1 Reagents

Jian-Pi-Gu-Shen-Hua-Yu decoction (JPGS) was prepared from the pharmacy department of Cangzhou Hospital of Integrated Traditional Chinese and Western Medicine. Streptozotocin (S17049), and irbesartan (S42406) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. 1S,3R-RSL 3 (R873890) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Assay kits for total protein, creatinine (Cr) and blood urea nitrogen (BUN), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Biological Engineering Institute. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assay kit was purchased from Beyotime Biotechnology. ELISA kits for4-hydroxynonenal, Interleukin (IL) -6, IL-1β, and tumor necrosis factor α (TNF-α) were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. Primary antibodies for mouse TLR4 (ab22048), NF-κB P65 (ab32536), p-NF-κB P65 (ab76302), IL-1β (ab283818), IL-18 and ACTB (ab6276), Caspase 3 (ab184787), Caspase 9 (ab185719) and secondary antibody goat anti-rabbit IgG H&L (ab205718) were purchased from Abcam. Primary antibody for mouse NLRP3 (bs-10021R) was purchased from Boaosen Biotechnique Co., Ltd. Primary antibodies for mouse Caspase 1 (22915-1-AP), IL-18 (10663-1-AP), JNK (66210-1-Ig), p-JNK (80024-1-RR), P38 MAPK (14064-1-AP), and p-P38 MAPK (28796-1-AP) were purchased from Proteintech Co., Ltd. Total RNA extraction, first-strand cDNA reverse transcription, polymerase chain reaction (PCR) kits and primers were obtained from TianGen Biotechnology Co., Ltd. (Beijing, China).

2 UPLC-MS analysis of JPGS decoction

JPGS was obtained from Cangzhou Hospital of Integrated Traditional Chinese Medicine and Western Medicine. Ultra performance liquid chromatography (UPLC) coupled with mass spectrometer (MS) was conducted as the quality control of JPGS.

2.1 Sample preparation and extraction

400 μL solution (Methanol: Water = 7:3, V/V) containing internal standard was added into 20 mg sample, and vortexed for 3 min. The sample was sonicated in an ice bath for 10 min and vortexed for 1 min, and then placed in -20 °C for 30 min. The sample was then centrifuged at 12000 rpm for 10 min (4 °C). And the sediment was removed, then centrifuged the supernatant at 12000 rpm for 3 min (4 °C). 200 μL aliquots of supernatant were transferred for LC-MS analysis.

2.2 HPLC Conditions

All samples were for two LC/MS methods. One alipuot was analyzed using positive ion conditions and was eluted from T3 column (Waters ACQUITY Premier HSS T3 Column 1.8 μm, 2.1 mm * 100 mm) using 0.1 % formic acid in water as solvent A and 0.1 % formic acid in acetonitrile as solvent B in the following gradient: 5 to 20 % in 2 min, increased to 60 % in the following 3 mins, increased to 99 % in 1 min and held for 1.5 min, then come back to 5 % mobile phase B witnin 0.1 min, held for 2.4 min. The analytical conditions were as follows, column temperature, 40 °C; flow rate, 0.4 mL/min; injection volume, 4 μL; Another alipuot was using negative ion conditions and was the same as the elution gradient of positive mode.

