Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary materials

Plasma untargeted metabolomic study of Chinese Medicine *Zhi-Zi-Da-Huang* decoction intervention to alcohol-induced hepatic steatosis

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Text S1. Preparation of *ZZDHD*

The medicinal plants used to prepare *ZZDHD* were commercially available dry matter, which were purchased from Jiangsu Simcere Pharmacy Ltd. (Nanjing, China), and identified by Professor Minjian Qin (Department of Chinese Materia Medica, China Pharmaceutical University). All voucher specimens were deposited at the Herbarium of China Pharmaceutical University for future reference. The mixture, including *Gardenia jasminoides* Ellis (batch number: 141212; collection in Jiangxi, China), *Rheum palmatum* L. (batch number: 141214; collection in Gansu, China), *Citrus aurantium* L. (batch number: 150131; collection in Jiangxi, China) and Sojae Semen Praeparatum (batch number: 141231; collection in Henan, China) with a ratio of 3:1:4:8 in weight were immersed in 10 volumes of distilled water (v/w) for 30 min and then decocted by boiling for 30 min. The extract solution was filtered through four layers of gauze and the procedure was repeated two times. Combined the three filtrates and concentrated to a final density of 1.0 g/mL in a decompression condition at 48˚C.

Text S2. Quality evaluation of *ZZDHD*

Preparation of the standard solutions and samples of ZZDHD:

The standard stock solutions of geniposide, naringin, hesperidin and neohesperidin were separately prepared in methanol. Then, a mixed standard working solution was obtained by diluting stock solutions to desired concentrations of 23.57 μg·mL^{-1} for geniposide, 59.12 μg·mL^{-1} for naringin, 11.76 μg·mL^{-1} for hesperidin and 37.34 μg·mL^{-1} for neohesperidin.

An aliquot of 1.0 mL of *ZZDHD* was diluted into 10 mL with water, and then 2.5 mL aliquot of the mixture was added with 7 mL of ethanol/water (95:5, v/v), centrifuged at 8 000×*g* for 5 min. An aliquot of 3 mL of the supernatant was diluted into 10 mL with ethanol/water (70:30, v/v) and filtered through a 0.45 μm membrane.

LC-UV conditions:

LC–UV systems consisted of Agilent-1260 LC coupled with DAD detector (Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation was performed on a Phecda C_{18} column (250 mm × 4.6 mm, 5 μm, Hanbon Science & Technology Co., China). A mobile phase of 0.1% acetic acid (solvent A) and methanol (solvent B) was used. The linear elution gradient was optimized as follows: (i) 5–30% B (0–12.5 min); (ii) 30–36% B (12.5–27 min); (iii) 36–45% B (27–50 min); (iv) 45–75% B (50–58min); (v) (vi) 75–95% B (58–65 min); (vii) 95% B (65–80 min); (viii) 5% B (81–90 min). The flow rate was 1.0 mL·min^{-1}. The column temperature was maintained at 25°C. A 20 μL aliquot was injected for LC analysis. The detection wavelength was 258 nm.

Results:

The LC chromatogram of blank solvent (70% ethanol), the mixed standard working solution and *ZZDHD* sample were shown in Fig. S1. Quality study on three batches of *ZZDHD* was shown in Table S1. Quality study of the *ZZDHD* at three storage times of 0 d, 4 d and 8 d was shown in Table S2. Based on the analysis of variance (ANOVA) performed by SPSS 17.0 (SPSS Inc., USA), the content diferences of geniposide, naringin, hesperidin and neohesperidin among three batches of *ZZDHD* showed no statistical significance (P >0.05), respectively. Moreover, the content diferences of geniposide, naringin, hesperidin and neohesperidin among the three storage time points of 0 d, 4 d and 8 d were not significantly different (P >0.05), respectively.

