Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Analysis of Blood Components and Target Prediction for the Combined Use of *Dendrobium officinale* Compound and Western Medicine in Antihypertensive Therapy Based on Network Pharmacology and Molecular Docking

Renzhao Wu[1,2,*], Jie Wang[1,*], Huifang Zhou[1,*], Xiaomin Xue[2,*], Cheng Tong[3,*], Lisha Zhao[2], Yue Wu[4], Xiyu Mei[2], Zhongxiu Guo[2], Caicai Xi[5]

[1]School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, People's Republic of China; [2]Institute of Basic Medicine, Zhejiang Academy of Traditional Chinese Medicine, Hangzhou, People's Republic of China; [3]Department of Laboratory Medicine, Linping Campus, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [4]Department of Cardiology Medicine, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou, People's Republic of China; [5]Clinical Pharmacy Department, Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Caicai Xi, Email xicaicaizyhx0122@163.com

Aim of the Study: To investigate the primary monomer components and the associated mechanisms of action of DOC in exerting antihypertensive effects with Western medicine.

Materials and Methods: In this study, UPLC-Q-TOF/MS technology was used to analyze the blood components of DOC and its interaction with Western medicine. The antihypertensive effects of serum monomer components were also evaluated. Blood pressure measurements for experimental animals were recorded before administration, at three hours and twenty-four hours after the first administration, as well as the same time of the last administration of the treatment cycle. Meanwhile, the antihypertensive mechanism was explored through the integration of network pharmacology and molecular docking technology.

Results: Blood component analyses revealed the identification of five compounds in the serum: Kaempferide, Rutin, Syringaldehyde, Paeoniflorin, and Hyperoside. In terms of serum drug concentrations, Irbesartan and Amlodipine concentrations were multiplied by 1.36 and 0.56, respectively, following the combination therapy of Chinese and Western medicine compared with the Western medicine alone group. The concentrations of Paeoniflorin, Kaempferin, and Syringaldehyde in the combination group were significantly higher than those observed in the DOC group. The results from pharmacodynamic experiments indicated that the antihypertensive effect achieved through the combined application of Chinese and Western medicine on SHRs was superior to that resulting from the dual administration of two types of Western medicines alone.

Conclusion: The combination of DOC with two Western medications has been shown to significantly enhance antihypertensive effects in SHRs. Furthermore, five major monomers present in the serum of experimental rats post-treatment exhibit notable antihypertensive properties.

Keywords: spontaneously hypertensive, seropharmacology, network pharmacology, *Dendrobium officinale*, *Paeonia lactiflora*

Introduction

According to the WHO, hypertension (high blood pressure) is when the pressure in your blood vessels is too high (140/90 mmHg or higher) or ongoing antihypertensive medication, and it is one of the most prevalent chronic diseases affecting the cardiovascular system. This condition can impair the structure and function of vital organs such as the

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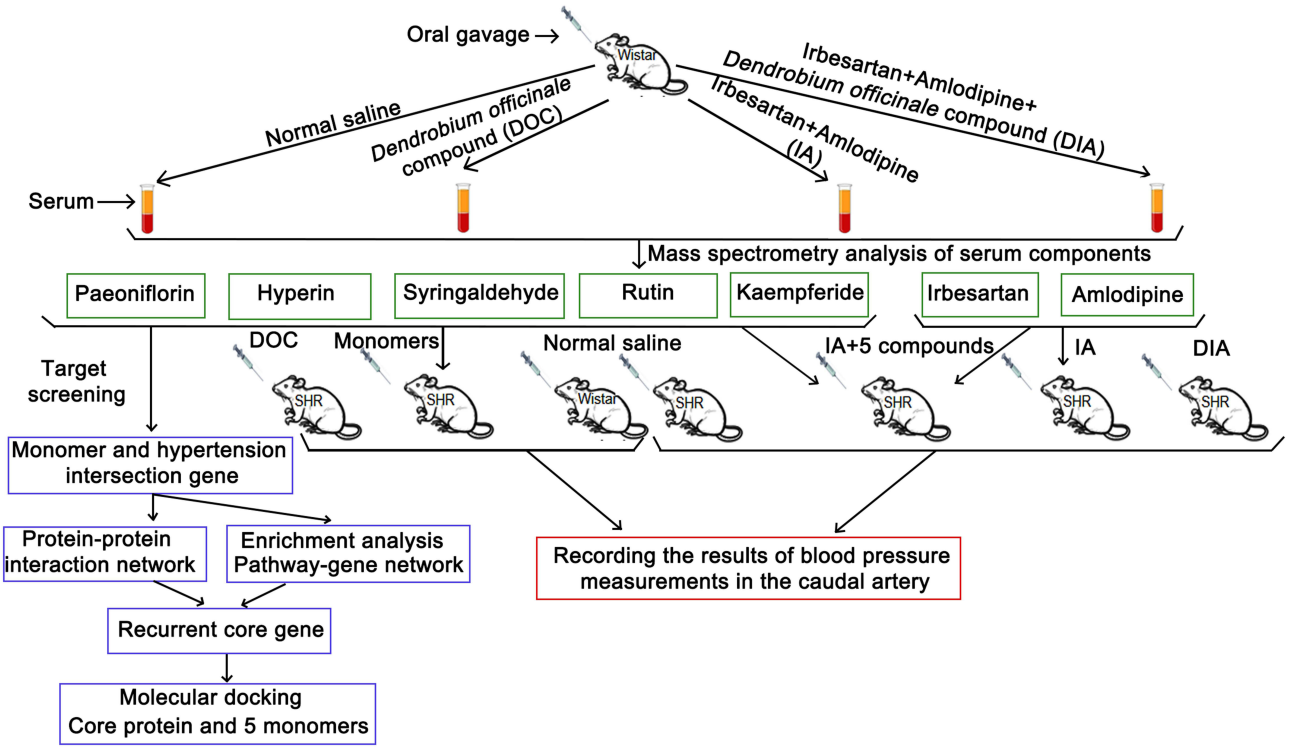
Graphical Abstract



