

Integrating serum pharmacochimistry, network pharmacology and metabolomics analysis to explore the possible mechanism of Qingjiehuagong decoction in the treatment of acute pancreatitis

Bai-Jun Qin^{1, 2#}, Min-Chao Feng^{2#}, Chi Zhang³, Xian-Zhong Bu², Wen-Hao Gong², Yue-Qiao Chen⁴, Yan Tang², Hui Li², Kun-Rong Liu⁴, Guo-Zhong Chen^{4*}, Xi-Ping Tang^{5*}

¹Department of Gastroenterology, Chongqing Traditional Chinese Medicine Hospital, Chongqing 400011, China. ²Guangxi Key Laboratory of Molecular Biology of Traditional Chinese Medicine and Preventive Medicine, Nanning 530000, China. ³Department of Orthopedics, Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Nanning 530000, China. ⁴Department of Gastroenterology, The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning 530000, China. ⁵Department of Endoscopy Center, Cancer Hospital Affiliated to Guangxi Medical University, Nanning 530000, China.

#These authors contributed equally to this work and are co-first authors for this paper.

*Corresponding to: Guo-Zhong Chen, Department of Hepatic, The First Affiliated Hospital of Guangxi University of Chinese Medicine, No. 89 Dongge Road, Nanning 530000, China. E-mail: cheninj@163.com; Xi-Ping Tang, Department of Endoscopy Center, Cancer Hospital Affiliated to Guangxi Medical University, No.71 Hedi Road, Nanning 530000, China. E-mail: cheninj@163.com.

Author contributions

The experiments were conceived and designed by Qin BJ and Chen GZ. The experiments were performed by Feng MC, Tang XP, Zhang C, and Bu XZ. The data was analyzed by Chen YQ, Gong WH, and Li H. The manuscript was written by Qin BJ, Tang Y, and Liu KR. Funding was acquired by Chen GZ. All the listed authors have read and approved the submitted manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

QJHGD, Qingjiehuagong decoction; UPLC-HRMS, ultra-performance liquid chromatography-high resolution mass spectrometry; UPLC-Q-TOF MS, ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry; TCMS, traditional Chinese medicine systems pharmacology; AP, acute pancreatitis; TCM, traditional Chinese medicines; PPI, protein-protein interaction; GO, Gene Ontology; PCA, principal component analysis; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable significance in the projection; H&E, hematoxylin and eosin; MG, model group; NG, normal group; MAP, mild acute pancreatitis; SAP, severe acute pancreatitis; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCODE, molecular complex detection; LC-MS, liquid chromatography-mass spectrometry; ESI, electrospray ionization; ATP, adenosine triphosphate.

Citation

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Abstract

Background: The Qingjiehuagong decoction (QJHGD), which has been used in clinical trials to treat acute pancreatitis (AP), has demonstrated encouraging results. **Methods:** In this particular investigation, we used both metabolomics and network pharmacology to investigate the fundamental processes and targets that QJHGD employs to cure AP. **Results:** Using a cerulein-induced rat model of AP, we showed that QJHGD effectively improved pancreatic tissue damage and reduced serum levels of AMY, LPS, IL-1 β , IL-6, IL-8 and TNF- α . In total, 28 blood entry compounds derived from QJHGD were identified by ultra-performance liquid chromatography-high resolution mass spectrometry technology. The intersecting target genes of 108 genes associated with identified compounds in QJHGD and AP disease genes were identified using a network pharmacology approach. The protein interaction network revealed AKT1, TNF- α , IL-6, VEGFA, and TP53 as important targets. Gene ontology analysis showed that response to stimulus, molecular function regulator and organelle part were the main functions, and Kyoto Encyclopedia of Genes and Genomes analysis showed that 20 pathways such as AGE-RAGE signaling pathway in diabetic complications and the IL-17 signaling pathway were the main pathways involved in the anti-AP effects of QJHGD. Thirty-two potential metabolic markers and 13 possible metabolic pathways were identified by metabolomics analysis. Combined network pharmacological analysis revealed that QJHGD affects four metabolic pathways (tryptophan metabolism; glycolysis and gluconeogenesis metabolism; valine, leucine and isoleucine degradation metabolism; the urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine), five metabolites (indole-3-acetate, pyruvate, methylmalonate, L-citrulline, N-acetyl-L-glutamate) and four related targets (AKT1, ALDH2, NOS2, NOS3) to combat inflammation. The strong affinity of QJHGD's interactions with its primary targets was established by molecular docking and molecular dynamics simulations. **Conclusion:** This research investigate the critical targets and mechanisms of QJHGD for treating AP. The results of this investigation provide novel tactics and complementary techniques for the clinical treatment of AP.

Keywords: acute pancreatitis; Qingjiehuagong decoction; metabolomics; network pharmacology

Highlights

This study integrated serum medicinal chemistry, pharmacodynamics, network pharmacology, and serum metabolomics to comprehensively and systematically reveal the active ingredients of Qingjieshuang decoction and its pharmacological mechanism in the treatment of acute pancreatitis, providing new clinical drug selection and effective evidence for the treatment of acute pancreatitis with traditional Chinese medicine.

Medical history of objective

Qingjieshuang decoction is a clinical experience formula of Professor Chen Guozhong, a famous Chinese medicine practitioner in Guangxi, which is based on Xiaochengqi decoction, derived from Zhang Zhongjing's treatise on typhoid, article 213 (200 C.E.). In ancient times, this formula was often used to treat the actual symptoms of Intestine. In the current study, modern pharmacological studies have shown that this formula has the effects of protecting pancreatic cells, inhibiting inflammatory response, and promoting gastrointestinal motility.

Background

Acute pancreatitis (AP) is a digestive disease in which the pancreatic acini are abnormally activated by pancreatic enzymes, leading to local inflammation, edema and necrosis [1, 2]. Disease progression triggers an accelerated release of inflammatory mediators and cytokines, and ultimately a potentially fatal outcome for patients with severe acute pancreatitis. The incidence of AP ranges from 13/100,000 to 45/100,000 per year worldwide, and the incidence in China has increased from 0.19% to 0.71% in the past 20 years [3, 4]. Current clinical treatments for AP include antibiotics, surgical interventions, oral pancreatic enzyme inhibitors, and lavage of the peritoneal cavity, although recent meta-analyses have shown that poor clinical benefit [5, 6]. Therefore, reliable and effective complementary alternative therapies for AP are required.

Traditional Chinese medicines (TCMs) have shown effectiveness and safety in the treatment of AP in clinical practice, and are now included in the official Chinese for the *Diagnosis and Treatment of Acute Pancreatitis* (2021) [7, 8]. According to TCM theory, the core pathogenesis of AP is “dampness, heat, stasis and toxicity”. We formulated Qingjieshuang decoction (QJHGD) for the treatment of AP according to the principles of “clearing heat, resolving dampness and detoxification, resolving stasis and attacking the lower part of the body to preserve Yin (The substantive material substances inside the human body, such as bodily fluids)” based on Xiao Cheng Qi Tang (A classical Chinese medicine formula) to combine Qi (the intangible, high-mobility nutritive substance that maintains vital activities)-regulating, dampness-transforming, blood-vitalizing and blood-stasis-eliminating herbs that eliminate toxicity and preserve Yin and protect Yang (Substances with protective effects on the body). QJHGD contains *Bupleurum chinense* DC, *Glycyrrhiza uralensis* Fisch., *Salvia miltiorrhiza* Bge., *Rheum officinale* Bail., *Scutellaria baicalensis* Georgi., and *Citrus aurantium* L. [9, 10]. QJHGD is used for the treatment of AP in the First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine and has been granted a national invention patent (batch number: ZL201811021893.2).

QJHGD has shown good clinical results in the treatment of AP, effectively reducing the serum inflammatory index of patients, promoting pancreatic function repair and reducing the incidence of complications [11–15]. The use of this formulation as a TCM (No. S2019021) for the treatment of AP is supported by the Guangxi Administration of Traditional Chinese Medicine. Modern pharmacological studies have shown that QJHGD regulates the TLR4/NF- κ B/MyD88 signaling pathways and the intestinal flora to inhibit inflammatory responses and alleviate AP [16–18].

In this study, we used a cerulein-induced model of AP in rats to

assess the therapeutic efficacy of QJHGD based on histopathology and analysis of serum biochemical and inflammatory factors. After characterization of the potential active components of QJHGD by ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS), we used a network pharmacology approach to identify the main targets and potential mechanisms underlying the therapeutic effects of QJHGD in AP. After that, we used a serum metabolomics approach based on ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) technology to search for related endogenous metabolites and metabolic pathways. Furthermore, by integrating the endogenous metabolites and the exogenous active ingredients, we were able to systematically elucidate the pharmacodynamic material basis and mechanism of the therapeutic effects of QJHGD in AP.

Materials and methods**Materials**

The following materials and reagents were used in this study: cerulein (S62702; Yuanye Bio-Technology Co., Shanghai, China), ulinastatin for injection (032005103; Temple Biochemical Pharmaceutical Co., Guangzhou, China), pentobarbital sodium (AM00469; Sigma Pharmaceuticals Co., ST. Louis, MO, USA), hematoxylin and eosin (H&E) staining kit (G1120; Solarbio Science & Technology Co., Beijing, China), serum lipase activity assay kit (BC2345; Solarbio Science & Technology Co., Beijing, China), serum amylase (AMY) activity assay kit (BC5055; Solarbio Science & Technology Co., Beijing, China), rat interleukin 1 β (IL-1 β) ELISA kit (RA20020; Bioswamp Biotechnology Co., Wuhan, China), rat IL-6 ELISA kit (RA20607; Bioswamp Biotechnology Co., Wuhan, China), rat IL-8 ELISA kit (RA20553; Bioswamp Biotechnology Co., Wuhan, China), rat tumor necrosis factor α (TNF- α) ELISA kit (RA20035; Bioswamp Biotechnology Co., Wuhan, China), acetonitrile (1499230-935; Merck & Co., Darmstadt, Germany), formic acid (F0654; Tokyo Chemical Industry Co., Tokyo, Japan), ammonium acetate (70221; Sigma Pharmaceuticals Ltd., ST. Louis, MO, USA), methanol (A456-4; Fisher Scientific Co., Shanghai, China), aqueous ammonia (A470-500; Fisher Scientific Co., Shanghai, China). The Chinese medicinal materials in QJHGD formula comprised: CH (batch number: 17010267), GC (17010163), DS (16060117), DH (16060431), HQ (16182144), and ZS (16080375), all purchased from Guangxi Xianzhu traditional Chinese Medicine Technology (Nanning, China).

Animals

Specific pathogen-free male Sprague-Dawley rats (200 $\pm$ 20 g) were purchased from Hunan Slaughter Jingda Laboratory Animal Company (Changsha, China; Certificate No. SCXK(Xiang)2019-0004). All rats were maintained in the laboratory of the Experimental Centre for Medical Molecular Biology, First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine (license No. SYXK(Gui)2019-0001) under the following conditions: 12-hour light/dark cycle, constant temperature (24 $\pm$ 1 $^{\circ}$ C), and relative humidity (50 $\pm$ 10%). This study was approved by the Experimental Animal Ethics Committee of Guangxi University of Chinese Medicine (No. DW20220310-020) conducted in accordance with the requirements of the *Principles of Laboratory Animal Care*.

QJHGD preparation

The drug and dose composition of the formula are *Bupleuri Radix* (20 g), *Fructus Aurantii Immaturus* (10 g), *Scutellaria baicalensis* Georgi (10 g), *Radix Salviae Miltiorrhizae* (10 g), *Rheum palmatum* (5 g), and *Radix Glycyrrhizae* (5 g) in a 4:2:2:2:1:1 proportion by weight. The preparation was performed in accordance with the “Criteria for TCM Decoction Room Management in Medical Institutions” using previously described methods [19, 20]. The final yield was 1 g/mL of raw QJHGD (100% concentration).

Establishment of the cerulein-induced rat model of AP and evaluation of the anti-inflammatory effects of QJHGD

The AP animal model was induced by seven intraperitoneal injections of cerulein (50 µg/kg) administered at 1-h intervals [21]. Rats were divided into four groups (n = 6/group): normal (untreated), AP control (saline by gavage), QJHGD (9.45 g/kg by gavage); and ulinastatin (injected intraperitoneally with 5 U/kg ulinastatin). All treatments were administered once every 12 h for 24 h. Doses were selected based on previous reports of the therapeutic effects and conversion of the human dose based on body surface area [22]. Specifically, using this formula, the medium dosage of QJHGD used was 9.45 g/kg/d, equivalent to a dose in humans of 105 g/60 kg/d. According to the previous study of the research group, it was found that the medium-dose group had the best therapeutic effect, so the QJHGD group received 9.45 g/kg/d QJHGD intervention in study [17].

