Electronic Supplementary Material (ESI) for RSC Advances.

Huang-Lian-Jie-Du decoction treated sepsis via regulating ERK and SRC/STAT3 pathway and ameliorating metabolic status

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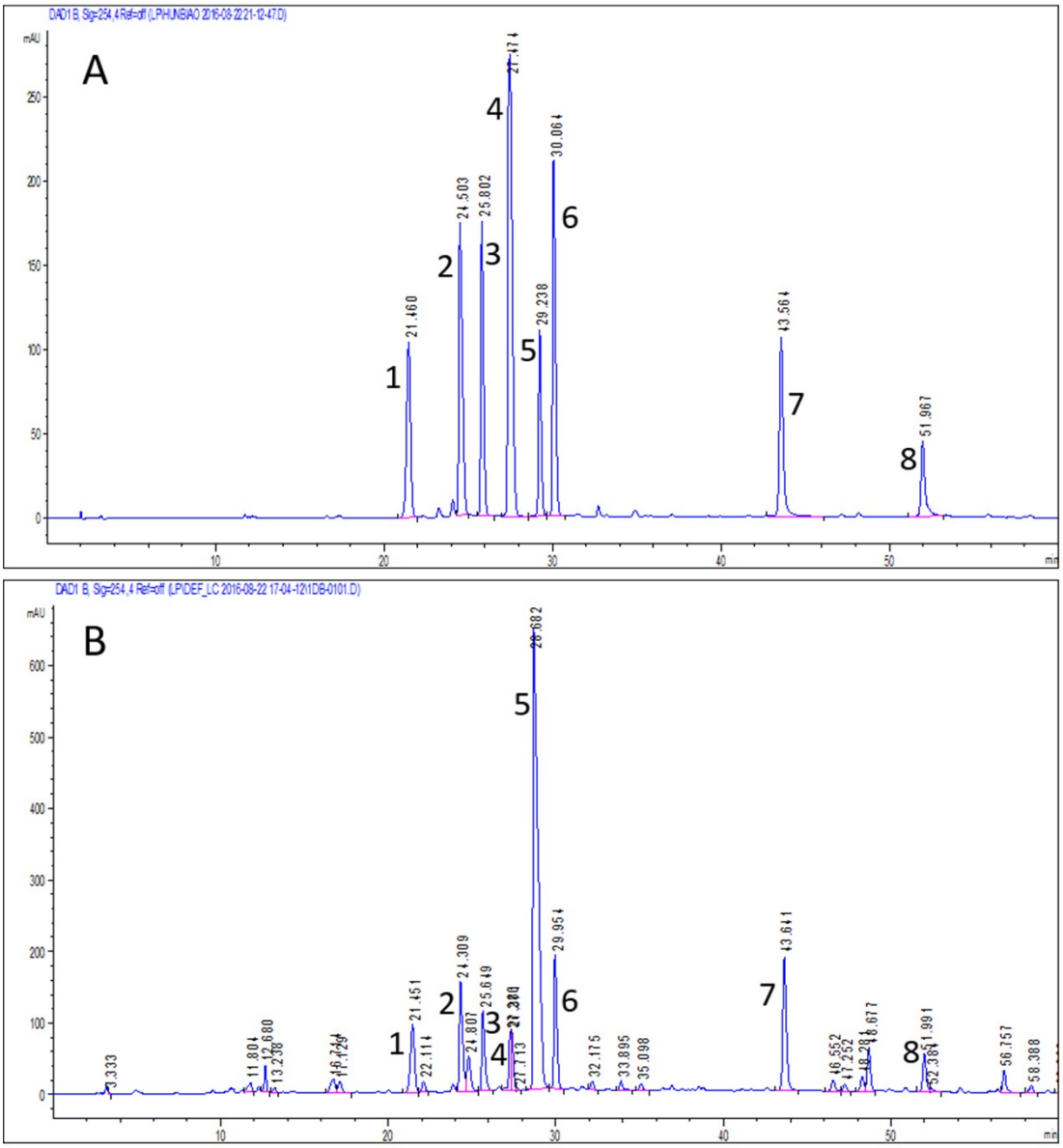


Fig. S1 Chromatograms of standard references and Huang-Lian-Jie-Du decoction (HLJDD). (A) HPLC-UV chromatogram of standard reference at 254 nm, (1) Geniposide; (2) Coptisine; (3) Epiberberine; (4) Jatrorrhizine; (5) Berberine; (6) Palmatine; (7) Baicalin; (8) Baicalein. (B) HPLC-UV chromatograms of HLJDD monitored at 254nm.