heart, brain, and kidneys, leading to severe complications including cerebral hemorrhage, coronary heart disease, and renal failure. Hypertension has become a major risk factor for cardiovascular diseases globally, significantly contributing to morbidity and mortality rates associated with these conditions.[1] The global prevalence of hypertension is rising annually. Some researchers predict that by 2025, the number of individuals affected by hypertension worldwide will reach 1.5 billion. Consequently, the risk of associated diseases and the severity of their impact are expected to increase as well.[2] Although the development of antihypertensive medications has allowed some patients to benefit from a combination of various Western drugs tailored to individual differences, evidence indicates that approximately 10% of patients fail to achieve adequate blood pressure control despite the use of more than three medications.[3] This condition is referred to as refractory hypertension. Furthermore, studies have demonstrated that 85% of patients experience adverse drug effects, which may include drug resistance and significant blood pressure fluctuations.[4] Additionally, some research has suggested that the long-term use of angiotensin-converting enzyme inhibitors (ACEIs) may be associated with an increased risk of lung cancer.[5] Therefore, exploring innovative methods and new targets for the treatment of hypertension has become an urgent need to control the current situation of global hypertension prevention and treatment.

Traditional Chinese medicine (TCM) has been demonstrated to significantly influence the management of hypertension. TCM and its associated compounds exhibit characteristics of multi-component, multi-pathway, and multi-target synergistic effects, enabling them to regulate blood pressure through a variety of distinct mechanisms.[6] Research has indicated that the integration of TCM with various classes of Western antihypertensive medications can enhance the synergistic effects of these drugs on blood pressure regulation.[7,8] This combination is beneficial for effective blood pressure control and presents significant prospects for further investigation. In our previous study, we demonstrated that *Dendrobium officinale* Kimura et Migo exhibited a stable and prolonged antihypertensive effect in spontaneously hypertensive rats (SHRs), accompanied by favorable safety profiles. Further studies have demonstrated that the antihypertensive effect of *Dendrobium officinale*

compound (DOC, with *D. officinale* and *Paeonia lactiflora* Pall. Reise as the primary ingredients) in combination with Irbesartan and Amlodipine is superior to that of these two Western medications when administered to SHRs. DOC formulated from *D. officinale* and *P. lactiflora* has been successfully developed using modern technology; however, the specific blood components and material basis underlying its pharmacological action remain unclear. This study aimed to analyze the principal blood components in SHRs following treatment with the DOC combined with Irbesartan and Amlodipine, and the specific efficacy of the measured single components was also verified by animal experiments and blood composition analyses. This analysis aims to elucidate the antihypertensive effects exerted by these blood components on SHRs and explore their gene targets through network pharmacology, thereby providing new insights into potential therapeutic targets for hypertension management.