After the drug intervention, rats were anesthetized by intraperitoneal injection of 1–2 mL sodium pentobarbital. The abdomen was cut along the midline under sterile conditions and blood was collected from the abdominal aorta. After coagulation (30 min), serum was separated by centrifugation at 3,000 rpm for 10 min. Serum levels of IL-6, IL-1β, IL-8, TNF-α, amylase and lipase were measured using kits according to the manufacturers' recommendations. The pancreas was quickly removed, fixed with 4% paraformaldehyde, and the tissue was encased in paraffin. The H&E stain was applied to sections that were 5 micrometers thick before being examined with a light microscope.

The experimental data were statistically analyzed using SPSS 25.0 software, and the measurement data are presented as $\pm$ s. Data that conformed to normal distribution and chi-square were analyzed by single analysis of variance, and least-significant difference-t test was used for two-way comparison between groups. The rank sum test was used for data not conforming to normal distribution and variance homogeneity. Test level: the test water level was $\alpha = 0.05$.

Qualitative identification of chemical components of QJHGD

Preparation of QJHGD samples. A total of 600 µL QJHGD was added to 400 µL precooled 40% methanol solution, and vortex mixed. Subsequently, 100 µL of this mixture was added to 700 µL precooled 40% methanol solution and vortex mixed. The supernatant from each sample was centrifuged at 16,000 rpm for 15 minutes at 4 °C, then kept at 4 °C until it was needed again.

Preparation of rat serum samples. Serum samples for analysis were prepared by adding 200 µL serum to 800 µL methanol. After vortexing for 60 s, the mixture was cooled at –20 °C for 30 min, and centrifuged at 16,000 rpm for 20 min at 4 °C. The supernatant was vacuum dried before 100 µL 40% methanol aqueous solution was added to the residue. Samples were centrifuged at 16,000 rpm for 15 min at 4 °C.

Blank serum + QJHGD samples were prepared by adding 200 µL blank serum and 40 µL QJHGD test solution to 800 µL methanol. The mixture was vortexed for 60 s and placed at –20 °C for 30 min before centrifugation at 16,000 rpm for 20 min at 4 °C. Thereafter, the supernatant vacuum dried and the residue was dissolved in 100 L 40% methanol aqueous solution, vortexed and centrifuged at 16,000 rpm for 15 min at 4 °C.

UPLC-HRMS conditions. Samples (4 L blank serum, drug-containing serum, blank serum + QJHGD) and 2 µL QJHGD solution were injected into the analytical apparatus. For the liquid chromatography-mass spectrometry (LC-MS) analysis, one injection of blank serum and one injection of drug-containing serum per batch, three repeated injections of blank serum + QJHGD per batch and five repeated injections of QJHGD group serum per batch were added. The chemical components of QJHGD were identified by UPLC-HRMS technology using a Thermo Q-Exactive HFX mass spectrometer. The samples were eluted with mobile phase A (H₂O-formic acid (99.9:0.1, v/v) and mobile phase B is ACN-FA (99.9:0.1, v/v) and scanned from m/z 90 to 1,300 in the negative and positive ion modes.

Network pharmacology analysis

Target prediction. Based on the QJHGD blood components identified by mass spectrometry of serum samples, their targets were acquired in

traditional Chinese medicine systems pharmacology (TCMSP) database (The full name and website address of the data websites involved in the network pharmacology section are detailed in [Supplementary Table S1](#), the same below), the ETCM database, the BATMAN-MAN database, and the SwissTargetPrediction database [23–26]. When searching for associated AP targets, we utilized the word “acute pancreatitis” as the search term in the DisGeNET database, the DrugBank Online database, the GeneCards database, and the OMIM database. Before retrieving the merged gene targets, the names of genes and proteins were standardized with the help of the UniProt database. This was done before the merged gene targets were obtained. The Venny tool was used to map and compare the target genes of QJHGD with the genes of AP in order to determine whether target genes overlapped with each other.

Based on the cross-target results, we then constructed a protein-protein interaction (PPI) network using the string database, with a species restriction of “Homo sapiens” and a medium confidence level of 0.4. The PPI network data were imported into Cytoscape 3.7.1 for CytoHubba and molecular complex detection (MCODE) algorithm prediction of the set of key targets of the network.

Pathway enrichment analysis. Pathways involved in the therapeutic effects of QJHGD in AP were predicted by Gene Ontology (GO) enrichment analysis of the cross-targets using the David database. We also performed Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, plotted pathway histograms based on P -value ≤ 0.01 , and selected the top 20 pathways to plot KEGG bubble maps. The component-pathway Sankey diagram was constructed by combining the QJHGD blood components identified by mass spectrometry analysis of serum samples. The data were visualized using OmicShare database.

Network construction. We used the active ingredients of QJHGD, the cross-targets between the active ingredients and the disease, and the first 20 KEGG signaling pathways to construct a key herbal-component-target-pathway network for QJHGD in the treatment of AP. The network was built using Cytoscape 3.7.1 software.

Metabolomics

Sample preparation. After collecting serum samples in Vacutainer tubes containing the chelating agent ethylene diamine tetra-acetic acid, the samples were centrifuged at 1,500 g for 15 minutes at 4 °C. The temperature of the storage space was maintained at –80 °C. For the UPLC-Q-TOF/MS analysis, the samples were frozen at 4 °C [27]. Then 100 µL aliquots were mixed with 400 µL cold methanol/acetonitrile (1:1, v/v) to eliminate the proteins. Before drying the supernatant in a vacuum centrifuge, the mixture was centrifuged at 14,000 g for 15 minutes at a temperature of 4 °C. In preparation for the LC-MS analysis, the samples were re-dissolved in 100 µL acetonitrile/water (1:1, v/v) solvent.

UPLC-Q-TOF MS analysis. Shanghai Applied Protein Technology carried out analysis with the assistance of a UPLC (1290 Infinity LC, Agilent Technologies) that was linked to a quadrupole time-of-flight (AB Sciex Triple TOF 6600).

A 2.1 mm 100 mm ACQUITY UPLC HSS T3 1.8 µm column (Waters, Ireland) was used for RPLC separation. The mobile phase in the electrospray ionization (ESI)-positive mode contains A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). In the ESI negative mode, the mobile phase contained A (0.5 mM ammonium fluoride in water) and B (acetonitrile). The gradient was 1% B for 1.5 minutes, then increased linearly to 99% in 11.5 minutes and maintained for 3.5 minutes. It was then reduced to 1% in 0.1 minutes, with a 3.4-minute re-equilibration period. The elution was done at a flow rate of 0.3 mL/min, and the column temperatures were maintained at 25 °C.

The ESI source conditions were set as follows: Ion Source Gas 1, 60; Ion Source Gas 2, 60; curtain gas, 30; source temperature, 600 °C; and Ion Spray Voltage Floating, $\pm$ 5500 V. The instrument was configured to do an acquisition using the m/z range of 60–1,000 Da, and the accumulation time for the TOF MS scan was set at 0.20 seconds per

spectrum for MS-only acquisition. The instrument was programmed to gather data throughout an m/z range of 25–1000 Da, and the accumulation time for the product ion scan was set to 0.05 seconds per spectrum to facilitate automatic MS/MS data collection. The high-sensitivity mode of information-dependent acquisition was used to capture the product ion scan. The parameters were set as follows: the collision energy was fixed at 35 V with ± 15 eV; declustering potential was 60 V (+) and -60 V (–); and isotopes excluded within 4 Da, with candidate ions monitored per cycle: 10 [28].

Data processing. Before being imported into freely available XCMS software, the raw MS data (wiff.scan files) were converted to MzXML files using the ProteoWizard MSConvert program. The following settings were used for peak picking: centWave m/z = 10 ppm, peak width = c (10, 60), and prefilter = c (10, 100). In order to group the peaks, the parameters bw = 5, mwid = 0.025, and minfrac = 0.5 were utilized. Collection of Algorithms of Metabolite pRofile Annotation was used for annotating isotopes and adducts. Only the variables that contained more than fifty percent of non-zero measurement values in at least one group were kept in the final set of extracted ion characteristics. Metabolites were identified by comparing the accuracy m/z value (< 10 ppm) and MS/MS spectra with an in-house database developed using accessible, genuine standards.

Statistical analysis. After being submitted to sum-normalization, the processed data were analyzed using multivariate methods, such as Pareto-scaled principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Both response permutation testing and seven-fold cross-validation were used in order to assess the model's degree of robustness. It was determined how much each variable in the OPLS-DA model contributed to the classification by computing the variable significance in the projection (VIP) value for each variable. The Student's *t*-test was used to ascertain whether there was a statistically significant gap in quality between two distinct sets of samples. For screening substantially altered metabolites, VIP > 1 and $P < 0.05$ were utilized. In order to establish the degree of connection that exists between two variables, Pearson's correlation analysis was carried out.

Integrated analysis involving metabolomics and network pharmacology

Construction of the compound-reaction-enzyme-gene network. The mechanism of action of QJHGD for treating AP was investigated using a combination of network pharmacology and metabolomics methods. A compound-reaction-enzyme-gene network was generated to highlight the relationships between metabolites, pathways, enzymes, and genes. The differential metabolites found by metabolomics were imported into MetScape database for metabolic pathway analysis. In order to identify intersecting hub genes as key metabolites and targets, the targets predicted by MetScape were compared with those anticipated by network pharmacology.

Molecular docking. The intersecting hub genes were selected for molecular docking with the active ingredients of QJHGD, for which 2D structures were downloaded from the PubChem database and mechanistic structure was optimized using ChemBio3D. The crystal structures of the target proteins were obtained from the RCSB database. Ligand and receptor files are converted to pdbqt format by AutoDockTools 1.5.6. Their structures were improved by replacing water molecules with hydrogen atoms. Finally, molecular docking was performed in DS software (BIOVIA Discovery Studio 2021).

Molecular dynamics simulation. In order to prepare for the study, the complexes were run through a 50 ns all-atom MD simulation using the GROMACS version 2019.3 software package. In order to handle XOD, an Amber99sb-ildn Force Field was used. Multiwfn was used to determine the restrained electrostatic potential of the ligands, and the General Amber Force Field was used to parameterize the results [29, 30]. The acubic box was given a layer dimension of 12 angstroms, and the TIP3P water model was used to solvate the complexes. After adding K⁺ counterions to the system for the necessary time to accomplish charge neutralization, more Na⁺ and Cl[–] ions were

introduced. The Particle Mesh Ewald technique was used to calculate the long-range electrostatic interactions, and the grid was configured with a spacing of 1.0 in the periodic boundary conditions [31]. Both canonical ensemble and constant-pressure, constant-temperature were brought into equilibrium via a 0.1-ns ensemble prior to the MD simulation. The MD simulation was used to investigate the 50 ns trajectory, and the integration time step was set at 2 fs.

Results

Effects of QJHGD on pancreatic pathology and serum inflammatory factors in AP rats

As shown in Figure 1, H&E staining of the pancreas showed diffuse destruction of the tissue structure, focal dilatation of the lobular septa, atrophy of glandular vesicles and infiltration of inflammatory cells in the model group. Compared with the model group, the pancreatic tissues of rats treated with QJHGD were more compact and intact, with orderly arrangement of acinar cells, less inflammatory cell infiltration and edema and fewer hemorrhagic foci in the lobules. Furthermore, while serum levels of AMY, LPS, TNF- α , IL-1 β , IL-6 and IL-8 were significantly higher in the model group ($P < 0.001$) (Figure 2a–2f), the levels were significantly decreased after QJHGD treatment ($P < 0.001$). A similar pattern of changes in these indexes were observed in the ulinastatin group.

QJHGD constituents

51 chemical compounds were identified from QJHGD by mass spectrometry analysis (Figure 3a, 3b). These chemical compounds were classified as shown in Supplementary Table S2 and the proportions are shown in Figure 3c. The top six classes of compounds were classified as follows: benzene and substituted derivatives (12.9%), carboxylic acids and derivatives (20.5%), fatty acyls (11.1%), flavonoids (22.1%), organo-oxygen compounds (18.1%), and prenol lipids (15.3%). Details of these compounds and the originating sources are shown in Table 1.

