 (dd), 3.23 (m), 3.77(s), 4.48 (dd) 7.08(s),
22	tyrosine	H_3/H_5, C_5H/C_6H	3.06 (m), 3.20 (m), 3.94 (m), 6.91 (d), 7.20 (d)
23	tryptophan	CH=CH, $H_2N-CH-CH_2$	3.47 (m), 4.03 (m), 7.23 (t), 7.27 (t), 7.33 (s), 7.55 (d)
24	phenylalanine	CH=CH, CH_2, $CH-NH_2$	3.13 (m), 3.28 (m), 4.00 (m),7.32 (m),7.42 (m)
25	niacinamide	$H_2/H_4/H_5/H_6$	7.58 (dd), 8.21 (d), 8.7 (d), 8.93 (s)
26	uridine	H_5, H_6, H_1'	5.9 (d), 7.9 (d)
27	adenosine	CH-OH, N=CH-N	8.20 (s), 8.34 (s)

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets, dt, doublet of triplets.

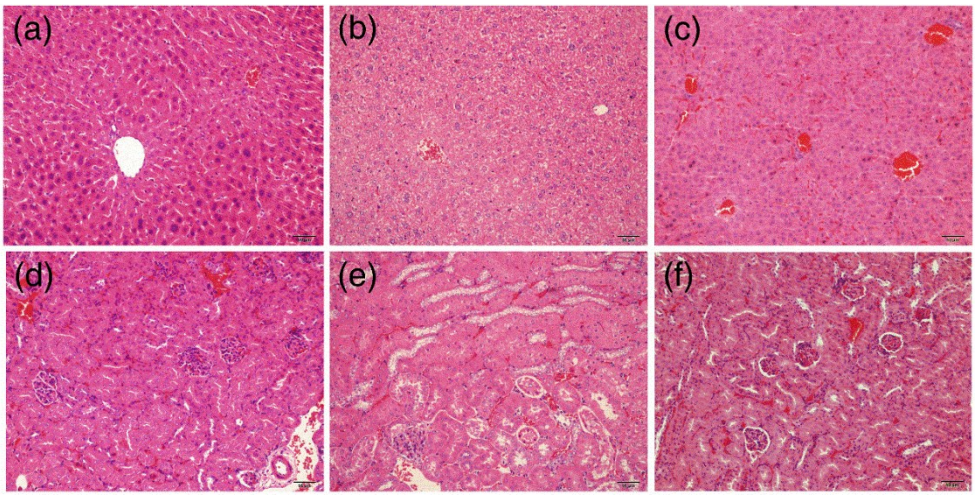

Fig. S1 Histopathological photomicrographs of mice liver (a, b and c) and kidney sections (d, e and f) of NC, LPS and Bai group.

The sliced sections were stained with hematoxylin and eosin (H&E), and examined by light microscopy (200 x magnification).

Compared with NC group (a and b), LPS treated group revealed glycogen cavity and inflammatory cell infiltration in livers (d), and congestion and renal tubular epithelial cell necrosis in kidneys (e). Compared with LPS group, Bai group (c and f) showed apparently attenuated damage.