Network pharmacology is an emerging discipline that integrates systems biology and network informatics. It leverages extensive biological information databases to investigate the pathogenesis of diseases and the mechanisms of drug action.[9] Due to the complex composition of TCM, its action targets are numerous, and it interacts with various signaling pathways. This complexity presents challenges for a deeper exploration of the mechanisms underlying TCM's effects. However, network pharmacology can be employed to analyze the impact of multi-component drugs on the human body at a systemic level, which aids in identifying therapeutic targets for the effective components of these medications.[10] Molecular docking is another technique that has gained popularity based on the rise of cyberpharmacology, which is capable of modeling molecule-protein interactions at the atomic level.[11,12] In this study, we intend to conduct an in-depth analysis of the major blood entry components in SHR rats after treatment with compound dendrobium iron combined with irbesartan and amlodipine benzenesulfonate, to clarify the antihypertensive effects of these blood entry components on SHR rats, to predict the relevant targets of action of DOC in combination with network pharmacology and molecular docking technology, to elaborate the functional components and biochemical pathways, and to draw clearer boundaries between the biological bases of hypertension, providing new directions for exploring the therapeutic targets of hypertension, as well as further deepening the understanding of how to regulate these targets.

Materials and Methods

Equipment and Materials

Equipment

SCIEX X-500R four-stage pole time-of-flight mass spectrometer (AB SCIEX, USA); TurboIonSpray Ion source (AB SCIEX, USA); Waters ACQUITY I-Class Plus UPLC ultra-performance liquid chromatography system (Waters Inc., USA); QTRAP5500+ triple quadrupole mass spectrometer (AB SCIEX, USA); Thermo ST40R cryogenic high-speed centrifuge (Thermo Fly, USA); IKA micro Vortex Mixer (Aika, Germany); AUW220D electronic balance (Shimadzu Co., Japan); CentriVap anti-acid centrifugal concentrator (LABCONCO, USA); Centrifuge 5810R high-speed freezing centrifuge (Eppendorf, Germany); KQ-400DE CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., LTD).; BP-98A non-invasive tail artery blood pressure measuring instrument (Japan Soft and Soft Co., LTD). DK-450B electric thermostatic water tank (Shanghai Senxin Experimental Instrument Co., LTD).

Reagents

Methanol, acetonitrile and formic acid (Merck Company, Germany), Milli-Q ultrapure water (Millipore Company, USA), and other reagents were analytically pure.

Experimental Drugs

DOC (specification: 1.5g/ tablet), each gram containing about 0.777 g compound crude drugs. Batch number: 201905002, provided by Zhejiang Academy of Traditional Chinese Medicine. DOC was composed of *D. officinale* and *P. lactiflora* in a 4:3 ratio, and the powdered form was mixed with excipients and pressed into tablets. Irbesartan tablets (specification: 0.15 g/ tablet, batch number: 20042611, 22013911) were provided by Hanhui Pharmaceutical Co., LTD.; Amlodipine tablets (specification: 5 mg/ tablet, batch number: 200751724, 220210534) were provided by Suzhou Donorui Pharmaceutical Co., LTD. Control substance of the qualitative experiment: Hydroxytyrosol (batch number: 170038–202112, mass fraction 98.11%), Corilagin (batch number: 110046–202109, mass fraction 98.11%), The content by mass fraction: is 100%), Fumaric acid (batch number:

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060020–20111, content by mass fraction: is 99.01%), Kaempferide (batch number: 190035–202111, content by mass fraction: 99.49%), Isoquercitrin (batch number: 250027–202111, content by mass fraction: 99.24%), Loureirin A (batch number: 120036–202111, content by mass fraction: 99.13%), Syringaldehyde (batch number: 040058–202112, content by mass fraction: 99.03%), Rutin (batch number: 120025–202104, content by mass fraction: 98.41%), Hyperoside (batch number: 100003–202110, mass fraction 98.4%) were purchased from Shanghai Hongyong Biological Technology Co., LTD. Paeoniflorin reference substance (batch number: 110736–202044, content by mass fraction 96.8%), Irbesartan (batch number: 100607–201804, content by mass fraction 100%); Amlodipine reference substance (batch number: 100374–201605, mass fraction 100%) were purchased from China Institute for Food and Drug Control. Efficacy test samples: Kaempferide (batch number: RFS-S06411912010, mass fraction 99.64%), Syringaldehyde (batch number: RDD-D04411801008, content mass fraction: 99.96%), Rutin (batch number: RDD-L00111804026, content by mass fraction: 98.17%), Hyperoside (batch number: RDD-J01202203011, content by mass fraction: 98.17%), and Paeoniflorin (batch number: RDD-S01002101022, content by mass fraction 99.43%) were purchased from Chengdu Remifensi Biotechnology Co., LTD.

Chromatographic and Mass Spectrometric Conditions

Chromatographic conditions: ACQUITY UPLC ® HSS T3 (100×2.1 mm, 1.8 μm) column, mobile phase was 0.1% formic acid acetonitrile (A) −0.1% formic acid water (B), gradient elution program: 0 ~ 1min, 99%–99%B; 1–14 min, 99%–50%B; 14–15 min, 50–40% B; 15–15.5 min, 40–1% B; 15.5–18 min, 1% B; 18.1–21 min, 99%B; Flow rate: 0.3 mL/min; Sample plate temperature: 8 °C; Column temperature: 40 °C; Injection volume: 4μL.