Network pharmacology analysis

Active ingredients of QJHGD and related targets. A total of 28 components identified in the serum were considered to be potential bioactive components of QJHGD (Table 1, Supplementary Table S3). Using a network pharmacology approach, we used these bioactive components to predict the potential targets and pathways involved in the therapeutic effects of QJHGD in AP. By searching the TCMSP database, BATMAN-TCM analysis platform, TCMIP v2.0 database, and Swiss target database, we identified 353 targets of potential bioactive components in QJHGD and their gene symbols were defined according to the UniPort website. A total of 1,042 AP-related genes were identified in searches of multiple databases (GeneCards (relevance score ≥ 11.63538647); OMIM, DisGeNET (relevance score ≥ 0.1) and DrugBank). After importing the potential target genes of QJHGD and the AP-related genes into the OmicShare cloud platform, 108 overlapping target genes were identified (Figure 4a). To further explore the core targets of QJHGD in the mechanism underlying the therapeutic effects in AP, these overlapping target genes were used to construct the PPI network, shown in Figure 4b. This network included 108 nodes and 2,171 edges and the degree of correlation with factors such as AKT1, TNF- α , TP53, IL6 and VEGFA is shown in Supplementary Table S4. The network of core targets identified by the CytoHubba algorithm are shown in Figure 4c and Supplementary Table S5. In addition, using MCODE to analyze the PPI network, we identified the sub-network with the highest score (31.304), which included 55 nodes and 1212 edges, as shown in Figure 4d and Supplementary Table S6. The overlap between the targets identified by CytoHubba algorithm and MCODE algorithm are shown in Figure 4e. These five targets identified [AKT1 (94), TNF- α (86), TP53 (85), IL6 (84), and VEGFA (81)] were considered to be potential core targets of QJHGD in the mechanism underlying the therapeutic effects on AP.

GO and KEGG analyses. After doing a study on the 108 genes that

overlap, researchers identified the top 20 KEGG pathways and 52 associated GO enrichment keywords involved in the mechanism that underlies the therapeutic benefits of QJHGD in AP ($P < 0.001$). As shown in Figure 5a, the GO keywords for the therapy of AP contained 27 biological processes, 14 molecular activities, and 11 cellular components. The biological processes terms included positive and

negative regulation of biological processes, metabolic process, cellular component organization or biogenesis, immune system process, detoxification, and response to stimulus. The molecular function terms included molecular function regulator, molecular transducer activity, transcription regulator activity, antioxidant activity, and structural molecule activity. In terms of cellular components, the genes were

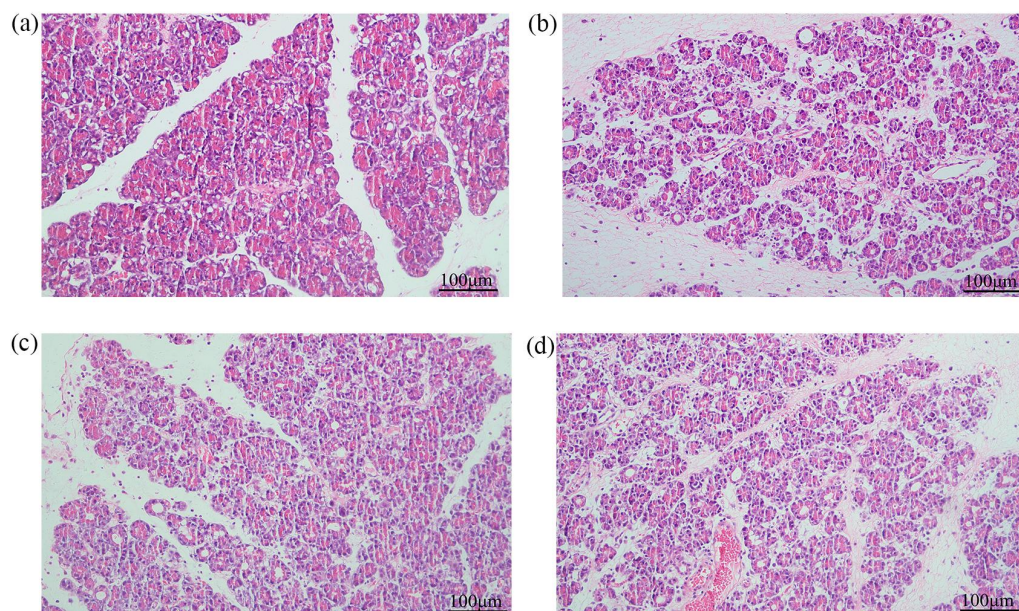


Figure 1 Pancreatic histopathological changes in AP rats observed by H&E staining (200 magnification). (a) Normal group. (b) AP model group. (c) QJHGD group. (d) WSTD group. AP, acute pancreatitis; H&E, hematoxylin and eosin; QJHGD, Qingjiehuagong decoction; WSTD, ulinastatin.

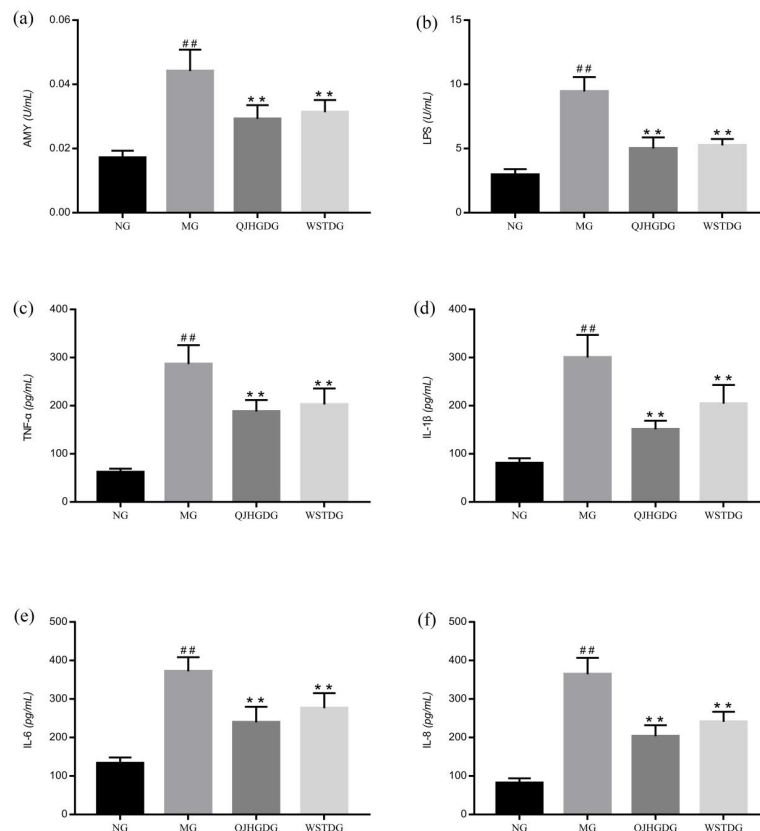


Figure 2 Serum biochemical and inflammatory factors in AP rats. (a) Amylase. (b) lipase. (c) TNF-α. (d) IL1-β. (e) IL-6. (f) IL-8. Data represent the mean $\pm$ SD ($n = 6$ /group). ^{##} $P < 0.01$ vs. normal group; ^{**} $P < 0.01$ vs AP model group. MG, model group; NG, normal group; QJHGD, Qingjiehuagong decoction; WSTD, ulinastatin.

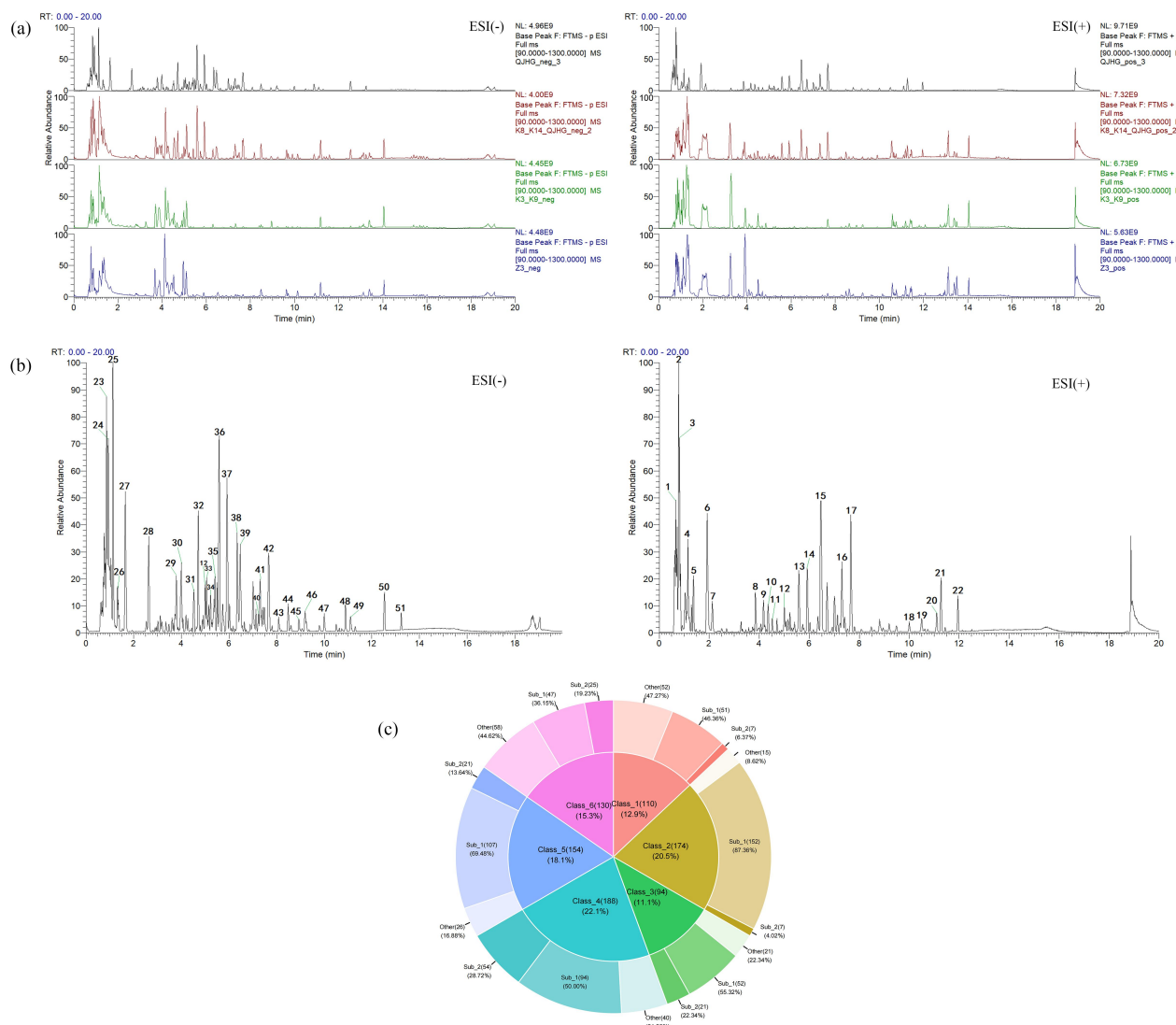


Figure 3 UPLC-HRMS total ion chromatograms of QJHGD. (a) The base peak chromatogram of blank serum, drug-containing serum and QJHGD test sample. The chromatogram represents QJHGD, blank serum + QJHGD, blank serum and administration serum (top to bottom panels). (b) Chromatogram of total ions in drug-containing serum; the spectrum peaks are marked. (c) The number proportion of compounds in each chemical classification based on comparison of secondary mass spectra. QJHGD, Qingjiehuagong decoction; UPLC-HRMS,

Table 1 Characterization of QJHGD constituents by UPLC-HRMS

No.	Identification	Model	Formula	tR/min	Score	Error/ppm	m/z	Class	Source	Into_Blood OR None	References
1	Scopoletin	$[M+H]^+$	$C_{10}H_8O_4$	0.67	0.9661	0.6	193.0496	Coumarins and derivatives	CH ₂ GC	Into_Blood	[32, 33]
2	Baicalein	$[M+H]^+$	$C_{15}H_{10}O_5$	0.80	0.9996	1	271.0599	Flavonoids	HQ	Into_Blood	[34]
3	5,7-Dihydroxy-6-methoxy-2-phenyl-4H-chromen-4-one	$[M+H]^+$	$C_{16}H_{12}O_5$	0.82	0.9991	0.1	285.0755	Flavonoids	HQ	Into_Blood	[35]
4	Chrysin	$[M+H]^+$	$C_{15}H_{10}O_4$	1.16	0.9993	0.2	255.0651	Flavonoids	HQ	Into_Blood	[36]
5	N-Methyltyramine	$[M+H]^+$	$C_9H_{13}NO$	1.48	0.9986	0.3	152.1070	Benzene_and_substituted_derivatives	ZS	None	[37]
6	Tanshinone IIA	$[M+H]^+$	$C_{19}H_{18}O_3$	1.92	0.9965	0.4	295.1328	Prenol lipids	DS	Into_Blood	[38]
7	Cryptotanshinone	$[M+H]^+$	$C_{19}H_{20}O_3$	2.16	0.9941	0.1	297.1485	Prenol lipids	DS	Into_Blood	[38, 39]

Table 1 Characterization of QJHGD constituents by UPLC-HRMS (continued)