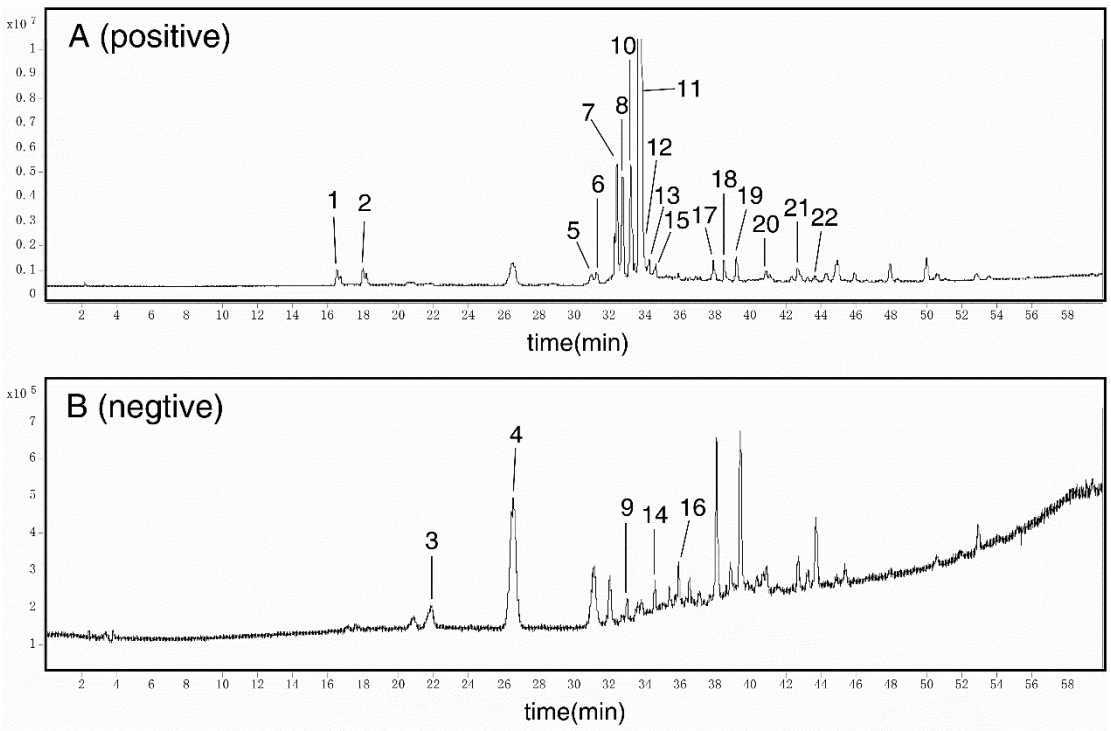


Fig. S2 The MS spectra of HLJDD in positive (A) and negative (B) ion mode.

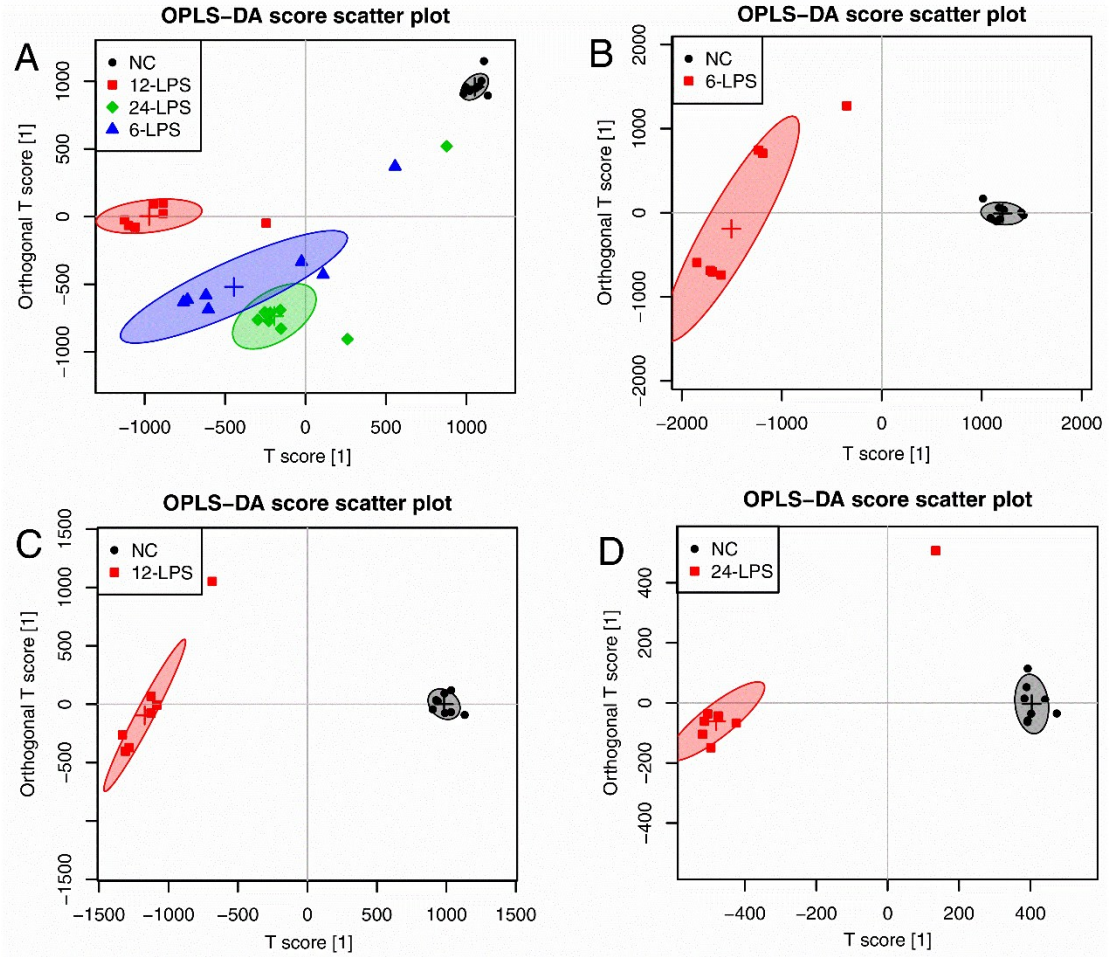


Fig. S3 Score plots from OPLS-DA analysis of NMR data from liver extracts of NC, 6-LPS, 12-LPS and 24-LPS group.