Mass spectrometric conditions: Waters time-of-flight mass spectrometer (Q-TOF/MS) was used. Time-of-flight mass spectrometry was performed with a TurboIonSpray ion source in the ESI-positive and -negative ion scan modes. The specific conditions were as follows: atomizing gas and heating gas 55 and 45 psi, curtain gas (CUR) 35 psi, ion source temperature 600 °C, source injection voltage (ISVF) 5500 V/-4500 V (positive and negative ion modes). The TOF MS scanning range (m/z) was 50–1500 Da. Secondary MS spectra were obtained using Multiple Reaction Monitoring (MRM), and the MRM parameters are shown in Table 1.

Data Analysis

Data were analyzed using SPSS 25.0 software. The one-way analysis of variance (ANOVA), LSD-t, and Dunnett T3 were used for normally distributed data, the independent-sample *t*-test was used for skewedly distributed data, and the Wilcoxon rank sum test was used for skewedly distributed data. Data were presented as the mean ± standard deviation ($\overline{X} \pm S$), and $P < 0.05$ was considered statistically significant. SCIEX OS software was used to collect and process the data. SCIEX OS software includes multiple confidence criteria, including mass accuracy, retention time, isotope, and matching use of the compound library. Based on the exact primary mass number, the isotope distribution ratio, and MS/MS of the compounds, the TCM MS/MS Library (which contains more than 1000 secondary data of TCM compounds) in SCIEX OS can be used to complete the screening of the target substances without the standard substance.

Table 1 MRM Parameters of Serum Detection Components

Index	Component Name	Formula	Precursorion (Da)	Fragment ion (Da)	Retention Time (min)	DP	CE
1	Kaempferide	$C_{16}H_{12}O_6$	285.10	117.00	12.02	−110	−53
2	Rutin	$C_{27}H_{30}O_{16}$	609.1	271.00	7.53	−110	−77
3	Syringaldehyde	$C_9H_{10}O_4$	181.1	151.00	7.64	−50	−28
4	Paeoniflorin	$C_{23}H_{28}O_{11}$	479.1	121.00	6.94	−88	−27
5	Hyperoside	$C_{21}H_{20}O_{12}$	463.1	300.00	7.67	−110	−40
6	Irbesartan	$C_{25}H_{28}N_6O$	427.10	193.00	12.5	−103	−38
7	Amlodipine	$C_{20}H_{25}ClN_2O_5$	409.00	238.00	11.65	80	30

Sample Preparation

Handling of Serum Samples

One thousand microliter serum samples were taken from each animal from the normal group, 2 Western medicine group, compound group, and 2 Western medicine + compound group, respectively. A 20 μL aliquot of phosphoric acid was added, sonicated for 1 min, and vortexed and mixed for 30s. The samples were loaded on the SPE column which was pre-balanced with 3 mL methanol and 3 mL water activation, eluted with 3 mL of water, discarded, and eluted with 3 mL of methanol. The eluate was collected and lyophilized in a lyophilization machine. The residue was redissolved in 100 μL of methanol, centrifuged at 14000 rpm for 20 min at 4 °C, and the supernatant was collected and continuously injected 3 times.

Sample Processing of DOC

Then, 2.0392 g of DOC were finely weighed, dissolved in 25 mL of 70% methanol, sonicated at 70 °C for 45 min, and left to stand until the samples were stratified. Then, 1 mL of the supernatant was taken and centrifuged at 14000 r for 20 min at 4 °C, after which the supernatant was collected and the sample was continuously injected 3 times.