Text S3. TOF/MS conditions

Capillary voltage, 4.0 KV/–3.5 KV; nebulizer pressure, 35 psi; flow rate of drying gas (N_2), 12.0 $L \cdot min^{-1}$; gas temperature, 350°C; skimmer voltage, 65 V; fragmentor voltage, 175 V; octopole radiofrequency, 750 V; scan range, 100–1200 Da. To ensure accuracy, the mass-to-charge ratio (*m/z*) of all ions in the mass spectra were real time corrected by reference ions (*m/z* 121.050873 (protonated purine) and 922.009798 (protonated hexakis, (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (HP-921)) for positive mode, *m/z* 112.985587 (proton-abstracted ammonium trifluoroacetate (TFA) anion) and 1033.988109 (TFA adduct of HP-0921) for negative mode). The acquisition and analysis of data were controlled by Mass Hunter B.04.00 software.

Text S4. Parameter settings for XCMS Online processing

Parameter settings for XCMS processing depend on the instrument platform in which the data were acquired. The parameters as follows: centWave for feature detection (Δ*m/z*= 5 ppm, minimum peak width = 10 s, and maximum peak width = 60 s); obiwarp settings for retention-time correction (profStep = 0.5); and parameters for chromatogram alignment, including mzwid = 0.025, minfrac = 0.5, and bw = 5.

Text S5. QqQ/MS conditions

QqQ/MS controlled by Xcalibur software was utilized to determine the product ion mass spectra (MS/MS). The spray voltage was 4.0 kV and −3.5 kV for positive and negative MS scan mode respectively, assisted by nitrogen sheath and auxiliary gas flow rate were 35 Arb and 5 Arb, respectively. The collision energy of 10–50 eV was used at a pressure of 1.3 mTorr for collision-induced dissociation to get distinct fragmentation for certain analyte.