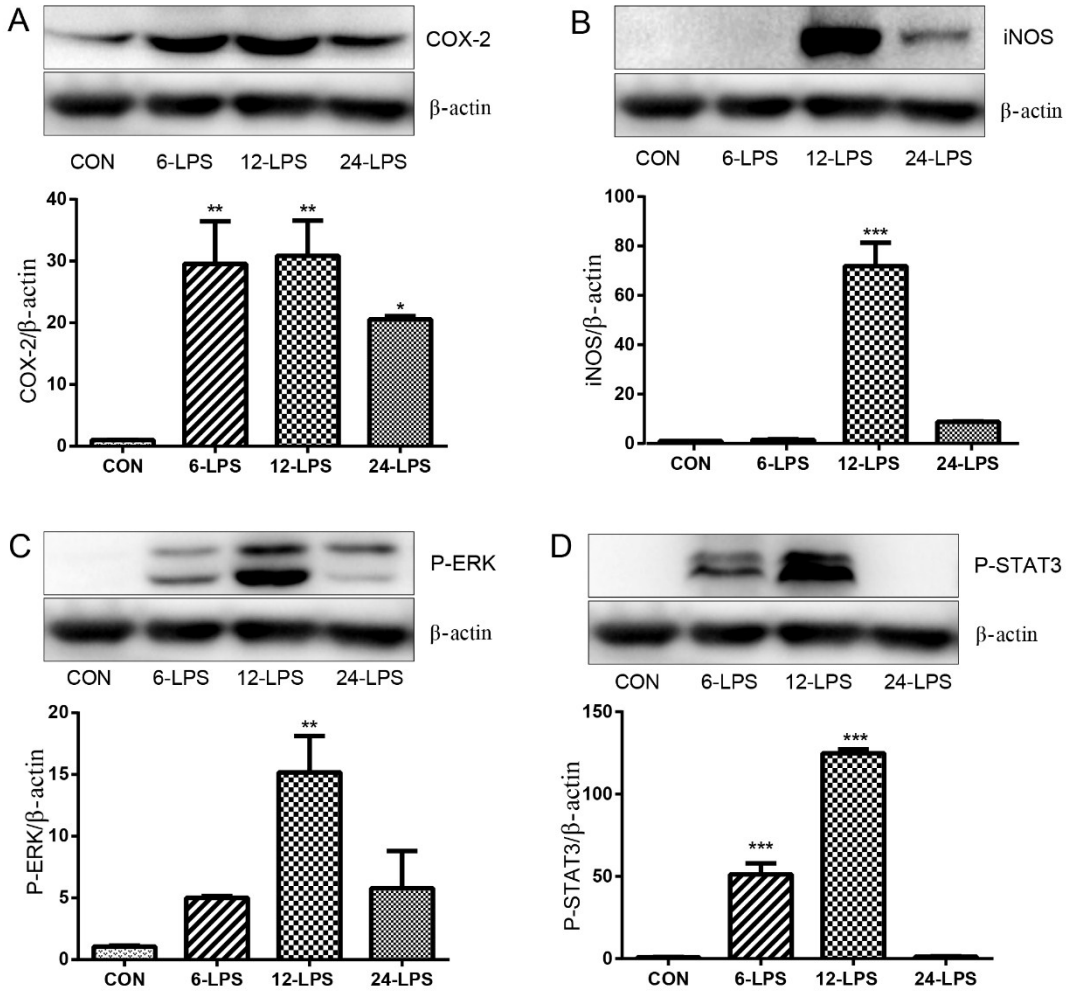


Fig. S4 LPS activated COX-2, iNOS, P-ERK and P-STAT3 expression at the 6th, 12th and 24th hr, and peaked at the 12th hr. Western blot results of COX-2, iNOS, P-ERK and P-STAT3 and their quantitative results were showed in A, B, C and D, respectively. In western blot analysis, β-actin was as loading control. Data are expressed as "mean ± SD.". * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for 6-LPS, 12-LPS and 24-LPS group vs. CON group.