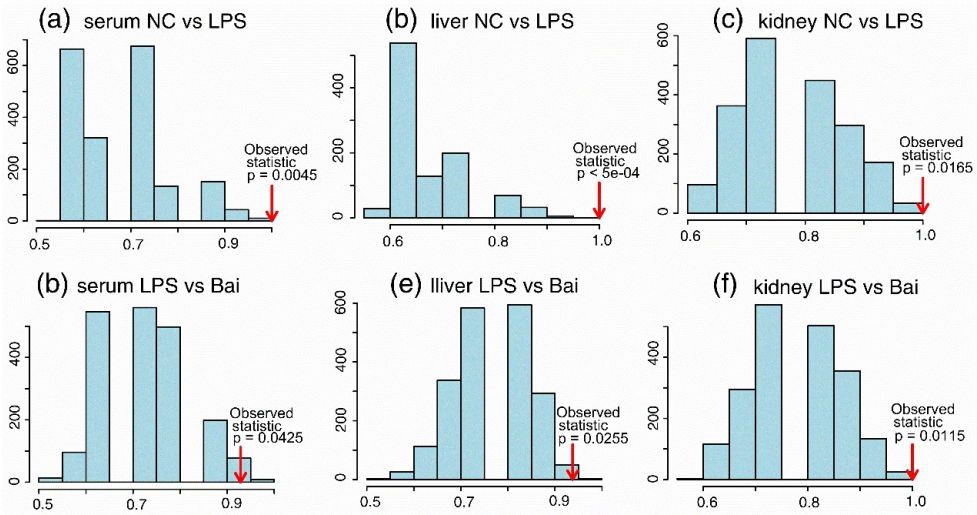

Fig. S2 Histograms for permutation test scores of OPLSDA models of NC, LPS and Bai group on the basis of 2000 permutations: the red arrows reveal the performance based on the original labels, significant for a p-value less than 0.05.

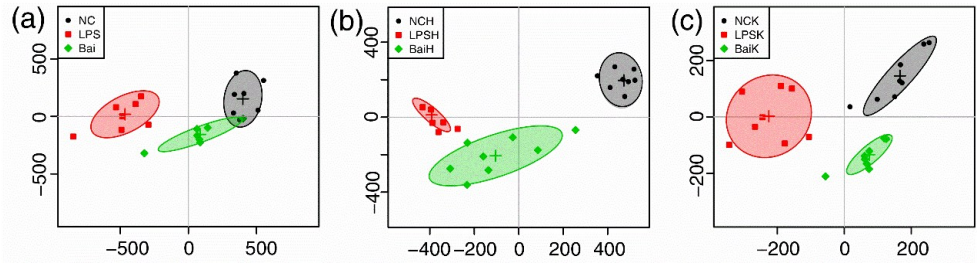

Fig. S3 Score plots from OPLS-DA analysis of NMR data from serum, liver extracts and kidney extracts of mice: a for serum, b for liver extracts and c for kidney extracts, respectively.

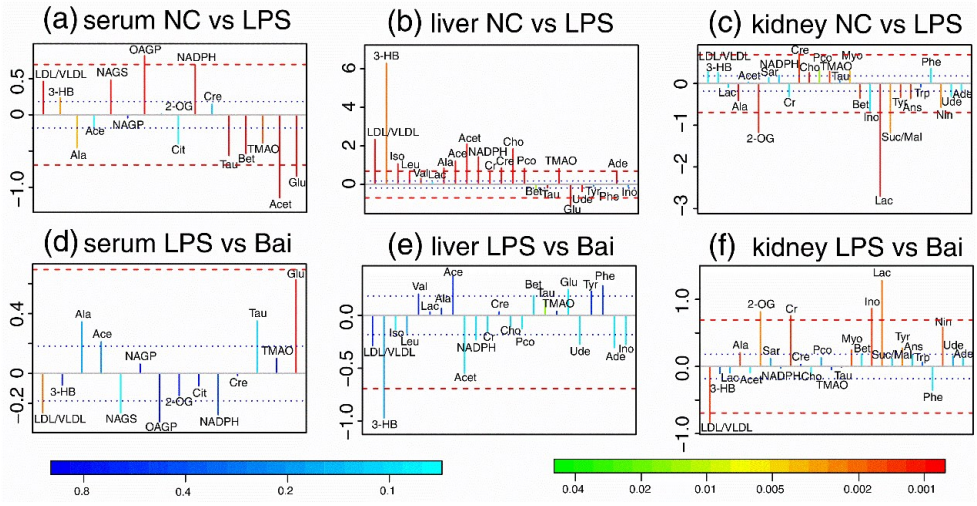

Fig. S4 Fold change plots color-coded according to the corresponding p-values adjusted by Benjamini-Hochberg method indicating significance of altered metabolites in serum (a and d), liver extracts (b and e) and kidney extracts (c and f). The blue dotted lines and red dashed lines representing an increase or decrease of 20% and 100%, respectively.