2.3 MS Conditions (AB)

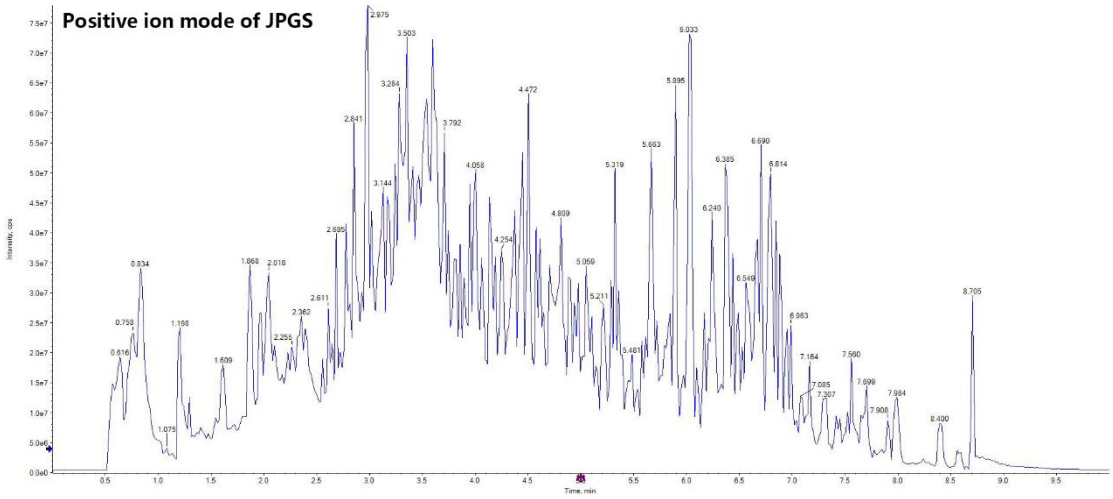
The data acquisition was operated using the information-dependent acquisition (IDA) mode using Analyst TF 1.7.1 Software (Sciex, Concord, ON, Canada). The source parameters were set as follows: ion source gas 1 (GAS1),50 psi; ion source gas 2 (GAS2), 50 psi; curtain gas (CUR), 25 psi; temperature(TEM), 550 °C; declustering potential (DP), 60 V, or−60 V in positive or negative modes, respectively; and ion spray voltagefloating (ISVF), 5000 V or−4000 V in positive or negative modes, respectively. The TOF MS scan parameters were set as follows: mass range, 50–1000 Da; accumulation time, 200 ms; and dynamic background subtract, on. The product ion scan parameters were set as follows:mass range, 25–1000 Da; accumulation time, 40

ms; collision energy, 30 or−30 V in positive or negative modes, respectively; collision energy spread, 15; resolution, UNIT; charge state, 1 to 1; intensity, 100 cps; exclude isotopes within 4 Da; mass tolerance, 50 ppm; maximum number of candidate ions to monitor per cycle, 18.

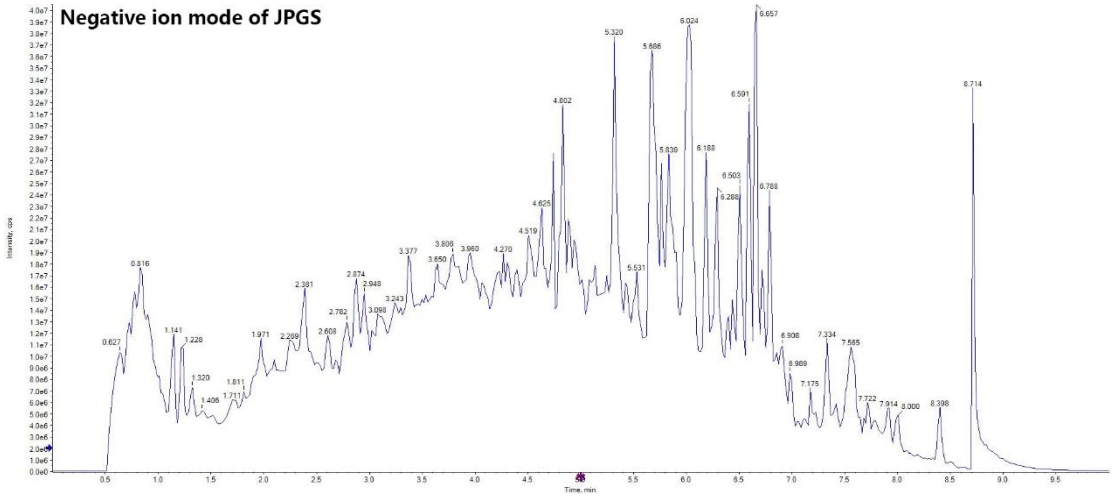
2.4 UPLC-MS analysis results of JPGS decoction

The JPGS test solution was firstly analyzed by the UPLC-MS system. **Figure S1** manifested the total ion chromatogram of JPGS in (A) positive ion mode and (B) negative ion mode. 28 kinds of components were identified (**Table S1**). We found that Astragaloside IV, Ginsenoside Ro, Ginsenoside RG1, Loganin, Dioscin, Gallic acid, Oleanolic acid, Atractylenolide III, Ferulic acid, Rosmarinic acid, Salvianolic acid A, Periandrin III, Emodin, Schisandrin, and others widely existed in JPGS.