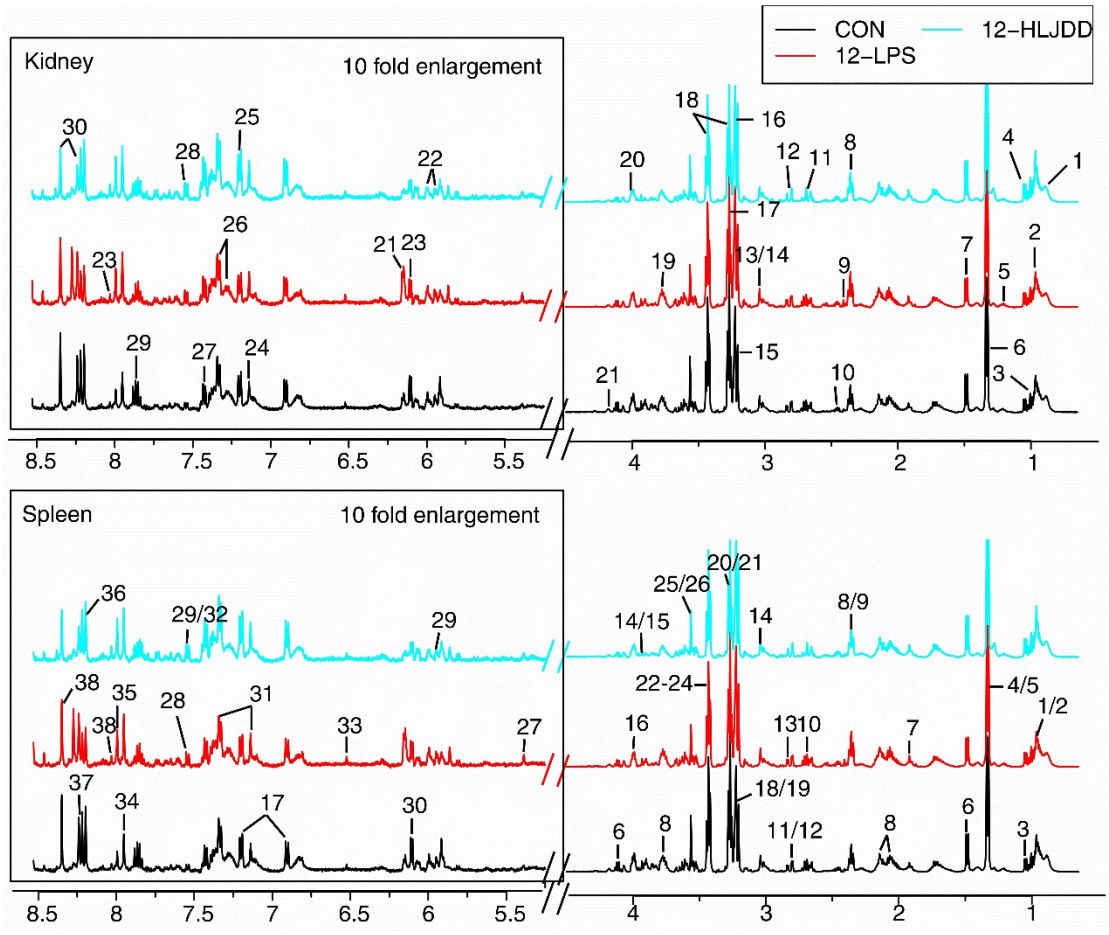


Fig. S5 Typical 500 MHz ^{1}H NMR spectra of liver and kidney tissue extracts obtained from the CON group (black line), 12-LPS (red line) and 12-HLJDD (green line). Metabolites in kidney extracts: 1. LDL/VLDL; 2. isoleucine (Ile); 3. leucine (Leu); 4. valine (Val); 5. maltose (Mal); 6. 3-hydroxybutyrate (3-HB); 7. lactate (Lac); 8. alanine (Ala); 9. acetate (Ace); 10. α-oxoglutarate (2-OG); 11. sarcosine (Sar); 12. nicotinamide adenine dinucleotide phosphate (NADPH); 13. creatine (Cr); 14. creatinine (Cre); 15. choline (Cho); 16. phosphocholine (Pco); 17. Trimethylamine N-oxide (TMAO); 18. taurine (Tau); 19. myo-inositol (Myo); 20. betaine (Bet); 21. inosine (Ino); 22. uracil (Ura); 23. guanosine (Gua); 24. anserine (Ans); 25. tyrosine (Tyr); 26. trptophan (Trp); 27. Phenylalanine (Phe); 28. niacinamide (Nin); 29. uridine (Ude); 30. Adenosine (Ade). Metabolites in spleen extracts: 1. Ile); 2. leucine Leu; 3. Val; 4. threonine (Thr); 5. Lac; 6. Ala; 7. Ace; 8. glutamate (Gln) 9. pyruvate (Pyr); 10 glutamine (Glu); 11. succinate (Suc); 12. trimethylamine (TMA); 13. N, N-Dimethylglycine; 14. Cr; 15. creatine phosphase (Pcr); 16. Cre; 17. Tyr; 18. ethanolamine; 19. histamine (His); 20. Cho; 21. o-Phosphocholine (OPC); 22. Tau; 23. TMAO; 24. Bet; 25. Myo; 26. glycine (Gly); 27. glucose (Glc); 28. Ade; 29. Ude; 30. N-Acetylserotonin (N-Ace); 31. Ino; 32. fumarate (Fum); 33. theophylline (The); 34. xanthine (Xan); 35. guanosine (Gua); 36. Nin; 37. AMP; 38. Phe.