No.	Identification	Model	Formula	tR/min	Score	Error/ppm	m/z	Class	Source	Into_Blood OR None	References
8	Isoferulic acid	$[M+H]^+$	$C_{10}H_{10}O_4$	3.74	0.9815	0.6	195.0652	Cinnamic acids and derivatives	DS	Into_Blood	[40]
9	Naringenin	$[M+H]^+$	$C_{15}H_{12}O_5$	4.18	0.9931	0.7	273.0754	Flavonoids	GC, ZS	Into_Blood	[41, 42]
10	Emodin	$[M+H]^+$	$C_{15}H_{10}O_5$	4.36	0.9985	0.3	271.0601	Anthracenes	DH	Into_Blood	[43]
11	trans-Cinnamate	$[M+H-H_2O]^+$	$C_9H_8O_2$	4.53	0.9992	1.2	131.0490	Cinnamic acids and derivatives	DH	None	[44]
12	Eugenol	$[M+H-C_2H_4]^+$	$C_{10}H_{12}O_2$	5.01	0.9938	0.1	137.0595	Phenols	CH, ZS	None	[45, 46]
12	Eugenol	$[M+H-C_2H_4]^+$	$C_{10}H_{12}O_2$	5.01	0.9946	0.2	140.0782	Phenols	CH, ZS	None	[45, 46]
13	L-Lysine	$[M+H]^+$	$C_6H_{14}N_2O_2$	5.59	0.999	0.1	147.1128	Organic acids and derivatives	DS	None	[47]
14	Isoliquiritigenin	$[M+H]^+$	$C_{15}H_{12}O_4$	5.92	0.9932	2.4	257.0800	Linear 1,3-diarylpropanoids	CH, GC	None	[48, 49]
15	Baicalin	$[M+H]^+$	$C_{21}H_{18}O_{11}$	6.46	0.9998	1.4	447.0919	Flavonoids	CH, DS	Into_Blood	[50, 51]
16	Liquiritigenin	$[M+H]^+$	$C_{15}H_{12}O_4$	7.32	0.9985	1.2	257.0807	Flavonoids	GC	None	[34, 52]
17	Gallate	$[M+H]^+$	$C_7H_6O_5$	7.67	0.9256	0.5	171.0288	Benzene and substituted derivatives	DH	None	[43]
18	Glycyrrhetinate	$[M+H-H_2O]^+$	$C_{30}H_{46}O_4$	10.01	0.9844	0.1	453.3362	Prenol lipids	GC	None	[53]
19	Serotonin	$[M+H]^+$	$C_{10}H_{12}N_2O$	10.45	0.9997	0.3	177.1023	Indoles and derivatives	DH	None	[54]
20	Wogonin	$[M+H]^+$	$C_{16}H_{12}O_5$	11.10	0.9985	0.9	285.0750	Flavonoids	HQ	Into_Blood	[34, 55]
21	Nobiletin	$[M+H]^+$	$C_{21}H_{22}O_8$	11.27	0.9977	0.3	403.1385	Flavonoids	ZS	Into_Blood	[42]
22	Tangeretin	$[M+H]^+$	$C_{20}H_{20}O_7$	11.95	0.9869	0.1	373.1280	Flavonoids	ZS	Into_Blood	[42]
23	Scopoletin	$[M-H]^-$	$C_{10}H_8O_4$	0.86	0.9349	0.7	191.0343	Coumarins and derivatives	CH, GC	Into_Blood	[32, 33]
24	Isoliquiritigenin	$[M-H]^-$	$C_{15}H_{12}O_4$	0.88	0.9923	0.2	255.0661	Linear 1,3-diarylpropanoids	CH, GC	Into_Blood	[48, 49]
25	Kaempferol	$[M-H]^-$	$C_{15}H_{10}O_6$	1.08	0.9935	0.9	285.0404	Flavonoids	CH, GC	Into_Blood	[56, 57]
26	Baicalin	$[M-H]^-$	$C_{21}H_{18}O_{11}$	1.31	0.9989	0.3	445.0776	Flavonoids	CH, DS	Into_Blood	[50, 51]
27	Isorhamnetin	$[M-H]^-$	$C_{16}H_{12}O_7$	1.66	0.9978	0.5	315.0510	Flavonoids	CH, GC	Into_Blood	[41, 57]
28	(R)-3-(3,4-Dihydroxyphenyl)- -actate	$[M-H]^-$	$C_9H_{10}O_5$	2.62	0.9938	2.2	197.0445	Phenolic compounds	DS	Into_Blood	[58]
29	3,4-Dihydroxybenzaldehyde	$[M-H]^-$	$C_7H_6O_3$	3.78	0.9976	1.6	137.0240	Organooxygen compounds	DS	Into_Blood	[59, 60]
30	Chrysin	$[M-H]^-$	$C_{15}H_{10}O_4$	3.98	0.9984	1.2	253.0503	Flavonoids	HQ	Into_Blood	[36]
31	Wogonin	$[M-H-CH_3]^-$	$C_{16}H_{12}O_5$	4.52	0.9811	1.6	268.0372	Flavonoids	HQ	Into_Blood	[34, 55]
32	Scutellarin	$[M-H]^-$	$C_{21}H_{18}O_{12}$	4.71	0.9986	0.3	461.0726	Flavonoids	HQ	Into_Blood	[36]
33	Viscidulin I	$[M-H]^-$	$C_{15}H_{10}O_7$	5.06	0.9596	0.7	301.0353	Flavonoids	HQ	Into_Blood	[61]
34	Salvianolic acid A	$[M-H]^-$	$C_{26}H_{22}O_{10}$	5.23	0.986	0.4	493.1141	Stilbenes	DS	Into_Blood	[58]
35	Narirutin	$[M-H]^-$	$C_{27}H_{32}O_{14}$	5.41	0.9953	0.2	579.1719	Flavonoids	ZS	Into_Blood	[42]
36	Naringin	$[M-H]^-$	$C_{27}H_{32}O_{14}$	5.59	0.932	0.7	579.1718	Flavonoids	GC, ZS	None	[42, 62]
37	Glycycoumarin	$[M-H-C_5H_{10}]^-$	$C_{21}H_{20}O_6$	5.92	0.9333	0	297.0381	Isoflavonoids	GC	Into_Blood	[63]
38	Liquiritin	$[M-H]^-$	$C_{21}H_{22}O_9$	6.35	0.9817	0.5	417.1191	Flavonoids	GC	Into_Blood	[49]
39	Formononetin	$[M-H]^-$	$C_{16}H_{12}O_4$	6.47	0.9897	0.7	267.0662	Isoflavonoids	GC	Into_Blood	[49]

Table 1 Characterization of QJHGD constituents by UPLC-HRMS (continued)

No.	Identification	Model	Formula	tR/min	Score	Error/ppm	m/z	Class	Source	Into_Blood OR None	References
40	Maleic acid	[M-H] ⁻	C ₄ H ₄ O ₄	7.24	0.9965	0.3	115.0026	Carboxylic acids and derivatives	DH	Into_Blood	[64]
41	Syringin	[M + HCO ₂] ⁻	C ₁₇ H ₂₄ O ₉	7.31	0.8741	0.7	417.1403	Organooxygen compounds	ZS	None	[65]
42	Wogonoside	[M-H] ⁻	C ₂₂ H ₂₀ O ₁₁	7.67	0.9974	0.6	459.0932	Flavonoids	CH, HQ	Into_Blood	[55, 66]
43	Apigenin 7-O-neohesperidoside	[M-H] ⁻	C ₂₇ H ₃₀ O ₁₄	8.09	0.9978	0.4	577.1567	Flavonoids	ZS	None	[67]
44	Naringenin	[M-H] ⁻	C ₁₅ H ₁₂ O ₅	8.48	0.9976	0.8	271.0610	Flavonoids	GC, ZS	Into_Blood	[41, 42]
45	Physcion	[M-H] ⁻	C ₁₆ H ₁₂ O ₅	8.94	0.9827	0.6	283.0610	Anthracenes	DS	None	[68]
46	Baicalein	[M-H] ⁻	C ₁₅ H ₁₀ O ₅	9.19	0.9986	0.9	269.0456	Flavonoids	HQ	Into_Blood	[34]
47	Poncirin	[M + HCOO] ⁻	C ₂₀ H ₂₄ O ₁₄	10.00	0.9799	0.3	639.1934	Flavonoids	ZS	None	[69]
48	Rhein	[M-H] ⁻	C ₁₅ H ₈ O ₆	10.89	0.9096	0.5	283.0246	Anthracenes	DH	Into_Blood	[43]
49	5,7-dihydroxy-6-methoxy-2-p-henyl-4H-chromen-4-one	[M-H] ⁻	C ₁₆ H ₁₂ O ₅	11.10	0.9919	0.4	283.0610	Flavonoids	HQ	Into_Blood	[35]
50	Emodin	[M-H] ⁻	C ₁₅ H ₁₀ O ₅	12.54	0.9986	0.2	269.0454	Anthracenes	DH	Into_Blood	[43]
51	Scolymoside	[M-H] ⁻	C ₂₇ H ₃₀ O ₁₅	13.24	0.9981	0.8	593.1515	Flavonoids	ZS	None	[70]

Into_Blood OR None: Compounds contained in QJHGD enter into blood or not. QJHGD, Qingjiehuagong decoction.