Preparation of the Standard Working Solution

One milligram each of Kaempferide (KF), Rutin (RU), Syringaldehyde (SY), Paeoniflorin (PA), Hyperoside (HYP), Irbesartan (IR), and Amlodipine (AM) was weighed precisely, and 1 mL of methanol was added to make a 1 mg/mL mother liquor. Different amounts of mother liquor were prepared and finally mixed with 1 mL of 350 ng/mL, 260 ng/mL, 300 μg/mL, 42 μg/mL, 860 ng/mL, 500 ng/mL, and 5 μg/mL (Working Solution 7). Then, 200 L of Working Solution 7 was added to 800 μL of methanol solution and mixed to make Working Solution 6, which was diluted in turn to get Working Solutions 5, 4, 3, 2, and 1. A series of standard Working Solutions with KF concentrations of 0.00112 ng/mL, 0.0056 ng/mL, 0.028 ng/mL, 0.14 ng/mL, 0.7 ng/mL, 3.5 ng/mL, and 17.50 ng/mL were obtained. The RU concentrations were 0.000832 ng/mL, 0.00416 ng/mL, 0.0208 ng/mL, 0.104 ng/mL, 0.52 ng/mL, 2.6 ng/mL, and 13.00 ng/mL, respectively. The SY concentrations were 0.96 ng/mL, 4.8 ng/mL, 0.024 g/mL, 0.12 μg/mL, 0.6 μg/mL, 3.00 μg/mL, and 0.015 mg/mL, respectively. The concentrations of PA were 0.1344 ng/mL, 0.672 ng/mL, 3.36 ng/mL, 16.8 ng/mL, 0.084 μg/mL, 0.42 μg/mL, and 2.1 μg/mL, respectively. The concentrations of HYP were 0.002752 ng/mL, 0.01376 ng/mL, 0.0688 ng/mL, 0.344 ng/mL, 1.72 ng/mL, 8.6 ng/mL, and 0.043 μg/mL, respectively. The IR concentrations were 1.6 ng/mL, 8.00 ng/mL, 0.040 μg/mL, 0.200 μg/mL, 1.00 μg/mL, 5.00 μg/mL, and 0.025 mg/mL, respectively. The concentrations of AM were 0.016 ng/mL, 0.08 ng/mL, 0.4 ng/mL, 2 ng/mL, 0.01 μg/mL, 0.05 μg/mL, and 0.25 μg/mL, respectively. As the reserve solution for standard curve investigation, the supernatant was centrifuged at 14000 rpm for 20 min at 4 °C, and the supernatant was stored in the refrigerator at 4 °C with the numbers 1, 2, 3, 4, 5, 6, and 7 for further use.

Preparation and Processing of Standard Curve Working Solution

Take 50 μL blank serum into a 1.5 mL centrifuge tube, add 10 μL of the standard working solution with different mass concentrations and 140 μL methanol: Acetonitrile (1:1) was vortexed for 1 min, centrifuged at 14000 r/min for 20 min, the supernatant was collected, lyophilized, redissolved with 100 μL methanol, vortexed and mixed, centrifuged at 14000 r/min for 20 min, and the supernatant was removed to obtain seven standard curve samples.

Repeatability Experiment, Precision Experiment, Sample Recovery, and Matrix Effect Experiment

Six parallel samples of the standard curve working solution 7 were made repeatedly, each injection was made, and the RSD value was calculated. Sample 3 was injected continuously for six needles to calculate the RSD value. Three concentrations of the standard working solution (1, 4, and 7) were selected as the low-, medium-, and high-concentration samples. Sample 1: refer to the content mentioned above for preparation; Sample 2: 10 μL of 1, 4, and 7 standard working solution was added to 190 μL of blank serum supernatant (serum supernatant: blank serum: (methanol/acetonitrile =1:1) =1:3, and the supernatant was centrifuged); Sample 3: 10 μL 1, 4, 7 standard working solution was added to 190 μL methanol/acetonitrile = 1:1 solution, the above three samples were made 18 samples, and 6 parallel samples were made at high, medium, and low doses, a total of 54 samples. The peak area of each sample was recorded, and the sample recovery rate = $A_{\text{sample 1}}/A_{\text{sample 2}}$; Matrix effect ratio = $A_{\text{sample 2}}/A_{\text{sample 3}}$.

Animal Experiment Design

In this experiment, the determination of the drug content in DOC was based on results obtained from three repeated tests of the same sample. The blood component experiment and pharmacological efficacy experiment were conducted independently, utilizing separate groups of rats (six per group) for each experiment. This study adhered to the "3R" principles of humane care for all animal experiments involved. All experimental animals were SPF-grade male rats, housed in the SPF barrier laboratory of Zhejiang Chinese Medical University Research Institute【SYXK (Zhe) 2019–0010】. The environmental conditions were maintained at a temperature of 22°C to 26°C, humidity levels between 40% and 70%, and a light/dark cycle of 12 hours (light from 7:00 AM to 7:00 PM). The dosage selection for DOC was based on clinical experience regarding its effects on humans, as well as calculated using the body surface area conversion formula: [DB] = [DA] × K, where [DA] represents the human dosage and [DB] denotes the rat dosage. The value of K is the equivalent dose ratio based on body surface area between humans and rats. Specifically, for a human weighing 70 kg (K = 0.018) and a rat weighing 200 g (K = 1.0). The dosage selection for individual components was determined through preliminary experiments; when the concentration of an individual component reached a level that produced therapeutic effects comparable to those of DOC, this concentration was adopted as the experimental dosage for animals in this study.