A



B



Supplementary Figure 1 Total ion chromatogram of JPGS obtained by UPLC-MS analysis in (A) positive ion mode and (B) negative ion mode.

Supplementary Table 1 Chromatography (TIC) of JPGS based on UPLC-MS/MS.

Compounds	Formula	Mode	Molecular weight (Da)	RT (min)	Adduct	sc
Gallic acid	C7H6O5	Negative	170.0215	1.8141	[M-H]-	0.
Salvianolic acid A	C26H22O10	Negative	494.1213	1.8201	[M-H]-	0.
gomisin N	C23H28O6	Negative	400.1886	2.3266	[M-H]-	0.
3,6,8-Trimethylallantoin	C7H12N4O3	Positive	200.0909	2.6016	[M+HCOO+2H]+	0.
Rubschisandrin	C23H28O6	Negative	400.1886	2.8327	[M-H]-	0.
Loganin	C17H26O10	Negative	390.1526	3.0686	[M+CH3COO]-	0.
schidigera-saponin C1	C44H70O18	Positive	886.4562	3.7220	[M+]+	0.
Ferulic acid	C10H10O4	Negative	194.0579	3.9605	[M-H]-	0.
Isoastragaloside I	C45H72O16	Positive	868.482	4.5889	[M+K]+	0.
Astragaloside VI	C47H78O19	Positive	946.5137	4.6429	[M+H]+	0.
Astragaloside VII	C47H78O19	Positive	946.5137	5.0987	[M+H]+	0.

Astragaloside V	C47H78O19	Positive	946.5137	5.0994	[M+Na]+	0.
Ginsenoside F1	C36H62O9	Negative	638.4394	5.2038	[M+HCOO]-	0.
Astragaloside III	C41H68O14	Positive	784.4609	5.2266	[M+Na]+	0.
Astragaloside IV	C41H68O14	Negative	784.4609	5.3487	[M-H]-	0.
Schisandrin	C24H32O7	Positive	432.2148	5.4747	[M+Na]+	0.
Ginsenoside Rh6	C36H62O11	Positive	670.4292	5.4780	[M+H-H2O]+	0.
Astragaloside II	C43H70O15	Positive	826.4715	5.5879	[M+H-H2O]+	0.
Oleanolic acid	C30H48O3	Positive	456.3603	5.6222	[M+H-H2O]+	0.
Ginsenoside RG1	C42H72O14	Negative	800.4922	5.8487	[M+CH3COO]-	0.
Ginsenoside Ro	C48H76O19	Positive	956.4981	5.8752	[M+NH4]+	0.
Rosmarinic acid	C18H16O8	Positive	360.0845	5.9383	[M+H-2H2O]+	0.
Wuweizisu C	C22H24O6	Positive	384.1573	6.1950	[M+H]+	0.
Dioscin	C45H72O16	Negative	868.482	6.2685	[M-H]-	0.
Atractylenolide III	C15H20O3	Negative	248.1412	6.2797	[M-H]-	0.
Emodin	C15H10O5	Negative	270.0528	6.6038	[2M-H]-	0.
Periandrin III	C42H64O16	Positive	824.4194	7.1249	[M+]+	0.
Diosgenin	C27H42O3	Positive	414.3134	7.3879	[M+]+	0.

3 Metabolomics of kidney tissues

Metabolite extraction: Samples (100 mg) of kidney tissue grinded with liquid nitrogen were placed in Eppendorf tubes, to which was added 500 μL of 80% aqueous methanol solution. After vortexing, the tubes were placed in an ice bath for 5 min, followed by centrifugation for 20 min at 15000 *g* and 4℃. Aliquots of the resulting supernatants were diluted with mass spectrometry-grade water to a final methanol content of 53%. The diluent was then centrifuged for 20 min at 15,000 *g* and 4℃ and the resulting supernatants were collected for LC-MS analysis [1]. Equal volumes of each experimental sample were thoroughly mixed and used as a quality control sample. A 53% methanol aqueous solution was used as a blank sample, and the pretreatments were the same as those described for experimental samples.