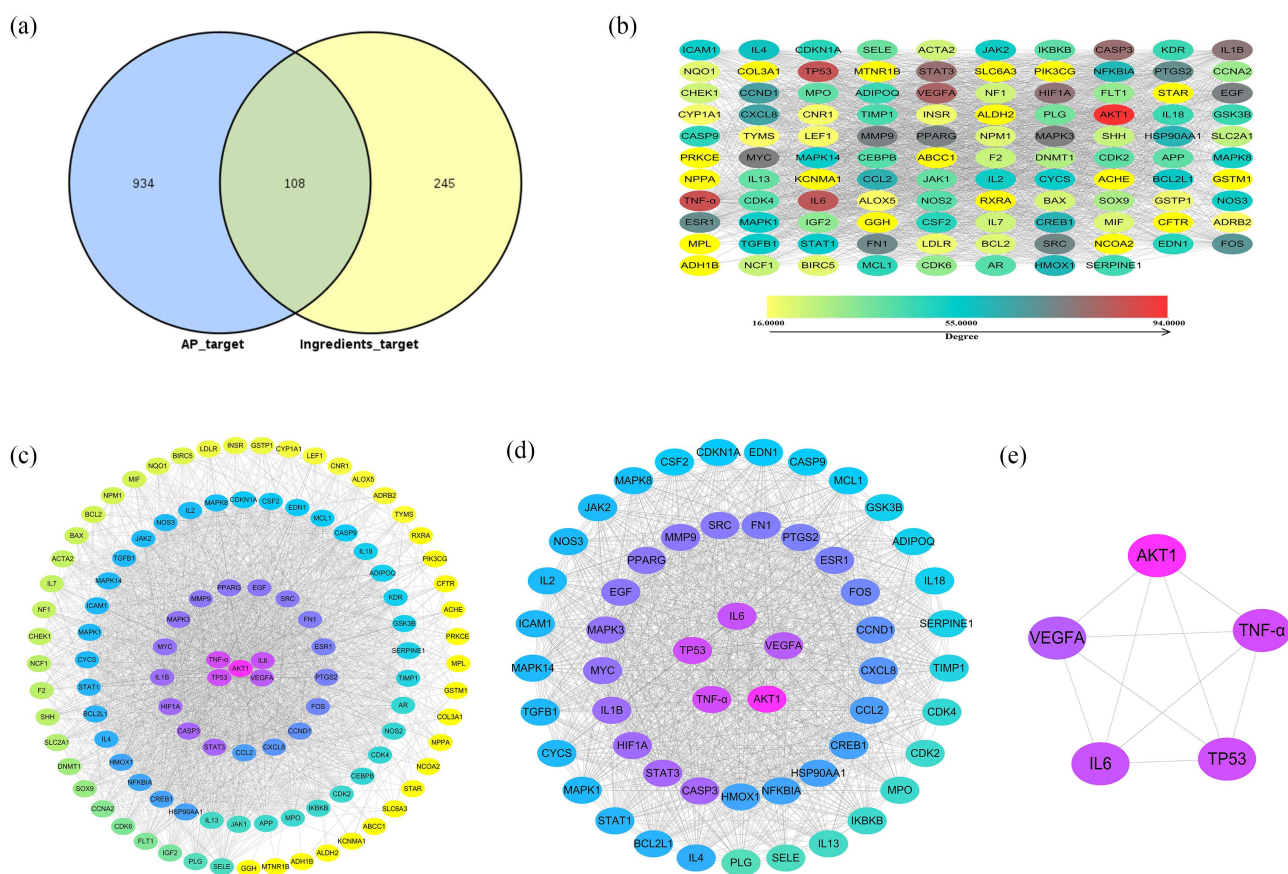


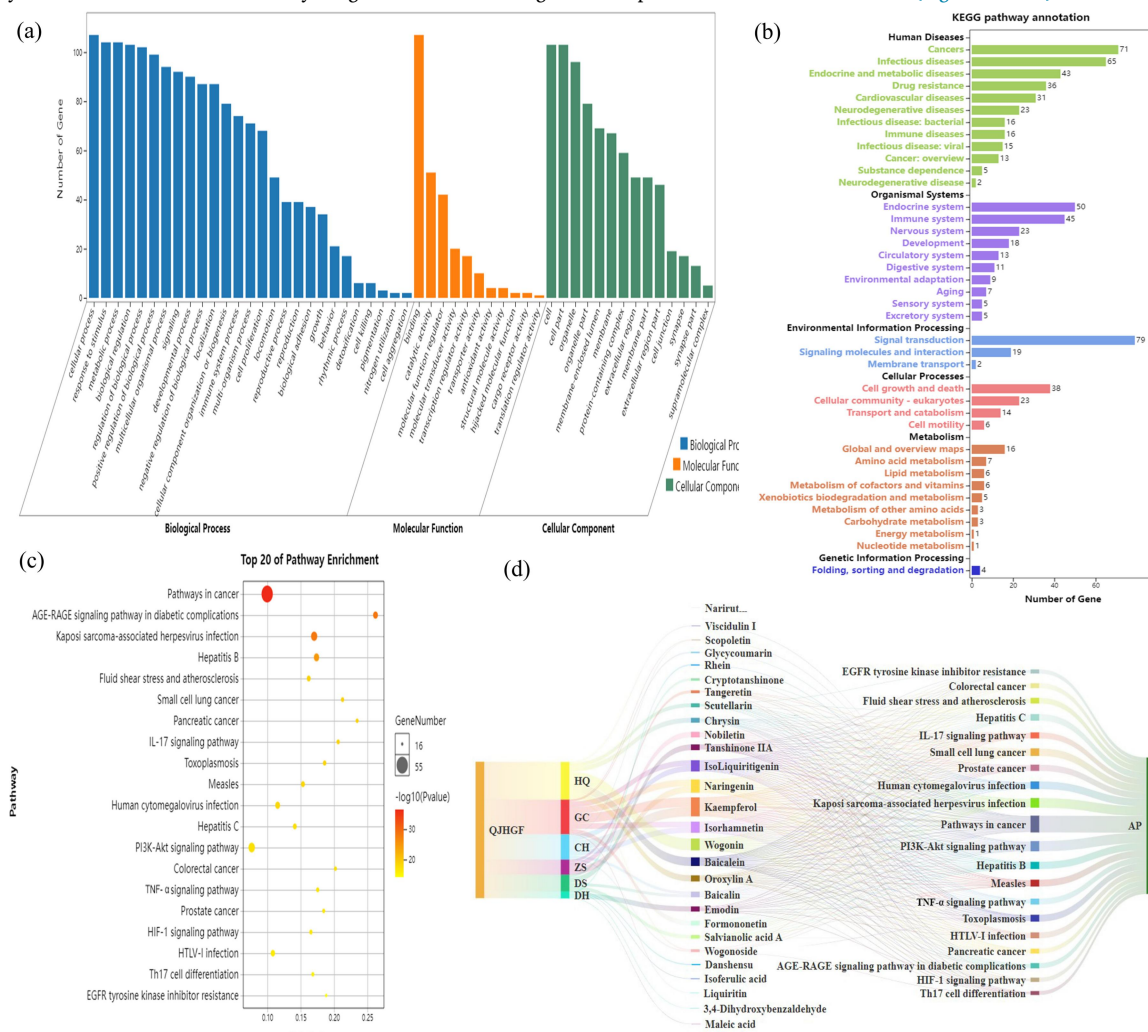
Figure 4 Network pharmacology target analysis. (a) Venn diagram showing the potential bioactive components of QJHGD in blood and AP-related disease targets. (b) PPI network diagram of the overlapping targets. (c) Network core targets based on CytoHubba (circle center). (d) Network core targets based on MCODE (circle center). (e) The overlap between the targets identified by CytoHubba algorithm and MCODE algorithm. AP, acute pancreatitis; QJHGD, Qingjiehuagong decoction; MCODE, molecular complex detection; PPI, protein-protein interaction network.

As shown in [Figure 5b](#) and [5c](#), the top 20 KEGG pathways included human diseases (e.g., infectious diseases and endocrine and metabolic diseases), organismal systems (e.g., immune system and digestive system), environmental information processing (e.g., signal transduction and signaling molecules and interaction), and metabolism (e.g., amino acid metabolism). The IL-17, TNF- α , HIF-1, Th17 cell differentiation, and PI3K-Akt signaling pathways were predicted to play predominant roles in the development of AP. In addition, as shown in the Sankey diagram in [Figure 5d](#), *Glycyrrhiza uralensis* Fisch., *Bupleurum chinense* DC., *Scutellaria baicalensis* Georgi, kaempferol, isoliquiritigenin, baicalin, wogonoside, baicalein, tanshinone IIA, naringenin were predicted to be particularly significant in the mechanism underlying the therapeutic effects in AP.

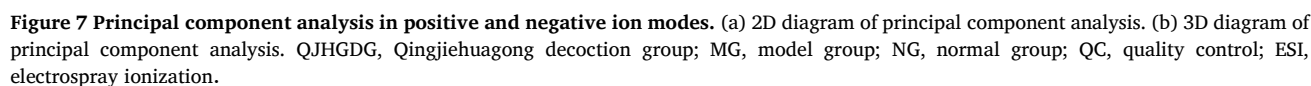
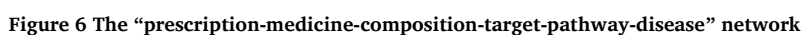
QJHGD-herb-composition-target-pathway-AP network. The QJHGD-herb-composition-target-pathway-AP network shown in [Figure 6](#) had 137 nodes, including QJHGD, 28 compounds in serum, 81 target genes, and the top 20 pathways in AP. Among them, 81 target genes were derived from the group of 108 overlapping genes overlapping with the top 20 pathway genes. The top five components were kaempferol (degree = 31), wogonin (degree = 31), emodin (degree = 23), isorhamnetin (degree = 22), and baicalein (degree = 22). The degrees of four targets [AKT1 (degree = 30), PTGS2 (degree = 27), JAK1 (degree = 22), and CASP3 (degree = 21)] exceeded 20. Pathways that exhibited a relatively high number of target

Results of metabolomics analysis

Multivariate data analysis. Analysis using the supervised OPLS-DA model showed the metabolites in each group were completely separated in both ion modes (Figure 8a–8d). The results of the



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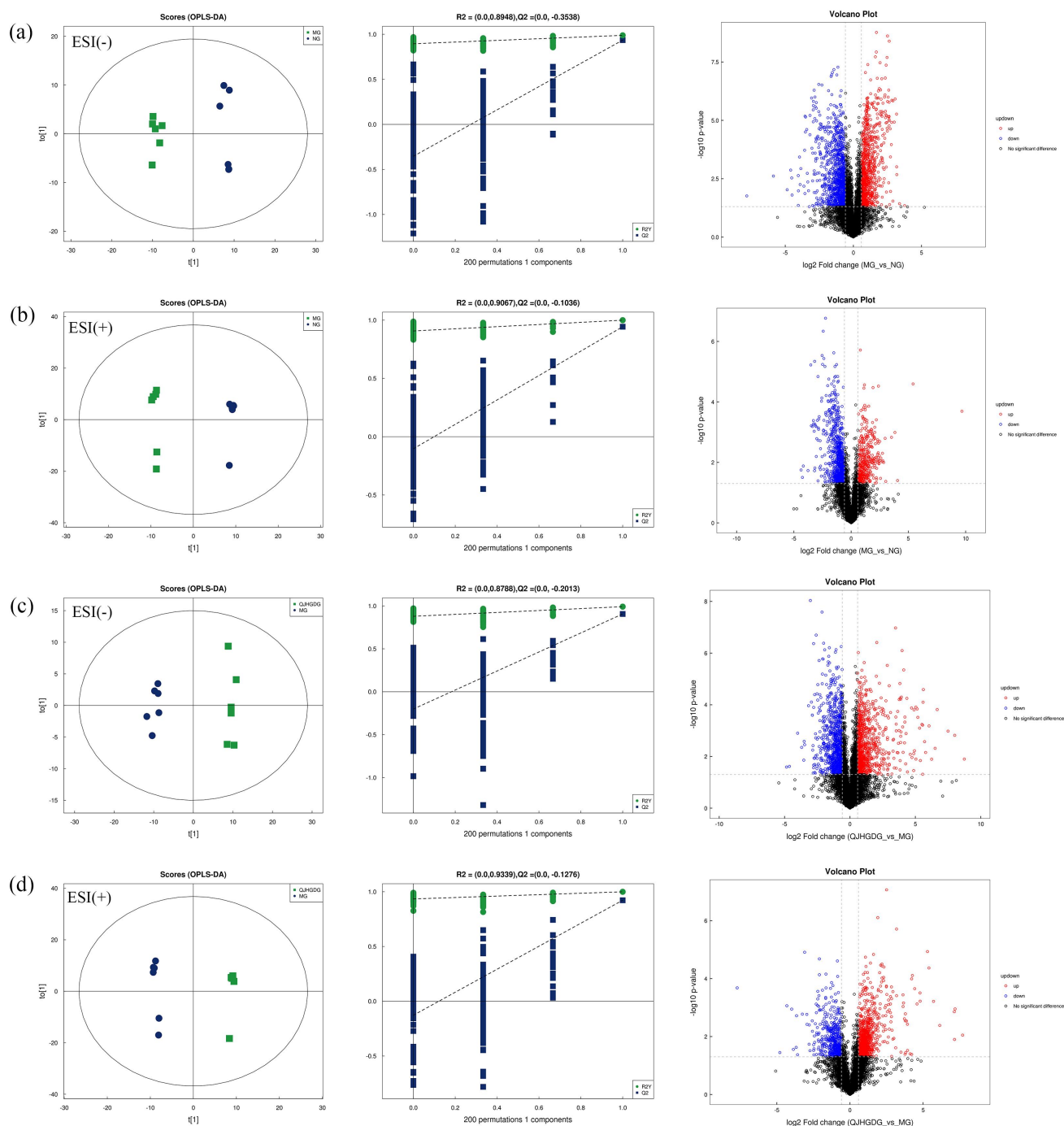


Figure 8 Results of multidimensional OPLS-DA analysis, permutation tests and volcano plots. (a) MG vs. NG in the negative ion mode. (b) MG vs. NG in the positive ion mode. (c) QJHGDG vs. MG in the negative ion mode. (d) QJHGDG vs. MG in the positive ion mode. OPLS-DA, orthogonal partial least squares discriminant analysis; MG, model group; NG, normal group; QJHGDG, Qingjiehuagong decoction group.

orthogonal partial least squares discriminant analysis [(NG versus MG: negative ion mode, $R^2Y = 0.8948$ and $Q^2 = -0.3538$; positive ion mode, $R^2Y = 0.9067$ and $Q^2 = -0.1036$) and (QJHGD versus MG: negative ion mode, $R^2Y = 0.8788$, and $Q^2 = -0.2013$; positive ion mode, $R^2Y = 0.9339$, $Q^2 = -0.1276$)] indicated that the predictive ability of the established model was reliable. Furthermore, Volcano plots revealed significant changes in the levels of multiple different metabolites by comparisons between the NG and MG groups or the QJHGD and MG groups.

Metabolic pathways analysis. In total, 22 significant differential metabolites (VIP scores > 1 and $P < 0.05$) were identified by comparison of the groups in the negative ion mode (Figure 9a). The levels of L-dihydroorotate and phenol were higher in the MG group

than those in the NG group. The levels of hippuric acid, glycocholic acid and dihydroisofuric acid were lower in the QJHGD group than those in the in the MG group. In total, 10 significant differential metabolites were identified by comparison of the groups in the positive ion mode (Figure 9b). Five differential metabolites, including indoleacetic acid, L-thiocitrulline, and niacinamide, were downregulated in the MG compared with the NG group. The levels of metabolites such as dimethyl sulfoxide and D-xylose were significantly upregulated in the QJHGD group compared with the MG group. A total of 32 differential metabolites were finally identified, as shown in Table 2 (Supplementary Table S7).

Moreover, KEGG functional class scoring revealed significantly enriched differential pathways (arginine biosynthesis, pyruvate

metabolism, nicotinate and nicotinamide metabolism) (Figure 9c). In over-representation analysis of the enrichment pathways of metabolites, the top 25 metabolic pathways included starch and sucrose metabolism and D-glutamine and D-glutamate metabolism (Figure 9c). For a more reliable and rigorous enrichment analysis, we considered 13 potential metabolic pathways (impact > 0.01) identified using these two approaches. As shown in Table 3, these were mainly multiple amino acid metabolic pathways such as arginine

biosynthesis, alanine, aspartate and glutamate metabolism, and valine, leucine and isoleucine degradation.