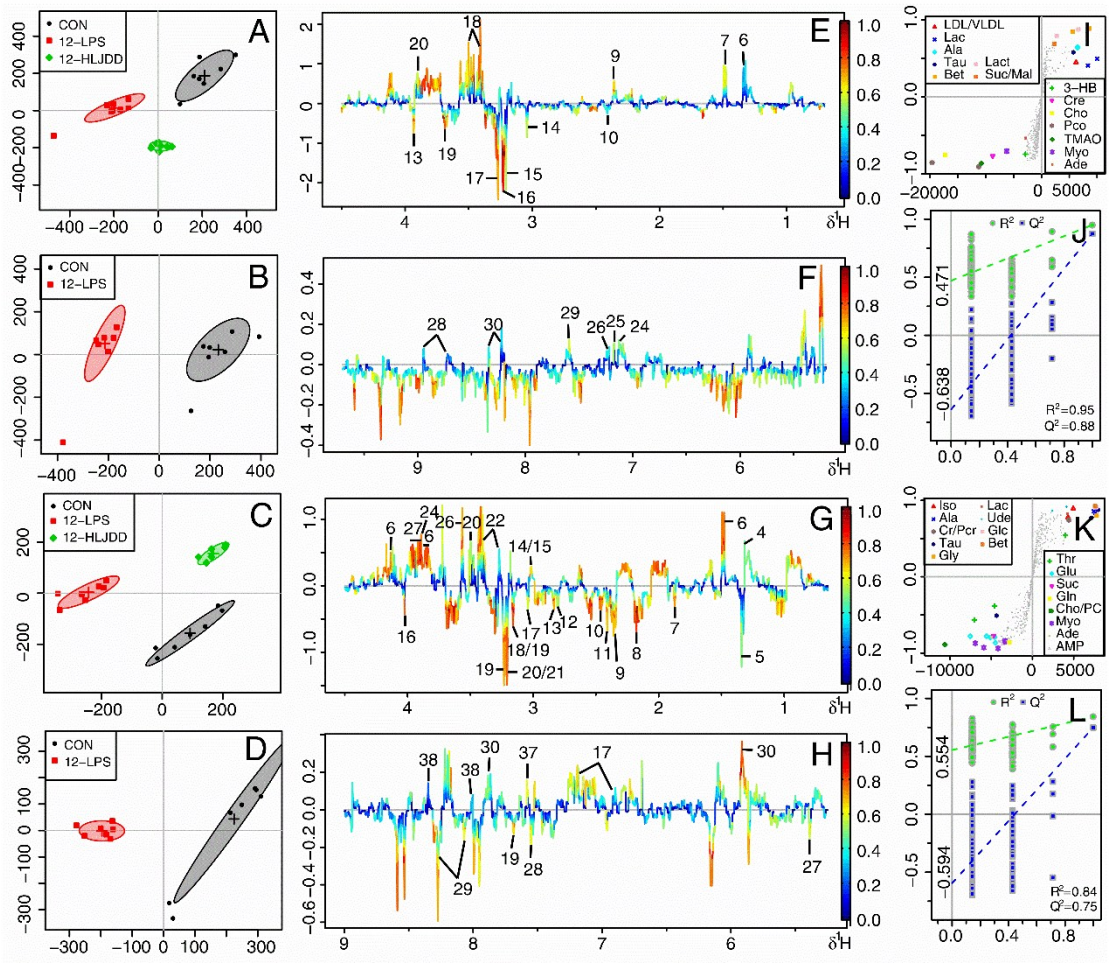


Fig. S6 Metabolomic profiles between CON, 12-LPS and 12-HLJDD groups in kidneys and spleens. A and B (for kidneys), C and D (for spleens): scores plots for OPLS-DA; E and F (for kidneys), G and H (for spleens): OPLS-DA loading plot color-coded according to the absolute value of correlation coefficients; I (for kidneys) and K (for spleens): S-plot; J (for kidneys) and L (for spleens): OPLS-DA scatter plots of statistical validation obtained by 200 times permutation test, with R^2 and Q^2 values in the vertical axis, the correlation coefficient (between the permuted and true class) in the horizontal axis, and the ordinary least squares (OLS) line for the regression of R^2 and Q^2 on the correlation coefficients.