Blood Component Experiment

Twenty-four Wistar rats (all male, 330—350 g) were provided by Zhejiang Weitong Lihua Laboratory Animal Technology Co., LTD. Tongxiang Branch, Laboratory animal production license number: SCXK (Zhejiang) 2021–0006; License number: SYXK (Zhejiang) 2019–0010; Qualified certificate number: 20211105Aazz0600067744. All experiments involved in this study were approved by the Experimental Animal Welfare Ethics Committee of Zhejiang Academy of Traditional Chinese Medicine (approval number: [2022]025 and [2022]026). After three days of adaptation, the animals were randomly divided into four groups of six animals. They were divided into the normal group, 2 Western medicine group, DOC group, and 2 Western medicine + DOC group. The rats were given 2 mL/100g of compound medicine twice a day by gavage. The normal group was given 0.9% normal saline each time, and the 2 Western medicine group was given 15 mg/kg IR and 0.5 mg/kg AM each time. The DOC group was given 2.8 g/kg DOC each time, and the 2 Western medicine + DOC group was given 15 mg/kg IR + 0.5 mg/kg AM + 2.8 g/kg DOC each time for five consecutive days. The administration began at 10 AM and 4 PM on the same day. On the morning of the fifth day, the rats were anesthetized half an hour after administration, blood samples were collected from the abdominal aorta, left at room temperature for 2 hours, centrifuged at 4 °C at 3000 r/min for 10 min, and 220 μL of the supernatant was separated and reloaded and placed at −80 °C for testing.

Design of the Drug Efficacy Experiment

Part I

Six Wistar rats (all male, 180–200 g) were used in this experiment. They were provided by Tongxiang Branch of Zhejiang Weitong Lihua Laboratory Animal Technology Co., LTD. Laboratory animal production license number: SCXK (Zhejiang) 2021–0006. License number: SYXK (Zhejiang) 2019–0010; Qualified certificate number: 20220706Aaaz0600999941.

Forty-eight SHRs (all male, 180–200g) were provided by Beijing Vitong Lihua Laboratory Animal Technology Co., LTD., Laboratory animal production license number: SCXK (Beijing) 2021–0006; License number: SYXK (Zhejiang) 2019–0010; Qualified certificate number: 110011220106686012. This study was approved by the Experimental Animal Welfare Ethics Committee of Zhejiang Academy of Traditional Chinese Medicine (approval number: [2022]026).

Six Wistar rats and 48 SHRs were fed for three days. The six Wistar rats were used as the normal group, while the 48 SHRs were randomly divided into the following eight groups: the model group, DOC group, KF group, RU group, SY group, PA group, HYP group, and monomer mixing group (MMG), with six rats in each group. The normal group and model group were administered 0.9% saline, the DOC group was administered 2.8 g/kg DOC, the KF group was given 723 μg/kg KF, the RU group was administered 2 mg/kg RU, the SY group was administered 138 mg/kg SY, the PA group was administered 75 mg/kg PA, the HYP group was administered 2 mg/kg HYP, and the PA group was administered 75 mg/kg PA. The MMG was administered 723 μg/mL (KF) + 2 mg/kg (RU) + 138 mg/kg (SY) + 75 mg/mL (PA) + 2 mg/kg (HYP). The patients were fasted for 12 hours before administration, and the drugs were administered once a day, 2 mL/100