Integrated analysis involving metabolomics and network pharmacology

Compound-reaction-enzyme-gene network. As shown in Figure 10, the compound-reaction-enzyme-gene network constructed using the differential metabolites contained four key metabolic pathways

Table 2 Differential metabolites in serum

NO	Name	Adduct	HMDB	KEGG	Formula	VIP	Fold change	P-value	m/z	rt(s)
1	Trehalose	[M-H] ⁻	HMDB000097 5	C01083	C ₁₂ H ₂₂ O ₁₁	1.135017729	0.24782 5852	4.15297E- 07	341.1 3117	412.25 6
2	L-citrulline	[M-H] ⁻	HMDB000090 4	C00327	C ₆ H ₁₃ N ₃ O ₃	2.757299525	0.49960 9111	4.0391E-0 6	174.0 8006	406.93 45
3	D-fructose	[M-H] ⁻	HMDB006253 8	C00095	C ₆ H ₁₃ O ₉ P	12.08329769	1.49196 142	4.53513E- 05	179.0 4817	306.97 1
4	Hippuric acid	[M-H] ⁻	HMDB000071 4	C01586	C ₉ H ₉ NO ₃	5.370360095	14.7082 8871	6.06968E- 05	178.0 4268	199.60 85
5	D-proline	[M-H] ⁻	HMDB000341 1	C00763	C ₅ H ₉ NO ₂	1.538059289	1.34024 7356	0.000135 586	114.0 5059	317.97 5
6	D-erythro-sphingosine-1-phosphate	[M-H] ⁻	HMDB000027 7	C06124	C ₁₈ H ₃₈ NO ₅ P	1.034269678	1.57037 3295	0.000163 606	378.2 2416	286.71 1
7	4,6-diamino-5-formamidopyrimidine	[M-H] ⁻	HMDB000481 6	C06502	C ₅ H ₇ N ₅ O	1.062073467	0.60091 9572	0.000175 642	152.0 3653	353.10 7
8	Apigenin 7-glucoside	[M-H] ⁻	HMDB003734 0	C04608	C ₂₁ H ₂₀ O ₁₀	1.073218426	0.53234 7247	0.000311 752	431.0 916	169.14 9
9	Glutamine	[M-H] ⁻	HMDB000064 1	C00064	C ₅ H ₁₀ N ₂ O ₃	6.859609727	0.57487 8256	0.000312 312	145.0 554	395.03 7
10	1-stearoyl-2-myristoyl-sn-glycerol-3-phosphocholine	[M + HC O ₂] ⁻	HMDB000803 1	C00157	C ₄₀ H ₈₀ NO ₈ P	3.552518318	2.00137 7186	0.000515 504	778.5 4058	143.24 9
11	Pantothenate	[M-H] ⁻	HMDB000021 0	C00864	C ₉ H ₁₇ NO ₅	4.104537444	0.50024 7061	0.000548 678	218.0 9336	280.98 9
12	N-acetyl-l-glutamate	[M-H] ⁻	HMDB000113 8	C00624	C ₇ H ₁₁ NO ₅	3.557234916	4.93321 9474	0.000817 636	188.0 4758	80.006
13	1-myristoyl-2-palmitoyl-sn-glycerol-3-phosphocholine	[M + Cl] ⁻	HMDB000786 9	C00157	C ₃₈ H ₇₆ NO ₈ P	2.190025332	0.56060 3501	0.002263 445	740.4 8794	149.39 85
14	Phenol	[M-H] ⁻	HMDB000022 8	C00146	C ₆ H ₆ O	7.500949479	0.08838 2689	0.002605 158	93.03 013	28.766 5
15	Hexadecanedioic acid	[M-H] ⁻	HMDB000067 2	C19615	C ₁₆ H ₃₀ O ₄	1.160411221	0.26762 6083	0.004103 894	285.1 9386	207.47 3

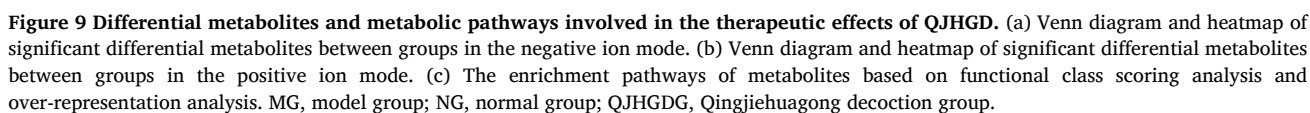
Table 2 Differential metabolites in serum (continued)

NO	Name	Adduct	HMDB	KEGG	Formula	VIP	Fold change	P-value	m/z	rt(s)
16	D-(+)-mannose	[M-H] ⁻	HMDB0062473	C00936	C ₆ H ₁₂ O ₆	1.074218424	1.19689 1466	0.004894 663	179.0 477	278.56 15
17	L-dihydroorotate	[M-H] ⁻	HMDB0003349	C00337	C ₅ H ₆ N ₂ O ₄	5.235827202	0.73938 7081	0.005294 297	157.0 3004	186.57 8
18	Dihydroisoferrulic acid	[M-H-CH ₂ O ₂] ⁻	HMDB0131138	C05619	C ₁₀ H ₁₂ O ₄	4.013963561	428.362 6388	0.012626 149	149.0 5326	101.78 35
19	Glycocholic acid	[M-H] ⁻	HMDB0000138	C01921	C ₂₆ H ₄₃ NO ₆	3.023678252	14.2678 7414	0.014464 457	464.2 8045	258.7
20	Methylmalonate	[M-H] ⁻	HMDB0000202	C02170	C ₄ H ₆ O ₄	1.152860644	0.62761 9944	0.014528 458	117.0 1348	396.27 8
21	Glycochenodeoxycholate	[M-H] ⁻	HMDB0000637	C05466	C ₂₆ H ₄₃ NO ₅	2.109689516	8.04040 4787	0.021470 018	448.2 8619	214.95 55
22	Pyruvate	[M-H] ⁻	HMDB0000243	C00022	C ₃ H ₄ O ₃	5.808363785	1.74172 1506	0.034322 237	87.00 469	135.59 3
23	5-methyl-2'-deoxycytidine	[M+H-C ₅ H ₈ O ₃] ⁺	HMDB0002224	C03592	C ₁₀ H ₁₅ N ₃ O ₄	3.032725179	0.55470 2932	0.006773 463	126.0 6521	201.97 9
24	7.alpha.-hydroxy-4-cholesten-3-one	[M+H] ⁺	HMDB0001993	C05455	C ₂₇ H ₄₄ O ₂	1.784716608	4.01341 132	0.002259 01	401.3 4054	35.656
25	Crimidine	[M+H] ⁺	HMDB0000012	C19138	C ₉ H ₁₂ N ₂ O ₅	1.259108479	0.23131 878	0.007493 947	172.0 3879	114.03 7
26	Dimethyl sulfoxide	[M+H] ⁺	HMDB0002151	C11143	C ₂ H ₆ OS	19.06953973	0.00477 0387	0.000209 007	79.02 047	60.902
27	Indole-3-acetate	[M+H] ⁺	HMDB0003911	C05145	C ₄ H ₉ NO ₂	2.502768407	0.23487 5628	2.08427E- 05	104.0 6966	303.72 4
28	D-xylose	[M+H] ⁺	HMDB0060254	C00181	C ₅ H ₁₀ O ₅	2.834349787	0.52136 9481	0.028321 66	151.0 6019	105.60 1
29	Indoleacetic acid	[M+H-C ₂ H ₂ O ₂] ⁺	HMDB0000197	C00954	C ₁₀ H ₉ NO ₂	1.607345662	4.78060 4717	0.015169 316	130.0 4858	87.562
30	L-thiocitrulline	[M+H-N ₃] ⁺	HMDB0000904	C00327	C ₆ H ₁₃ N ₃ O ₃	1.752169787	6.81548 3563	0.000750 048	175.0 7001	327.75 8
31	Niacinamide	[M+H] ⁺	HMDB0001406	C00153	C ₆ H ₆ N ₂ O	5.876356738	1.94506 021	0.030287 09	123.0 5479	65.452
32	Trimethylamine n-oxide	[M+H] ⁺	HMDB0000925	C01104	C ₃ H ₉ NO	2.744405067	0.12933 0875	0.001714 915	76.07 474	334.50 1

Table 3 The potential metabolic pathways identified in the positive and negative ion modes

No.	Pathway name	Total	Hits	P	Holm P	FDR	Impact
1	Arginine biosynthesis	14	3	0.00076719	0.064444	0.06444	0.22843
2	Pyruvate metabolism	22	1	0.26692000	1.000000	1.00000	0.20684
3	Nicotinate and nicotinamide metabolism	15	1	0.09534500	1.000000	0.90221	0.19430
4	Alanine, aspartate and glutamate metabolism	28	2	0.04611100	1.000000	0.92473	0.11378
5	Glycolysis and Gluconeogenesis metabolism	26	1	0.30750000	1.000000	1.00000	0.10044

No.	Pathway name	Total	Hits	P	Holm P	FDR	Impact
6	Pyrimidine metabolism	39	2	0.10042000	1.000000	0.93729	0.03727
7	Glycerophospholipid metabolism	36	1	0.39980000	1.000000	1.00000	0.09446
8	Pentose and glucuronate interconversions	18	1	0.30627000	1.000000	1.00000	0.07812
9	Starch and sucrose metabolism	18	2	0.02468600	1.000000	0.91729	0.07306
10	Citrate cycle (TCA cycle)	20	1	0.24578000	1.000000	1.00000	0.04634
11	Primary bile acid biosynthesis	46	2	0.13242000	1.000000	1.00000	0.04570
12	Sphingolipid metabolism	21	1	0.25642000	1.000000	1.00000	0.02434
13	Valine, leucine and isoleucine degradation	40	1	0.43334000	1.000000	1.00000	0.02264



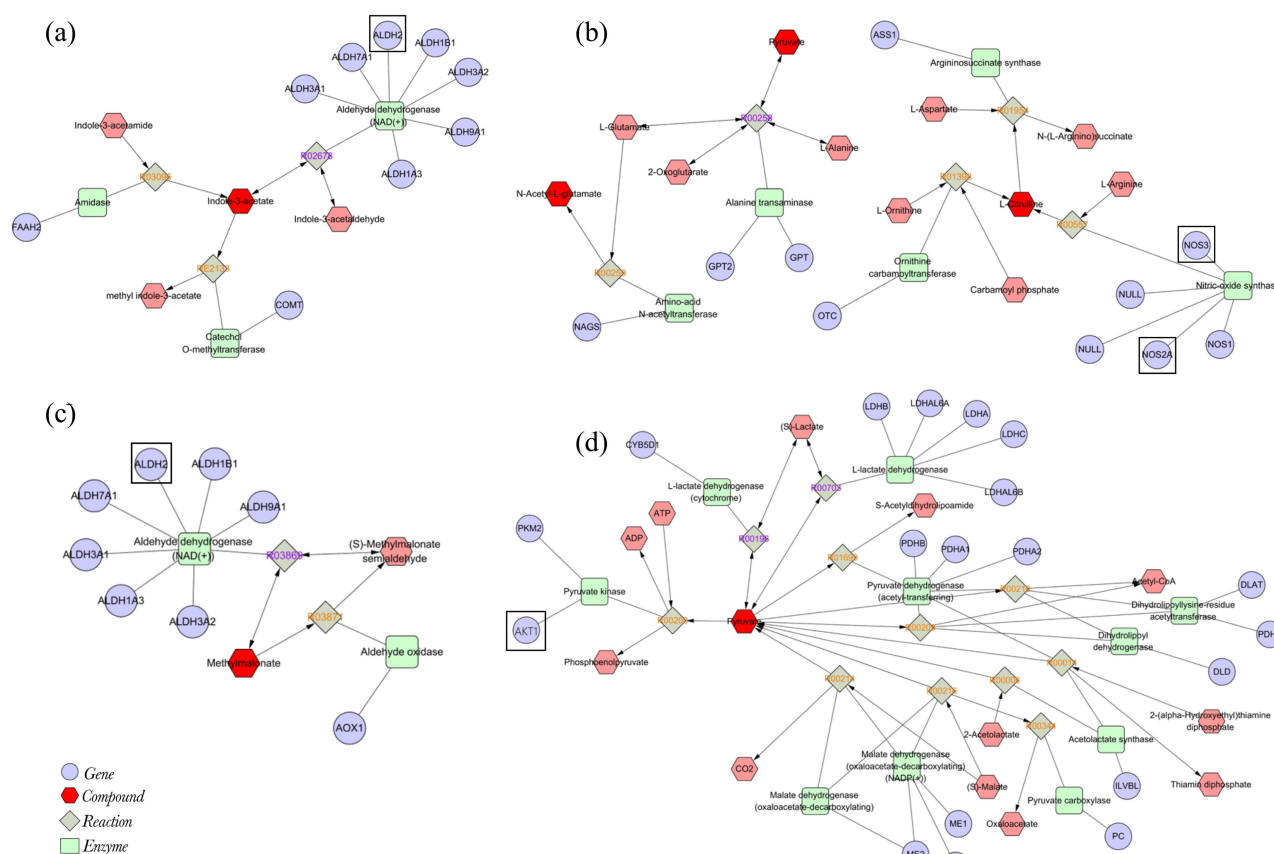


Figure 10 Compound-reaction-enzyme-gene network of key metabolites and targets. (a) Tryptophan metabolism. (b) Urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine. (c) Valine, leucine and isoleucine degradation metabolism. (d) Glycolysis and gluconeogenesis metabolism.