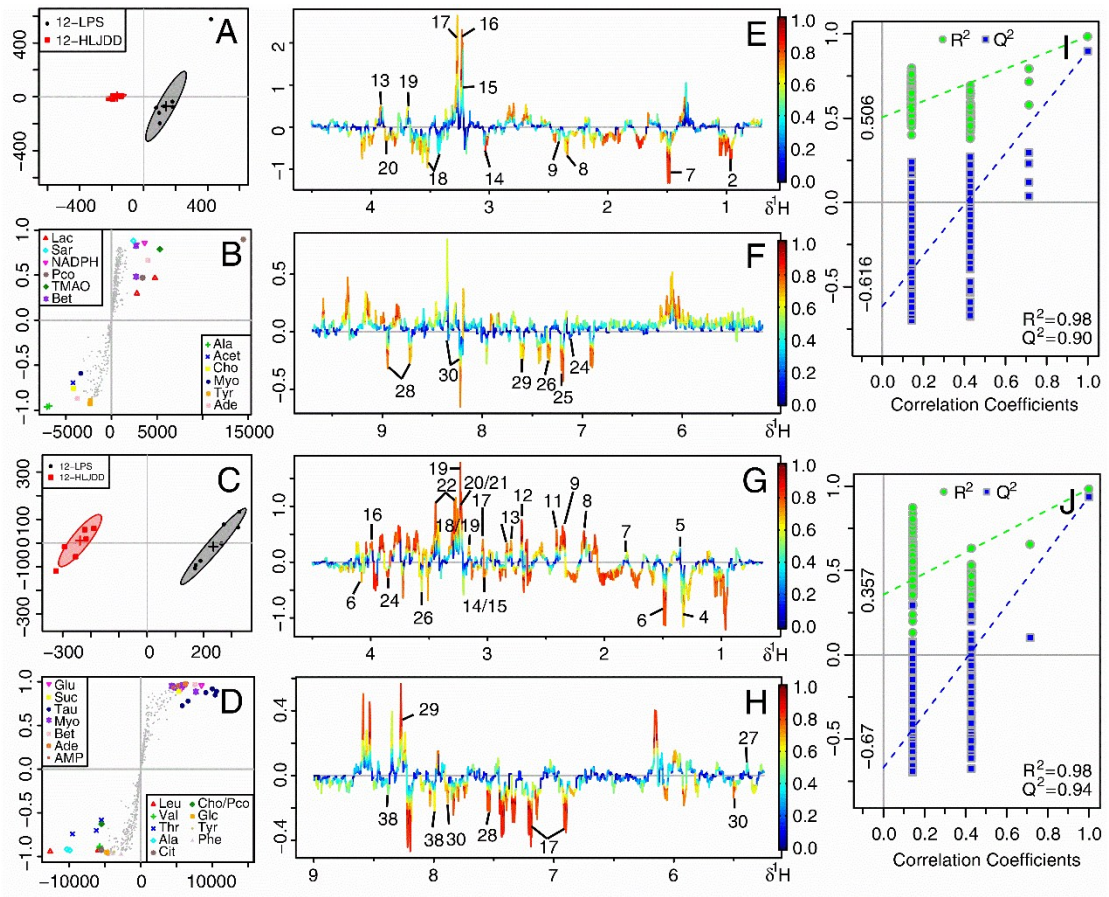


Fig. S7 OPLS-DA analysis of ^{1}H NMR data in kidneys and spleens for 12-LPS and 12-HLJDD groups. Scores plots on 12-LPS and 12-HLJDD (A for kidneys, C for spleens), loading plot (E and F for kidneys, G and H for spleens), color-coded S-plot (B for kidneys, D for spleens) for OPLS-DA; I (for kidneys), J (for spleens) OPLS-DA scatter plots of statistical validation obtained by 200 times permutation test, with R^2 and Q^2 values in the vertical axis, the correlation coefficients (between the permuted and true class) in the horizontal axis, and the OLS line for the regression of R^2 and Q^2 on the correlation coefficients.

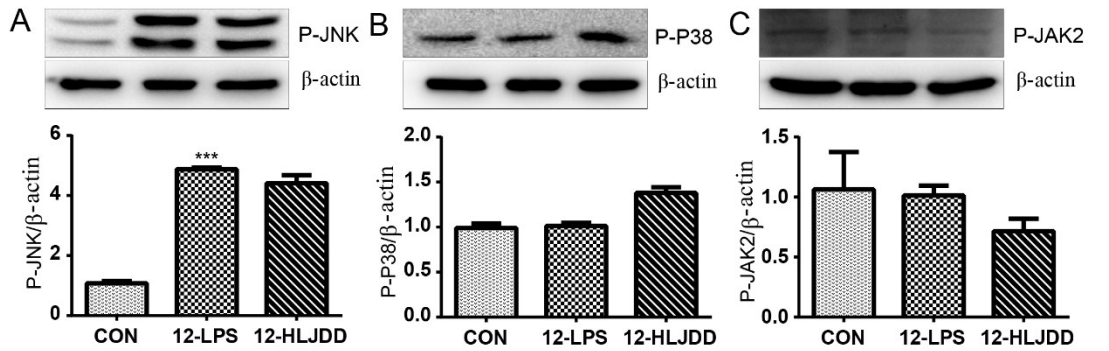


Fig. S8 Western blot results of P-JNK, P-P38, P-JAK2 expression and their quantitative results were showed in A, B and C, respectively. In western blot analysis, β-actin was as loading control. Data are expressed as "mean ± SD.". * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for 6-LPS, 12-LPS and 24-LPS group vs. CON group.

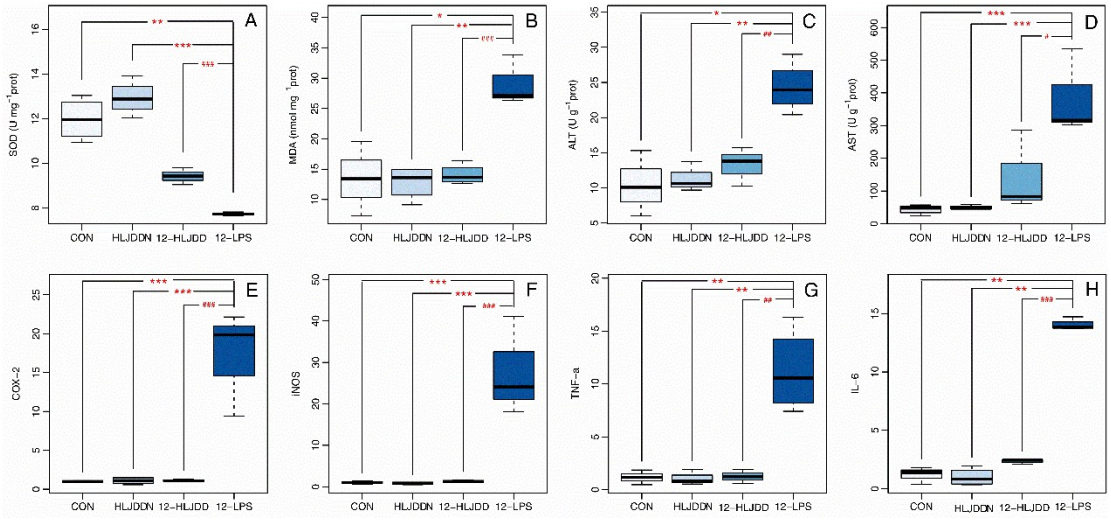


Fig. S9 Boxplots for mRNA expression levels of COX-2 (A), iNOS (B), TNF-α (C), IL-6 (D) and clinical chemistry results on SOD (E), MDA (F), ALT (G), AST (H). The bottom of each box, the line drawn in the box and the top of the box represent the 1st, 2nd, and 3rd quartiles, respectively. The bars (or "whiskers") represent the furthest observation lying within 1.5 times the interquartile range (from the 1st to 3rd quartile). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for 12-LPS group vs. CON group or HLJDDN group; # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ for 12-HLJDD group vs. 12-LPS group.