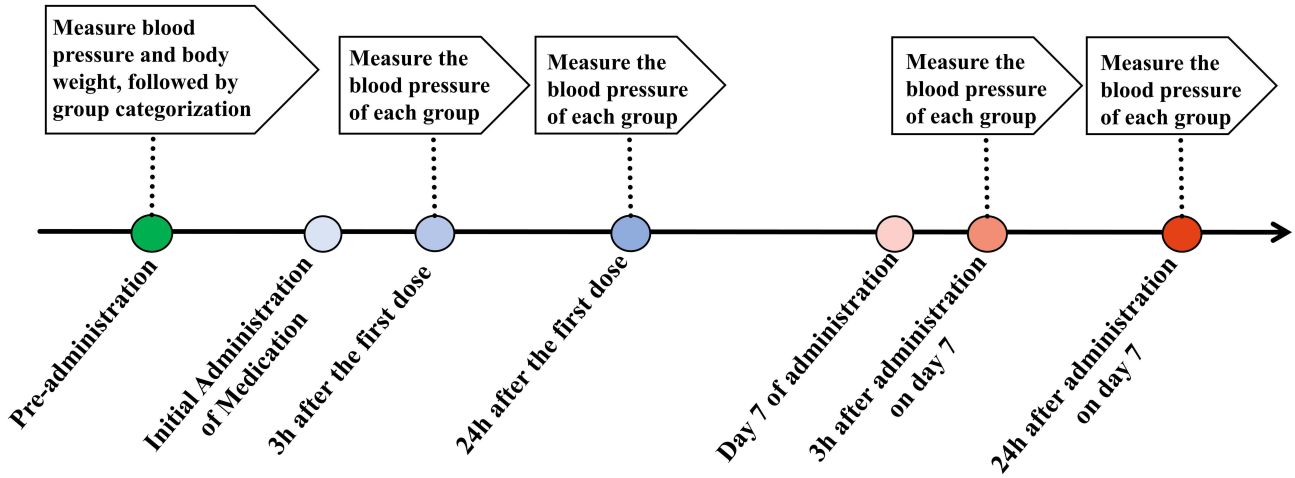

Figure 1 Timeline for Administration and Blood Pressure Measurement in Pharmacodynamic Animal Experiments.

g each time, for one week. Blood pressure was measured before administration, at 3 hours and 24 hours after the first time of administration, and at the same time after one week administration. The rats were weighed before administration (Figure 1).

Part II

Thirty rats (24 SHRs + 6 Wistar) were selected and blood pressure before administration was measured. Six Wistar rats were set as the normal group, and 24 SHRs were randomly divided into the following four groups: the model group, 2 Western medicine groups (IA), DOC + 2 Western medicine group (DIA), MM+ 2 Western medicine group (MIA), with six rats in each group. The normal group and the model group were given normal saline, the IA group was given 15 mg/kg IR and 0.5 mg/kg AM, and the DIA group was given 15 mg/kg (IR) + 0.5 mg/kg (AM) + 2.8 g/kg (DOC). The MIA group was given 15 mg/kg (IR) + 0.5 mg/kg (AM) + 723 μg/mL (KF) + 2 mg/kg (RU) + 138 mg/kg (SY) + 75 mg/mL (PA) + 2 mg/kg (HYP). The patients were fasted for 12 hours before administration, and the drug was given once a day, 2 mL/100g each time, and the drug was given for one week. Blood pressure was measured before administration, at 3 hours and 24 hours after the first time and the last time of administration, respectively. The rats were weighed before administration (Figure 1).

Methods of Blood Pressure Measurement

In this experiment, the blood pressure of all experimental rats was measured before drug administration as a control. After the commencement of treatment, the blood pressure was measured before drug administration, at the first time of drug administration, 3 hours after drug administration, and 24 hours after drug administration. During blood pressure measurement, the rats were heated on the iron plate of a 38.3 °C water bath. To avoid excessive heating caused by individual differences in each group of rats, the first heating time was 4 min, if the blood pressure data could not be obtained, the number of heating sessions was increased, and the second heating time was 2 min. The data were obtained on the computer through the BP-98A-type noninvasive tail artery blood pressure measuring instrument supporting software. The blood pressure of each rat was measured 6–10 times, and the mean value was used as the measurement value of that rat.

Network Pharmacology and Molecular Docking Experimental Methods

According to Article 32, Items 1 and 2 of the "Ethical Review Measures for Biological Life Sciences and Medical Research Involving Humans", promulgated in China on February 27, 2023, our research utilizes data from public databases involving human subjects. However, it does not inflict harm on individuals, nor does it involve sensitive personal information or commercial interests; therefore, it is exempt from ethical review.