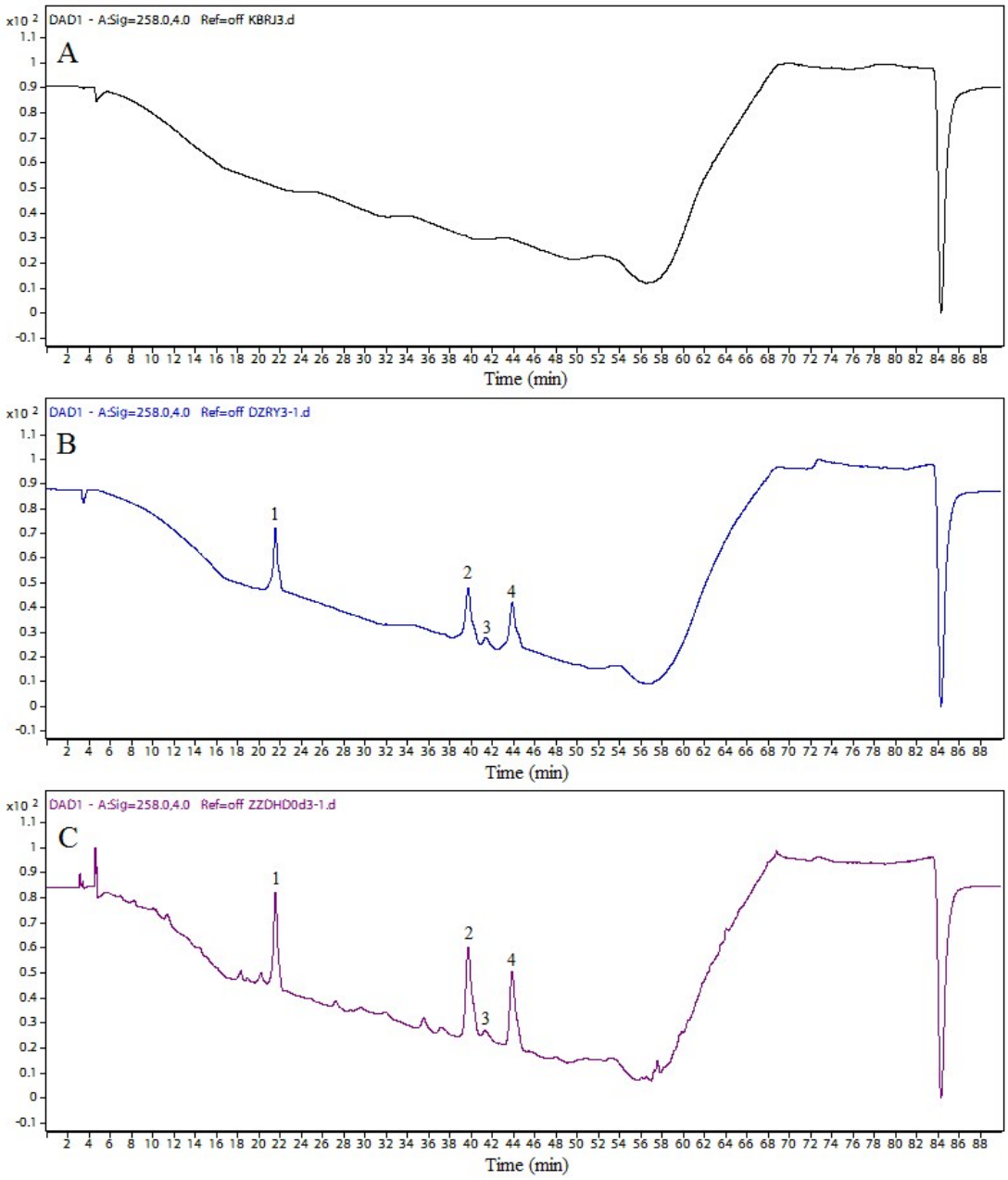
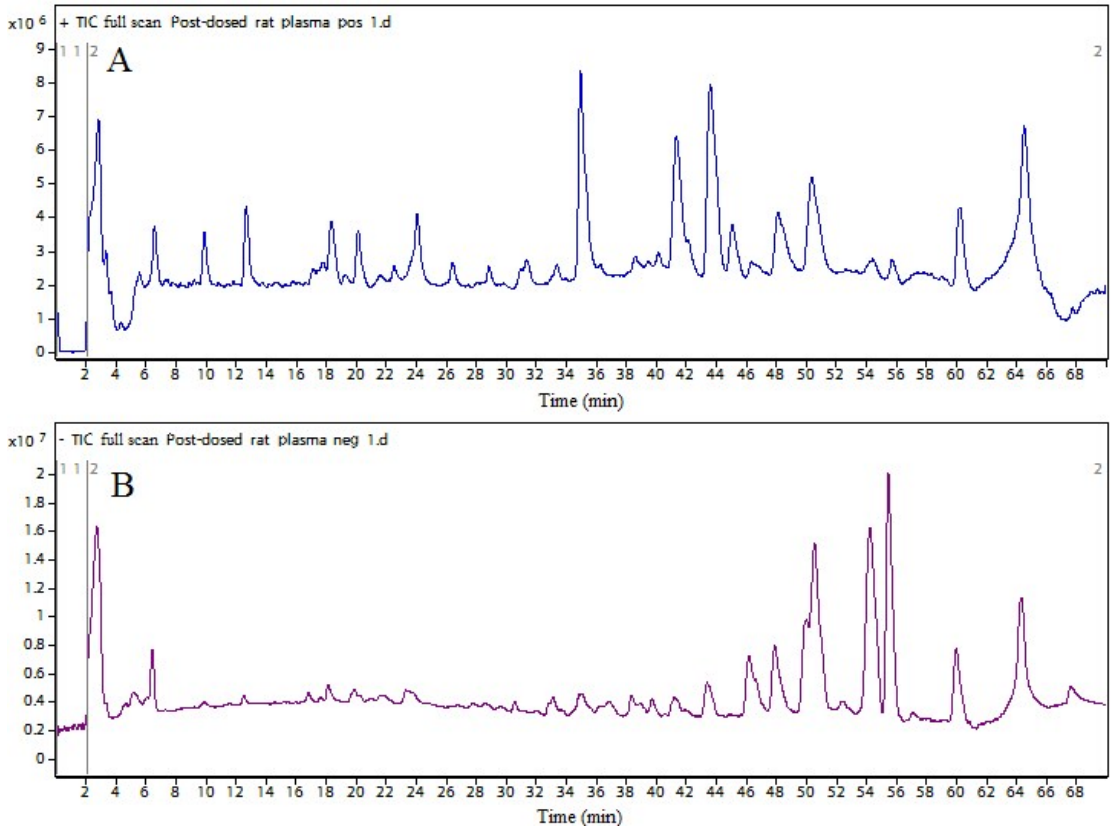