(tryptophan metabolism, glycolysis and gluconeogenesis metabolism, valine, leucine and isoleucine degradation metabolism, the urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine) with five associated key metabolites (indole-3-acetate, pyruvate, methylmalonate, L-citrulline, and N-acetyl-L-glutamate). Subsequently, a simplified interaction network based on our metabolomics and network pharmacology analyses was constructed to systematically understand the potential mechanisms underlying the effects QJHGD in the treatment of AP (Figure 11). Notably, glycolysis and gluconeogenesis and the valine, leucine, and isoleucine degradation metabolic pathways were among the 32 potential metabolic pathways in which metabolites were enriched (Table 4), suggesting that these two metabolic pathways are critical in the treatment of AP by QJHGD. Network pharmacology mapping indicated that the AP-related target genes ALDH2, AKT1, NOS2, and NOS3 play a crucial role in the therapeutic effect of QJHGD in AP through via these metabolic pathways.

Molecular docking. Based on the active components of QJHGD identified by UPLC-Q-TOF MS, molecular docking was performed with the key targets (Table 4) and the four pairs with the strongest binding capacity (Figure 12). AKT1-narirutin, ALDH2-naringenin, NOS2-salvianolic acid A, and NOS3-baicalin had good binding affinity with Libdock scores of 170.869, 128.045, 170.714 and 148.482, respectively (Supplementary Table S8, S9).

Molecular dynamics simulation. On the basis of the molecular docking studies, kinetic simulations of 50 ns were performed for each of the four pairs of protein-ligand complex systems to explore the potential interaction mechanisms within each system. In this study, root mean square deviation was first introduced to evaluate the stability of the complex system, with lower values indicating a more stable system. As shown in Figure 13, all three systems except ALDH2-naringenin reached equilibrium after 50 ns, at which point the

molecular dynamics simulation trajectories were available for further analysis, indicating that the constituted systems were stable. The mean root mean square deviation for the ALDH2-naringenin system ranged from 0.02 to 0.06, which was significantly lower than the ranges for the AKT1-narirutin, NOS2-salvianolic acid A, and NOS3-baicalin systems (0.05–0.2, 0.05–0.35, and 0.1–0.225, respectively). These data indicated the strength of this component anti-inflammatory activity, although this binding equilibrium state required further confirmation by introducing a longer simulation time into the analysis.

The root mean square fluctuation values of amino acid residues in the complex systems were calculated during the molecular dynamics simulations to analyze the relative changes in protein conformational flexibility in the different systems. The four systems had different root mean square fluctuation fluctuation trends and flexible regions. Moreover, the majority of the more flexible amino acid residues were identified as those that formed interactions with the compounds, indicating differences in the interactions of the four active components with their respective binding targets. Thus, the kinetic simulations confirmed the reliability of the molecular docking.

Discussion

AP is an abdominal emergency characterized by abdominal pain, nausea and vomiting, fever, and rapid increases in blood amylase and lipase within a short period of time and can be caused by stone obstruction, excessive alcohol consumption, high fat diet [71]. Mild acute pancreatitis (MAP) is self-limiting and usually resolves within 1 week. However, approximately 20% of patients develop moderately severe acute pancreatitis or severe acute pancreatitis (SAP) with pancreatic or peripancreatic tissue necrosis, organ failure or both and a mortality rate of 20% to 50% [72]. As a common inflammatory

Table 4 The information of key targets, metabolites and pathways

Related pathway	Key target	Key metabolite
Tryptophan metabolism	ALDH2	Indole-3-acetate
Glycolysis and gluconeogenesis metabolism	AKT1	Pyruvate
Valine, leucine and isoleucine degradation metabolism	ALDH2	Methylmalonate
Urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine	NOS2, NOS3	L-citrulline, N-acetyl-l-glutamate, pyruvate

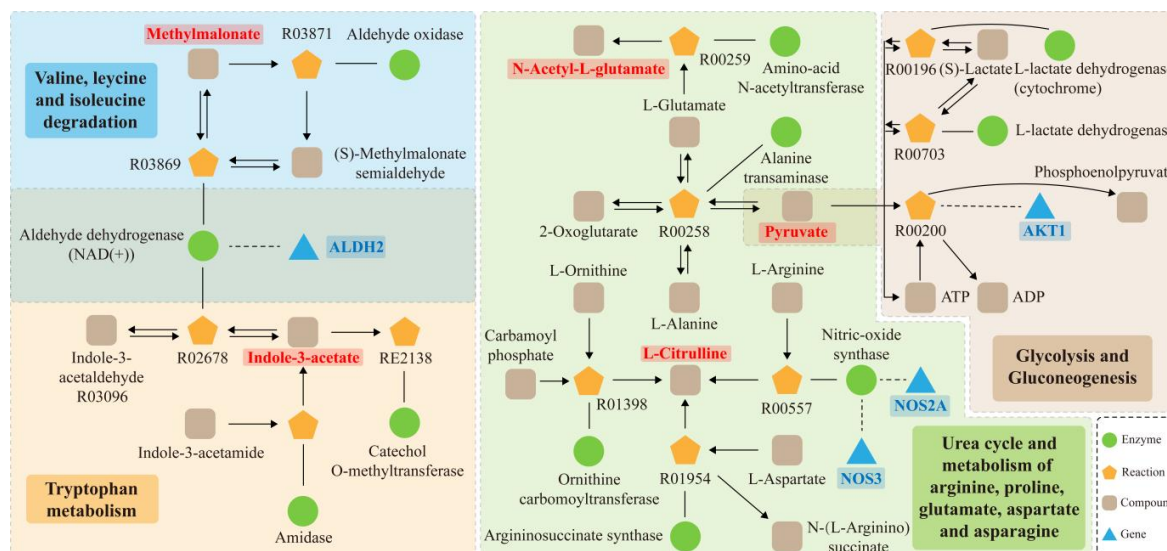


Figure 11 A simplified interaction network of the effects QJHGD in the treatment of AP based on targets, metabolites and metabolic pathways. QJHGD, Qingjiehuagong decoction; AP, acute pancreatitis.

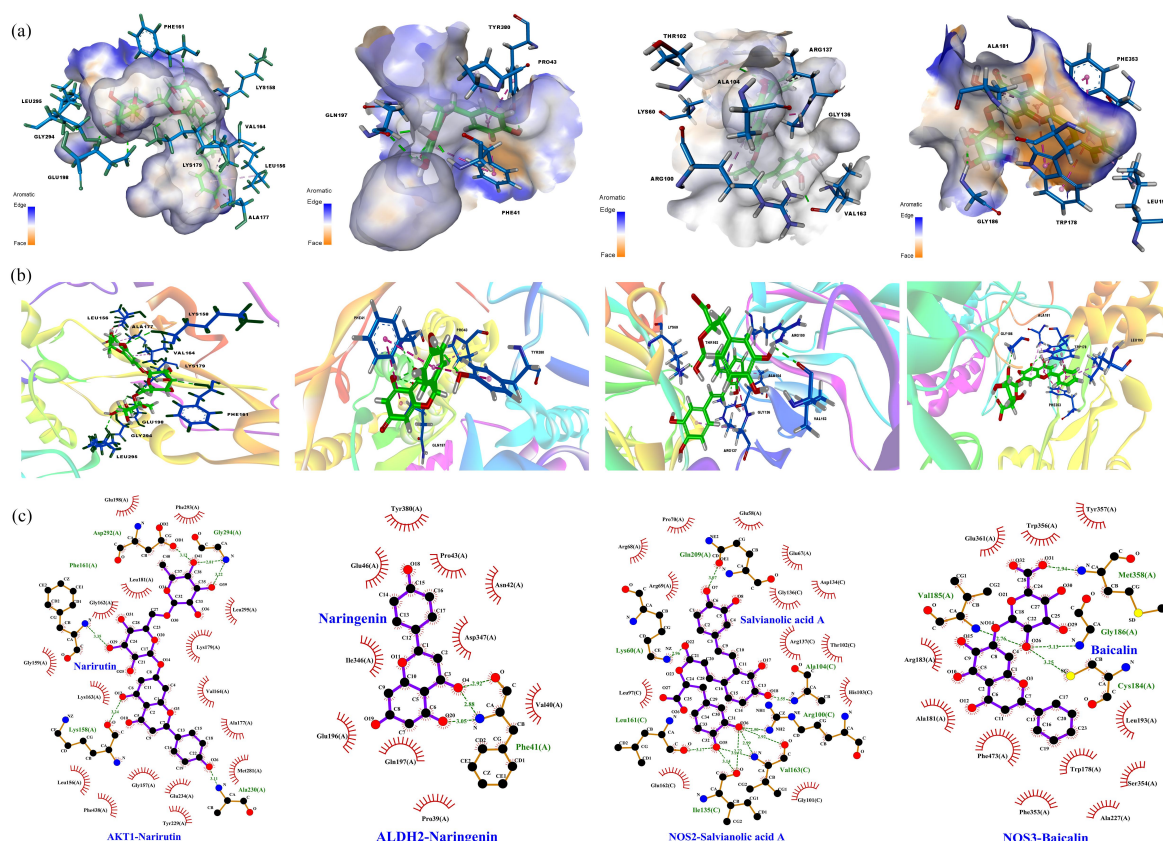


Figure 12 Molecular docking of active components and key target proteins (AKT1-narirutin, ALDH2-naringenin, NOS2-salvianolic acid A, and NOS3-baicalin from left to right). (a) Schematic aromatic 3D diagrams. (b) Schematic diagram of active pocket for target and receptor protein. (c) LigPlus schematic 2D diagrams.

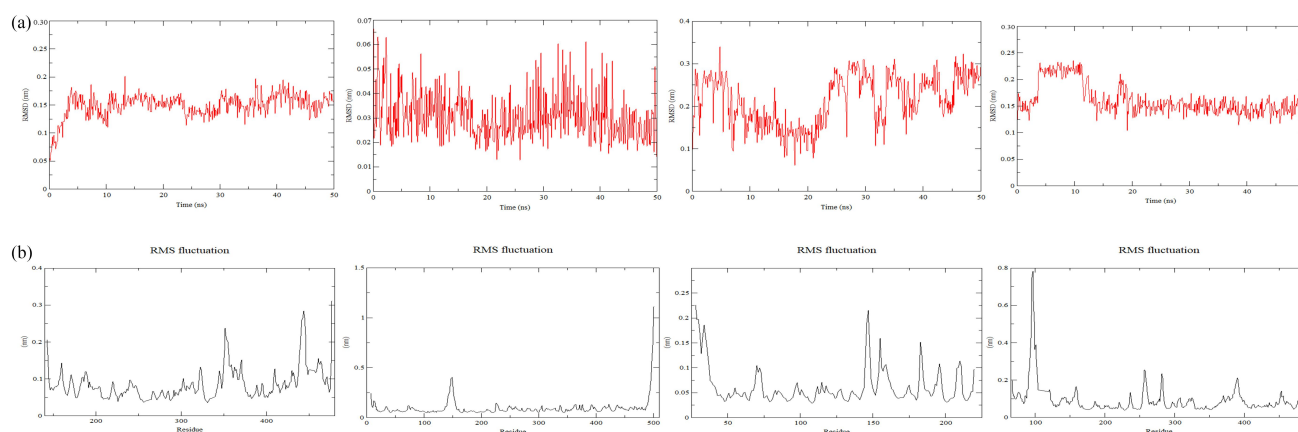


Figure 13 Molecular dynamics simulation of key active components and target proteins (AKT1-narirutin, ALDH2-naringenin, NOS2-salvianolic acid A, and NOS3-baicalin from left to right). (a) Root mean square deviation. (b) Root mean square fluctuation.

disease, the cause and pathogenesis of AP is not fully understood clinically and there are only a few effective therapeutic agents available. Although antisecretory drugs, antioxidants, protease inhibitors, platelet-activating factor inhibitors and anti-inflammatory immunomodulators have been found to reduce the severity of AP, there are limitations to their clinical use [73]. Chinese medicine treats diseases by regulating multiple pathways and targets, with the advantages of fewer side effects and greater holistic properties, which regulate the inflammatory response, greatly improve human immunity and provide effective prevention of diseases and their complications. Chinese herbal medicine has achieved excellent clinical efficacy in the treatment of AP and is being widely used [74].