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

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	ACCACACCTTCTACAATGAG	ACGACCAGAGGCATACAG
TNF-α	GACAGTGACCTGGACTGTGG	GAGACAGAGGCAACCTGACC
IL-6	CAGAAGGAGTGGCTAAGGACC	AACGCACTAGGTTTGCCGA
COX-2	TGAGTGGGGTGATGAGCAAC	TTCAGAGGCAATGCGGTTCT
iNOS	ATCCATCCCCTGAGCAATGTG	GACCGTCTAATGGGGAGCG

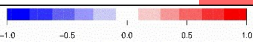
TNF-α: Tumor Necrosis Factor-α; IL-6: interleukin-6; COX-2: Cycloxygenase-2; iNOS: inducible nitric oxide synthase.

Table S2 Identified metabolites with the fold change between different groups and p-value [a].

	Metabolite	Assignments	Chemical shift (ppm)	12-LPS vs. CON		12-HLJDD vs. 12-LPS	
				Fold Change	P-value	Fold Change	P-value
Kidney	LDL/VLDL	γCH_3, βCH	0.89 (m),1.20-1.30 (m)	0.58		1.05	
	β-hydroxybutyrate	CH3, CH	1.19 (d)	1.60	**	0.89	
	lactate	βCH3, αCH	1.32 (d), 4.1 (q)	0.80		1.07	
	alanine	CH3	1.47 (d), 3.77 (q)	0.76	*	1.42	*
	acetoacetate	CH2	2.35 (s)	0.86		1.05	
	α-oxoglutarate	CH2	2.45 (t), 3.02 (t)	1.03		1.10	
	sarcosine	CH	2.71 (s)	1.01		0.84	
	NADPH	CH2, CH3	2.80 (dt)	1.34		0.21	**
	creatine	N-CH3, N-CH2-CO	3.02 (s), 3.92 (s)	0.99		2.00	**

	creatinine	N(CH3)3, N-CH2	3.02 (s), 3.92 (s)	2.15	**	1.08	
	choline	N-CH2	3.19 (s), 4.1 (d)	1.25	*	1.08	
	phosphocholine	CH3	3.21 (s)	1.45	**	0.87	
	Trimethylamine N-oxide	NH2-CH2, SO3-CH2	3.27 (s)	1.42	*	0.92	
	taurine	CH	3.25 (t), 3.41 (t)	1.01		0.99	
	myo-inositol	N(CH3)3, CH2	3.52 (dd), 3.61 (t), 4.1 (s)	1.59		1.07	
	betaine	O-CH-N, N-CH=N	3.25 (s), 3.88 (s)	0.65	**	0.70	*
	inosine	CH	4.27 (s), 4.42 (s), 6.09 (d), 8.22 (s)	0.48		0.43	
	lactose	CH, CH2	3.65 (m), 3.94 (m), 5.22 (d)	0.16	*	0.84	
	Maltose	CH	3.67 (s), 3.82 (m), 4.21d, 5.39 (d)	0.41		0.95	
	tyrosine	CH3, CH2, CH	3.06 (m), 3.20 (m), 6.91 (d), 7.20 (d)	1.34		1.43	**
	anserine	H3/H5, C5H/C6H	2.68 (m), 3.03 (dd), 3.77(s), 7.08(s)	1.29		1.03	
	tryptophan	CH=CH, H2N-CH-CH2	7.23 (t), 7.33 (s), 7.55 (d) 7.74(d)	1.51	*	1.22	
	phenylalanine	CH=CH, CH2, CH-NH2	3.13 (m), 3.28 (m), 7.32 (m),7.42 (m)	1.20		1.28	
	niacinamide	H2/H4/H5/H6	7.58 (dd), 8.21 (d), 8.7 (d), 8.93 (s)	0.93		1.46	*
	uridine	H5, H6, H1'	5.9 (d), 7.9 (d)	0.99		0.95	
	adenosine	CH-OH, N=CH-N	8.20 (s), 8.34 (s)	1.08		0.72	*
Spleen	isoleucine	δCH3, γCH3, αCH	0.93 (t), 1.00 (d)	0.88	***	1.08	*
	leucine	δCH3, δCH3, γCH, αCH	0.94 (t), 0.96 (t)	0.96		1.32	***
	valine	γCH3, γCH3	0.99 (d), 1.04 (d)	1.08		1.21	***
	threonine	CH3, CH	1.34 (d), 3.60 (d)	1.08		1.07	**
	alanine	βCH3, αCH	1.48 (d), 3.78 (q)	0.75	***	1.35	***
	acetate	CH3	1.91 (s)	1.13	*	1.14	**
	glutamate	βCH2, γCH2, αCH	2.04(m), 2.12(m), 2.34(m), 3.75(m)	1.15	**	0.92	***
	succinate	CH2	2.41 (s)	1.69		0.64	***
	glutamine	βCH2, γCH2, αCH	2.16(m), 2.46(m), 3.77(t)	1.27	*	1.07	
	citrate	CH_2, CH_2	2.55 (d), 2.66 (d)	1.06		1.03	
	trimethylamine	CH3	2.88 (s)	1.12		1.51	**
	Creatine/Pcr	CH_2, CH_3	3.04 (s), 3.93 (s)	0.98		0.90	***
	Choline/PC	N(CH3)3, N-CH2	3.21 (s)	1.25	*	1.07	**
	taurine	NH2-CH2, SO3-CH2	3.25 (t), 3.43 (t)	0.94		0.94	**
	myo-inositol	CH	3.27 (t), 4.07 (t)	1.30	*	0.85	***
	glycine	CH2	3.565 (s)	0.83	**	1.01	
	glucose	CH2	3.7-4.0 (m), 5.30 (s)	0.42	*	1.31	***
	betaine	CH2	3.26 (s), 3.90 (s)	0.62	**	0.53	***
	lactate	CH3	1.33 (d), 4.11 (q)	0.69	*	0.83	*
	inosine	CH3, CH2	6.10 (d), 8.34 (s)	0.81		0.87	
	uracil	CH, NH	5.81 (d), 7.54 (d)	1.20		1.09	
	adenosine	CH-OH, N=CH-N	8.25 (s), 8.35 (s)	1.44	*	0.59	***
	AMP	CH, CH2	8.23 (s), 8.58 (s)	3.32	*	0.28	***
	fumarate	CH	6.50 (s)	1.39		0.87	
	tyrosine	CH3, CH2, CH,	6.91 (d), 7.20 (d)	1.07		1.53	***
	anserine	CH3, CH2, CH, CH-	2.68 (m), 3.77(s), 7.08(s), 8.22(s)	1.22		1.02	
	phenylalanine	CH=CH, CH2, CH-NH2	7.32 (m), 7.40 (m)	1.36	*	1.09	