Chromatographic conditions: Chromatographic column: Hypersil Gold column (C18); column temperature: 40℃; flow rate: 0.2 mL/min; positive mode: mobile phase A: 0.1% formic acid; mobile phase B: methanol; negative mode: mobile phase A: 5 mM ammonium acetate and pH 9.0; mobile phase B: methanol. The gradient elution program used is shown in **Table S2**.

Supplementary Table 2 The gradient elution program

Time (min)	A%	B%
0	98	2
1.5	98	2
3	15	85
10	0	100
10.1	98	2
11	98	2
12	98	2

Mass spectrometry conditions: For the purposes of spectrometric analysis, we used a scan range of m/z 100–1500. The ESI source settings were as follows: spray voltage: 3.5 kV; sheath gas flow rate: 35 psi; aux gas flow rate: 10 L/min; capillary temperature: 320℃; lens RF level: 60; aux gas heater temp: 350℃; polarity: positive, negative; MS/MS secondary scans were data dependent.

Data preprocessing and metabolite identification: For processing, the raw data file was imported into CD 3.1 compound discoverer software. For each analyzed metabolite, we performed simple screening based on retention time, mass-to-charge ratio, and other parameters. To align the peaks of different samples to enhance the accuracy of identification, the deviation of retention time was set to 0.2 min and the deviation of mass was set to 5 ppm. The peaks were then extracted by setting the mass deviation to 5 ppm, the signal intensity deviation to 30%, and the signal-to-noise ratio to 3, as well as setting the minimum signal intensity and the addition ion. Having quantified the peak area, the target ion was integrated, and the molecular formula was predicted based on the detected molecular ion peak and fragment ion patterns, which were compared with those of reference spectra in the mzCloud

(https://www.mzcloud.org/), mzVault, and the Masslist databases. Blank samples were used to remove the background ions, and the raw quantitative results were normalized to facilitate identification and relative quantification results of the analyzed metabolites. Data processing was based on the Linux operating system (CentOS version 6.6) and R and Python software.

Statistical analysis: For the purposes of metabolite annotation and identification, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/pathway.html), Human Metabolome (https://hmdb.ca/metabolites), and LIPID MAPS (http://www.lipidmaps.org/) databases. For multivariate analysis, we used metaX metabolomics data processing software [2] for data conversion. The same software was used for principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to determine the variable importance in projection (VIP) scores of each metabolite. For univariate analysis, the statistical significance (P value) of difference in each metabolite between two groups was determined using *t* tests, and we calculated metabolite fold changes (FC) between the two groups. The default criteria for screening differential metabolites were a VIP >1 and $P < 0.05$, with an FC ≥ 1.2 or FC ≤ 0.83. Finally, the differential metabolites identified via screening were imported into MetaboAnalyst 5.0 for analysis of metabolic pathway enrichment.

4 Primer sequence used in this study

Supplementary Table 3 Primer sequence

		Sequence (5'->3')
Nlrp3	Forward primer	TTGGGAGACTCAGGAGTCCAA
	Reverse primer	TCTCCAAGGGCATTGCTTCG
Asc	Forward primer	TGAGCAGCTGCAAACGACTA

Casp1	Reverse primer	CACGAACTGCCTGGTACTGT
	Forward primer	CCTGTCAGGGGCTCACTTTT
	Reverse primer	TCCAAGTCACAAGACCAGGC
Il1b	Forward primer	ATGCCACCTTTTGACAGTGATG
	Reverse primer	TGTGCTGCTGCGAGATTTGA
Il18	Forward primer	GACAACTTTGGCCGACTTCA
	Reverse primer	CTGGGGTTCACTGGCACTTTG
Actb	Forward primer	CCCCTGAACCCTAAGGCCA
	Reverse primer	ATGGCTACGTACATGGCTGG

Reference

[1] Want EJ, Masson P, Michopoulos F, Wilson ID, Theodoridis G, Plumb RS, Shockcor J, Loftus N, Holmes E, Nicholson JK. Global metabolic profiling of animal and human tissues via UPLC-MS. *Nature Protocols* 2012; 8: 17–32. [DOI: 10.1038/nprot.2012.135]

[2] Wen B, Mei Z, Zeng C, Liu S. metaX: a flexible and comprehensive software for processing metabolomics data. *BMC Bioinformatics* 2017; 18. [DOI: 10.1186/s12859-017-1579-y]