QJHGD is derived from the ancient classical TCM formula, Xiao Cheng Qi decoction, with the addition of *Bupleuri Radix* and *Fructus Aurantii Immaturus*, which enriches the efficacy of the formula in regulating Qi (the intangible, high-mobility nutritive substance that maintains vital activities) and resolving dampness on the basis of clearing heat and the lower part of the body in response to dampness, heat, depression and knots as the core pathogenesis of early AP according to TCM.

The AP model used in this study was induced by cerulein, a cholecystokinin mimetic with strong stimulatory effects on gallbladder contraction and pancreatic enzyme secretion, leading to trypsinogen activation and self-digestion of the pancreatic tissue. Cerulein-induces AP that is mostly of the edematous type and is used predominantly in studies of MAP and its conversion to SAP [75]. Pany created a novel AP mouse model by injection of cerulein [76]. In this study, pathological analysis of pancreas tissues revealed obvious edema and bleeding, and dense inflammatory cell infiltration. This pathological state appeared to be consistent with the early stage of AP, especially MAP and moderately severe acute pancreatitis, and is therefore an ideal and stable animal model that is convenient to establish with good repeatability. However, herbal prescriptions are used for different patients according to the combination of syndromes and diseases, which means that there is heterogeneity in efficacy for the whole population suffering from a disease. Thus, the cerulein-induced rat model of AP, which is the only animal model that conforms to the characteristics of the clinical disease and syndrome, is a powerful tool to study the essence of this condition [77]. Therefore, we plan further studies to develop a rat AP disease syndrome combination model of response to stress, heat, depression and knots as the core TCM pathogenesis to further clarify the therapeutic effects of QJHGD.

In our pharmacodynamic assessment of the model, traditional histopathological evaluation of pancreatic tissue sections showed that QJHGD effectively alleviated pancreatic edema, inflammatory cell infiltration, and abnormal glandular lobule structure, while the degenerated and atrophied regional glands were repaired and the severity of AP was improved. Furthermore, QJHGD significantly

reduced the expression levels of serum AMS and LPS and inhibited the inflammatory response in AP model rats. As a common diagnostic indicator of AP, serum AMS levels peak within 1–2 days after the onset of pancreatitis and returns to normal levels in around 3–5 days. High AMS levels are associated with pancreatic complications. LPS is a common biochemical indicator of the severity of AP [78]. When the pancreas undergoes an inflammatory response, the permeability of the acinar cells is altered, leading to rapid increases in serum amylase and lipase levels [79]. In addition, the systemic inflammatory response caused by a variety of inflammatory factors is central to the onset and progression of disease. The inflammatory mediator TNF- α induces inflammatory factor production and its levels are positively correlated with the inflammatory response [80]. IL-6, IL-8 and IL-1 β are pro-inflammatory cytokines that also influence AP disease progression. After treatment with QJHGD, we discovered that the blood levels of TNF-, IL-8, IL-6, and IL-1 were dramatically lowered in AP model rats, which was the finding of this particular investigation. This is consistent with our previous study, and confirms the effective therapeutic effect of QJHGD on AP [16, 18].

TCMs contain many active ingredients with their different physicochemical properties. In this study, we used UPLC-HRMS to identify the components of QJHGD in the blood. This information is essential to clarify the effective components and mechanism of action of the formula. Based on our serum pharmacological chemistry analysis, we identified 1,396 chemical components of QJHGD in solution and in serum after administration to AP model rats. These included 103 classes of compounds identified according to the ClassyFire classification [81]. The high abundance peaks in the base peak chromatogram were confirmed by secondary spectrograms before the positive and negative ion diagrams were labeled with the peak numbers in numerical order (Figure 3). As shown in Table 1, 51 peaks were identified in the QJHGD solution, of which 28 peaks were blood-incorporated active components (the peak response intensity in the serum was more than three times higher than that in the blank serum). These active components and their derivatives may play important roles in the anti-AP effects of QJHGD.

The intersection of the 28 active ingredients and AP disease targets contained 108 major targets. AKT1, TNF- α , TP53, IL6 and VEGFA were identified as key targets of QJHGD in the PPI networks. AKT1 is expressed in almost all tissues and protects cells against injury and AKT gene knockout inhibits the protection of cells against apoptosis [82]. Studies have shown that inhibitors of the PI3K-AKT axis downregulate the extent of the inflammatory responses in animal models of MAP and furthermore, genetic ablation or pharmacological inhibition of the PI3K-AKT axis may prevent the tissue damage caused by the inflammatory response through regulation of PI3K-dependent activation of trypsinogen [83]. Small molecule inhibitors of PI3K-AKT signaling have also been shown to dampen the inflammatory response during AP. Systemic reduction in AKT1 activity does not protect the

pancreas from initial injury, but only temporarily delays leukocyte recruitment; however, AKT1 activity reduces acinar cell proliferation and exacerbates acinar-to-ductal cell metaplasia (ADM) [84].

p53 is located on chromosome 17p and functions as a molecular switch for cell survival or apoptosis, regulating DNA repair, cell proliferation and apoptosis, playing an important role in DNA damage-induced apoptosis [85]. When activated, p53 protein accumulates to a level that causes transcriptional activation of pro-apoptotic genes and also represses the transcription of anti-apoptotic genes, thereby inducing apoptosis via the mitochondrial pathway and death receptor pathway. Wei showed that p53 protein expression is closely related to the proliferation and apoptosis of alveolar cells in AP model rats [86]. TNF- α is the main cytokine that causes pancreatic and extra-pancreatic tissue damage in pancreatitis by stimulating the production of oxygen free radicals by inflammatory cells and the release of inflammatory factors such as IL-6 from alveolar cells into the blood. IL-6 is released by monocytes and endothelial cells, stimulates the synthesis of acute response proteins and promotes lymphocyte differentiation and maturation. Clinical studies have shown that the TNF- α and IL-6 levels correlate positively with the severity of AP [87, 88]. In AP, high levels of VEGF family factors, such as VEGFA, are expressed in pancreatic tissues and are significantly elevated in acute necrotizing pancreatitis. These changes reflect increased vascular microcirculatory disturbances in patients with pancreatitis [89].

Using the UPLC-Q-TOF MS metabolomics technique, we identified 32 potential serum biomarkers (22 in the negative ion mode and 10 in the positive ion mode) that were significantly modulated by QJHGD. Using functional class scoring and over-representation enrichment analyses, we identified 13 common metabolic pathways that were potentially to the effects of QJHGD in the treatment of AP. We then integrated the network pharmacology and metabolomics results to map the network of key metabolites, major metabolic pathways and related targets involved in the effects of QJHGD in the treatment of AP. The map contained five key metabolites, including indole-3-acetate, four metabolic pathways, including tryptophan metabolism, and four AP-related targets, including ALDH2.

Some of the metabolites produced by the tryptophan metabolic pathway can enhance oxidative stress, exacerbating pancreatic necrosis and subsequently releasing more inflammatory factors and causing multi-organ failure [90, 91]. Tryptophan metabolites have been shown to cause acute lung injury in rats with AP [92]. However, some metabolites play a positive role in this pathway and inhibit oxidative stress. We found that the tryptophan metabolite indole-3-acetate attenuated the expression of pro-inflammatory cytokines such as TNF- α and IL-1 β [93]. Biczó reported that the pathogenesis of AP in model mice was related to mitochondrial dysfunction and Ca²⁺ overload [94]. However, excessive loss of adenosine triphosphate (ATP) leading to pancreatic acinar cell lesions represents the key mechanism underlying both conditions [95, 96]. Glycolysis and gluconeogenesis are the processes by which glucose is metabolized and synthesized and involve the release of a large amount of ATP [97]. Bruce reported that the released ATP prevented intracellular Ca²⁺ overload and reduced the damage to pancreatic acinar cells induced by mitochondrial dysfunction [98]. Peng found that the administration of pyruvate, which is a metabolite of glycolysis that restores the energy supply, to the AP model significantly reduced the loss of ATP and prevented further necrosis of the pancreas [99]. Additionally, Wiesław-Ziółkowski showed that cerulein-induced AP was effectively prevented in rats by pre-intravenous injection of pyruvate, which may be related to its antioxidant activity [100]. AP is associated with infection, stress and pain, leading to an increase in basal metabolic rate and abnormally high rates of protein or amino acid degradation [101–103]. Yu found that valine, leucine, and isoleucine degradation metabolism pathways play an important role in SAP [104]. Furthermore, serum valine levels have been reported to be significantly reduced in patients with chronic pancreatitis, thus implicating valine as a potential biomarker for early-stage AP [105, 106, 107]. Methylmalonate is a product of the valine, leucine, and

isoleucine degradation pathway [108]. Part of the urea cycle is involved in the induction of the AP process, causing inflammatory cell infiltration and necrosis of pancreatic acinar cells [109]. N-acetyl-L-glutamate is an essential activator of the urea cycle, which plays a critical role in the elimination of toxic substances such as ammonia released in AP [110]. Intraperitoneal injection of excessive L-arginine in rats or mice can cause AP, although the mechanism of pancreatic injury is unclear, although its metabolites (L-ornithine) or L-citrulline, may play key roles [111–113]. Proline has the ability to resist oxidative stress, increase cell viability and inhibit apoptosis [114, 115]. Glutamate regulates the proliferation and/or activation of T cells, macrophages and B cells, and it has been demonstrated that aspartate and asparagine metabolism enhances IL-1 β production from inflammatory macrophages by boosting metabolic remodeling and activating HIF-1 α and NLRP3 inflammasome signaling, which has been shown to induce AP via multiple signaling pathways [116, 117]. Thus, there is compelling evidence that the urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine are involved in the development of AP. These metabolic pathways involve four related targets, including ALDH2, which is an essential enzyme for the process of removing lipid peroxides from the body. It has been reported that ALDH2 may have a protective effect on cerulein-induced AP [118]. Akt1 plays a key role in controlling the proliferation of acinar cells and the formation of acinar-to-ductal metaplasia during the development of AP [119]. While NOS2 and NOS3 are expressed in the pancreas, NOS2 appears only after stimulation by endotoxin or pro-inflammatory cytokines. Furthermore, subtypes of NOS synthesize NO, which is a powerful oxidant and participates in inflammatory reactions [120]. NO has been reported to protect locally injured pancreatic cells through regulation of the pancreatic vascular microcirculation [121, 122]. In conclusion, AKT1, ALDH2, NOS2, and NOS3 were identified as key target proteins of QJHGD, and these four proteins may be key regulators in the pathogenesis of AP.

In this study, we showed that QJHGD may inhibit cerulein-induced AP by protecting pancreatic tissue and suppressing the expression of inflammatory factors. The main active components of QJHGD were identified in LC-MS analysis, which in combination with the network pharmacology and metabolomics analyses showed that QJHGD affects four metabolic pathways (tryptophan metabolism, glycolysis and gluconeogenesis metabolism, valine, leucine and isoleucine degradation metabolism, urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine), five metabolites (indole-3-acetate, pyruvate, Methylmalonate, L-citrulline, and N-acetyl-L-glutamate) and four related targets (AKT1, ALDH2, NOS2, and NOS3) to combat inflammation. Molecular docking and molecular dynamics simulations validated the high affinity of the key targets for QJHGD. However, the following limitations of this study should be noted: (1) the functions of metabolites identified by metabolomics *in vivo* remain to be fully clarified; (2) key targets are required to be validated in experiments; (3) in the GeneCards disease-related gene screening, we set a similarity threshold (correlation score ≥ 11.63538647), which reduced the number of genes included in the network pharmacological analysis, resulting in noise in the gene list, which may not necessarily reflect the true association with the disease; (4) the targets for obtaining potential bioactive components of QJHGD from the TCMSP, ETCM, BATMAN-MAN database, and SwissTargetPrediction database are derived from literature reports and molecular predictions. Given the different credibility of the targets obtained through the two different methods, this study did not differentiate the study and may have some confounding bias.

Conclusion

We showed that QJHGD alleviates AP by affecting key target proteins (AKT1, ALDH2, NOS2, NOS3) to regulate multiple biological and metabolic pathways. This study provides experimental evidence of the therapeutic potential of QJHGD in AP in addition to a deeper understanding of the underlying mechanism. Thus, our findings indicate that QJHGD is suitable for development as a complementary

treatment of AP.

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