	nicotinamide	CH	7.60 (dd), 8.94 (s)	1.25	*	1.06	
	tryptophan	CH=CH, H_2N-CH-CH_2	7.23 (t), 7.27 (t), 7.33 (s), 7.74(d)	1.33		0.95	
	uridine	H5, H6, H1'	5.90 (d), 7.81 (d)	0.64		1.44	*
	histamine	CH2, CH	3.24 (dd), 7.12 (s)	1.60		1.02	

[a] Color coded according to $\log_2$ (FoldChange) using color bar 

. p-Value: *p < 0.05, **p < 0.01 and ***p < 0.001. Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Table S3 Compounds detected in HLJDD obtained by HPLC-Q-TOF-MS.

Peak	t_R(min)	Experimental $[M+H]^+$	Calculated $[M+H]^+$	Error (ppm)	Molecular formula	Proposed compound
1	16.470	342.1769	342.1700	0.09	$C_{20}H_{23}NO_4$	Phellodenrine
2	17.938	342.1701	342.1700	-0.1	$C_{20}H_{23}NO_4$	Magnoflorine
5	30.881	322.1078	322.1074	-1.33	$C_{19}H_{15}NO_4$	Berberubine
6	31.219	322.1078	322.1074	-1.34	$C_{19}H_{15}NO_4$	3-ofernloyquini acid
7	32.229	320.0919	320.0917	-0.56	$C_{19}H_{13}NO_4$	Coptisine
8	32.698	336.1233	336.1230	-0.89	$C_{20}H_{17}NO_4$	Epiberberine
10	33.156	338.1392	338.1387	-1.49	$C_{20}H_{19}NO_4$	Jatrorrhizine/Columbamine
11	33.724	352.1554	352.1543	-3.16	$C_{21}H_{21}NO_4$	Palmatine
12	33.756	336.1231	336.1230	-0.08	$C_{20}H_{17}NO_4$	Berberine
13	34.133	350.1390	350.1387	-0.99	$C_{21}H_{19}NO_4$	Unknown
15	34.531	528.1658	528.1653	-0.94	$C_{30}H_{25}NO_8$	Unknown
17	37.849	447.0921	447.0922	0.19	$C_{21}H_{18}O_{11}$	Baicalin
18	38.536	447.0924	447.0922	-0.5	$C_{21}H_{18}O_{11}$	Baicalinisomer
19	39.218	461.1087	461.1078	-1.76	$C_{22}H_{20}O_{11}$	Oroxylin A 7-O-glucuronide
20	40.828	375.1082	375.1074	-1.95	$C_{19}H_{18}O_8$	5,6-Dihydroxy-6,8,2',3'-tetramethoxyflavone
21	42.640	285.0768	285.0757	-3.75	$C_{16}H_{12}O_5$	Oroxylin A
22	43.600	285.0766	285.0757	-2.83	$C_{16}H_{12}O_5$	wogonin

Table S4 Compounds detected in HLJDD obtained by HPLC-Q-TOF-MS.

Peak	t_R(min)	Experimental $[M-H]^-$	Calculated $[M-H]^-$	Error (ppm)	Molecular formula	Proposed compound
9	32.970	338.1385	338.1398	3.7	$C_{20}H_{21}NO_4$	Canadine
14	34.520	547.1448	547.1457	1.69	$C_{26}H_{28}O_{13}$	Chrysin 6-C-arabinoside 8-C-glucoside
16	35.884	695.2179	695.2193	2.02	$C_{32}H_{40}O_{17}$	Phelloside

Table S5 Compounds detected in HLJDD obtained by HPLC-Q-TOF-MS.

Peak	t_R(min)	Experimental $[M+COOH]^-$	Calculated $[M+COOH]^-$	Error (ppm)	Molecular formula	Proposed compound
3	21.768	595.1860	595.1880	3.36	$C_{23}H_{34}O_{15}$	genipin-1-β-D-gentiobiose
4	26.493	433.1347	433.1351	1.01	$C_{17}H_{24}O_{10}$	Geniposide