Fig.S1 LC chromatograms of blank solvent (A), the mixed standard working solution (B) and *ZZDHD* sample (C) monitored at 258 nm. (1, geniposide; 2, naringin; 3, hesperidin; 4, neohesperidin)



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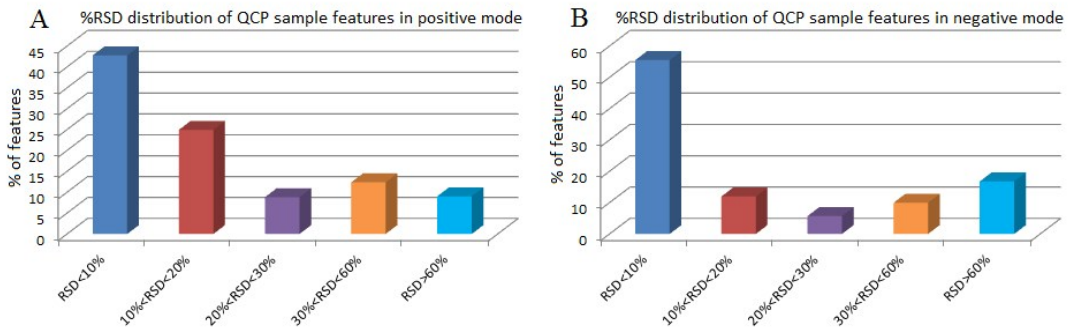
93 Fig.S2 LC–TOF/MS total ion chromatograms (TIC) of a rat plasma sample in positive-

94 ion mode (A) and negative-ion mode (B).



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96 Fig.S3 The %RSD distribution of all features detected from QCP sample in positive (A)

97 and negative-ion mode (B), respectively.

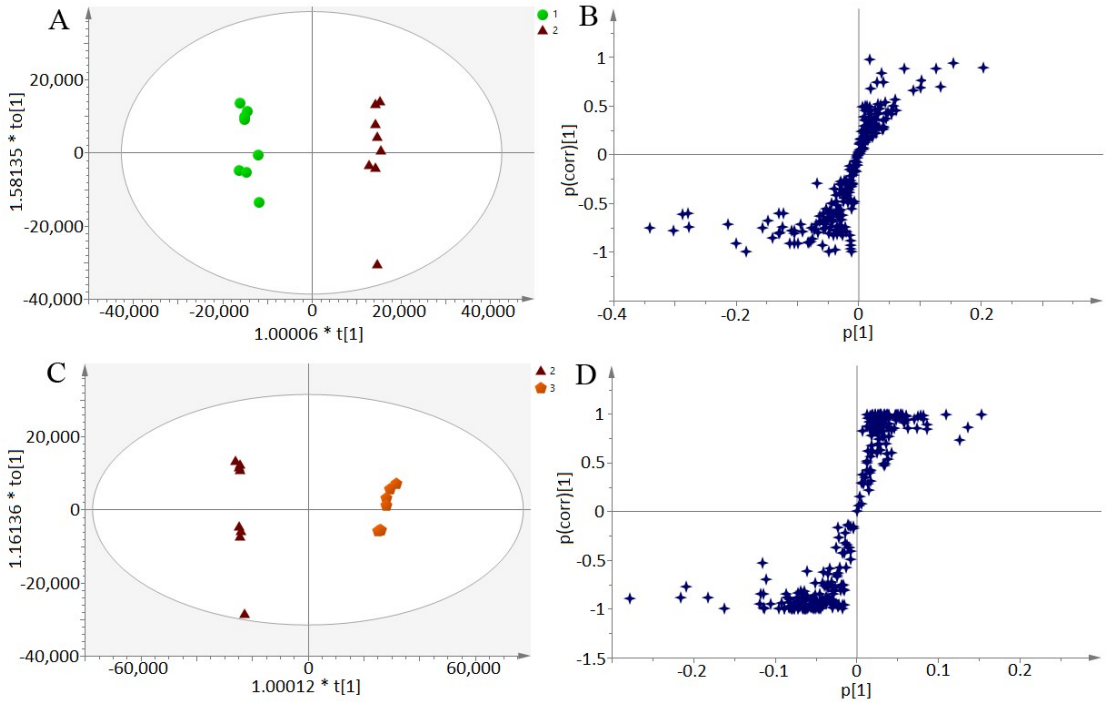

Fig.S4 OPLS-DA scores plot of plasma samples from control group and AHS group in negative-ion mode (A) with the statistical parameters (R^2X = 0.814, R^2Y = 0.995, Q^2 = 0.945, p[CV-ANOVA] = 2.0 × 10^{-4}), and corresponding S-plot from OPLS-DA model of the two groups (B). OPLS-DA scores plot of plasma samples from AHS group and *ZZDHD*-dosed group in negative-ion mode (C) with the statistical parameters (R^2X = 0.859, R^2Y = 0.996, Q^2 = 0.989, p[CV-ANOVA] = 1.1 × 10^{-9}), and corresponding S-plot from OPLS-DA model of the two groups (D). (1, control group, 2, AHS group; 3, *ZZDHD*-dosed group)

Table S1. Quality study on three batches of *ZZDHD.*

Compound	Content [a]			*P* [b]
	batch 1	batch 2	batch 3	
Geniposide	4.25±0.04	4.24±0.09	4.36±0.07	0.143
Naringin	9.13±0.07	9.09±0.05	9.24±0.07	0.066
Hesperidin	1.23±0.04	1.21±0.03	1.30±0.05	0.078
Neohesperidin	6.92±0.10	6.86±0.08	7.02±0.04	0.110

[a] Values are expressed in mg/g of the *ZZDHD* crud drug; and in mean ± SD, n=3.

[b] Content of geniposide, naringin, hesperidin and neohesperidin in three batches of *ZZDHD* showed no statistical significance (*P* >0.05) based on ANOVA, respectively.

111 Table S2. Quality study of the *ZZDHD* at three storage times of 0 d, 4 d and 8 d.

Compound	Content [a]			*P* [b]
	0 d	4 d	8 d	
Geniposide	4.25±0.04	4.19±0.02	4.21±0.04	0.178
Naringin	9.13±0.07	9.01±0.03	8.97±0.08	0.063
Hesperidin	1.23±0.04	1.19±0.01	1.24±0.02	0.125
Neohesperidin	6.92±0.10	6.50±0.04	6.76±0.03	0.133

112 [a] Values are expressed in mg/g of the *ZZDHD* crud drug; and in mean ± SD, n=3.

113 [b] Content of geniposide, naringin, hesperidin and neohesperidin in three storage

114 times showed no statistical significance (P >0.05) based on ANOVA, respectively.

Table S3. The detailed statistic values of *q* and *p* compared between each two groups for potential biomarkers (n=8 for each group)

Potential biomarkers	AHS *vs* Control		AHS *vs ZZDHD*-dosed		Control *vs ZZDHD*-dosed	
	q	*p*	*q*	*p*	*q*	*p*
L-valine	16.815	<0.001	20.316	<0.001	3.501	0.037
LysoPC (20:3)	8.527	<0.001	4.411	0.005	4.116	0.009
L-proline	10.151	<0.001	7.268	<0.001	2.883	0.054
L-leucine	25.988	<0.001	21.408	<0.001	4.579	0.004
LysoPC (18:0)	14.848	<0.001	13.395	<0.001	1.453	0.316
Phenylpyruvic acid	11.309	<0.001	7.516	<0.001	3.793	0.014
LysoPC (18:2)	9.624	<0.001	8.055	<0.001	1.569	0.280
LysoPC (18:1)	9.486	<0.001	6.427	<0.001	3.059	0.042
LysoPC (16:0)	10.217	<0.001	7.227	<0.001	2.990	0.047
L-tyrosine	5.561	<0.001	6.343	<0.001	0.782	0.586
GCDCA	6.518	<0.001	6.880	<0.001	0.362	0.801
L-tryptophan	11.089	<0.001	7.885	<0.001	3.204	0.034
Arachidonic acid	30.405	<0.001	20.628	<0.001	9.777	<0.001
GDCA	20.510	<0.001	30.326	<0.001	9.815	<0.001
Sphingosine-1-phosphate	25.333	<0.001	18.217	<0.001	7.116	<0.001
Palmitic amide	13.727	<0.001	8.481	<0.001	5.246	0.001