The study utilized three databases: GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), and DISGENET (https://disgenet.com/) to identify targets associated with Spontaneously Hypertension (SH). The SwissTargetPrediction website (http://swisstargetprediction.ch/) was employed to predict potential targets for Five Compounds (FC). Subsequently, the UniProt database (https://www.uniprot.org/) was used to standardize all identified targets and eliminate duplicates from both EH and FC. Using the Venny 2.1.0 tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html), we obtained the intersection of FC-EH targets and constructed a Venn diagram. The resulting intersecting targets were input into the STRING platform (https://cn.string-db.org/) to build a protein-protein interaction network (PPI). This PPI data was then imported into Cytoscape 3.9.1 software, where the built-in "Network Analyzer" feature provided network characteristic parameters for target proteins, allowing us to filter out core targets based on connectivity. Furthermore, we conducted functional annotation of the FC-SH intersecting targets using the DAVID database (https://davidbioinformatics.nih.gov/), obtaining GO and KEGG pathway enrichment analysis data. Enrichment bubble plots and bar charts were generated using an online bioinformatics platform (http://www.bioinformatics.com.cn/). We constructed a "Primary Hypertension - Pathway - Target - Component" network diagram in Cytoscape 3.9.1 software, utilizing its "Network Analyzer" function to derive effective components and target network characteristics by selecting high connectivity values for core components and targets. To further refine our selection of frequently occurring core targets, we took intersections between high-connectivity nodes from both the "FC-SH Intersection Targets" network diagram and those from the "SH-Pathway-Target-Component" network diagram. Finally, molecular docking validation was performed on selected core targets against FC compounds. We retrieved 3D structures of FC from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) while downloading protein structures of core targets from PDB database (https://www.rcsb.org/). Molecular docking analyses were conducted using the online CB-Dock2 platform (http://clab.labshare.co.uk/cb-dock/php/index.php) to evaluate cavity size and Vina scores.[13] CB-Dock2 is a blind docking tool that focuses on enhancing docking accuracy. It employs cavity detection-guided blind docking to investigate the interactions between proteins and ligands. This tool can predict the binding regions of a given protein by calculating the center and size using curvature-based cavity detection methods. Additionally, it is interconnected with the state-of-the-art docking software Autodock Vina, allowing CB-Dock2 to rank binding modes based on Vina scores and provide interactive 3D visualizations of various binding conformations.[14,15] A lower Vina score indicates reduced binding energy, which suggests more stable and effective interactions.[16] Therefore, we select the docking model with the lowest binding energy from each group for comparison and visualization purposes.

Results

Qualitative Experimental results of Blood Component Experiments

Rat serum was analyzed by the SCIEX X-500R four-pole time-of-flight mass spectrometer and compared with TCM MS/MS Library (which contains secondary data of more than 1000 Chinese medicinal compounds) in SCIEX OS, it was preliminarily determined that there may be 10 components in the compound serum: Hydroxytyrosol, Corilagin, Fumaric acid, Kaempferide, Isoquercitrin, Loureirin A, Syringaldehyde, Rutin, Hyperoside, Paeoniflorin, two Western medicine for Irbesartan and Amlodipine (Supplementary Figures 1–12).

Quantitative results of Blood Component Experiments

Methodology Investigation

Standard Curve

KF: y = 5371.68931 x + 118.77241 (r = 0.99896, r^2 = 0.99792) (weighting: 1 / x); RU: y = 14,103.19580x + 477.92603 (r = 0.99977, r^2 = 0.99955) (weighting: 1 / x); SY: y = 4532.48655x-1877.80267 (r = 0.99963, r^2 = 0.99926) (weighting: 1 / x); PA: y = 517.93625x + 1168.26144 (r = 0.99970, r^2 = 0.99939) (weighting: 1 / x); HYP: y = 37028.6x + 4921.47013 (r = 0.99998, r^2 = 0.99996) (weighting: 1 / x); IR: y = 91173.9x + 2998560 (r = 1.00000, r^2 = 0.99999) (weighting: 1 / x); AM: y = 43510.8x + 3190.96048 (r = 0.99996, r^2 = 0.99992) (weighting: 1 / x).

Precision Test

The RSD of KF, RU, SY, PA, HYP, IR, and AM were 9.79%, 11.59%, 10.48%, 11.10%, 6.23%, 1.13%, and 17.57%, respectively, which were less than 20%, and the precision was good.

The RSD of KF, RU, SY, PA, HYP, IR, and AM were 2.73%, 4.20%, 4.07%, 4.64%, 4.09%, 2.36%, and 4.77%, respectively, which were less than 15%. The repeatability was good.

Sample Recovery Experiment

KF low-, medium-, and high-concentration sample recovery rates were 89.53%, 93.26%, and 91.00%, respectively. The recovery rates of RU were 107.07%, 110.01%, and 93.01%, respectively. The recovery rates of SY were 90.38%, 103.90%, and 102.19%, respectively. The recovery rates of PA at low, medium, and high concentrations were 103.63%, 102.76%, and 101.86%, respectively. The recovery rates of HYP were 93.27%, 104.64%, and 95.52%, respectively. The recovery rates of IR at low, medium, and high concentrations were 95.59%, 103.56%, and 104.68%, respectively. The recovery rates of AM at low, medium, and high concentrations were 99.13%, 100.20%, and 95.86%, respectively, which were consistent with 85–115%. The analytical method had a good recovery rate, and the experimental method was accurate and reliable, which could be used for the determination of the components of DOC in blood.

Matrix Effect Experiment

KF low-, medium-, and high-concentration peak area ratios were 91.05%, 86.71%, and 97.97%, respectively. RU: 107.31%, 104.58%, and 105.08%, respectively. The ratios of low, medium, and high concentration peak areas of SY were 99.68%, 106.88%, and 101.48%, respectively. The ratios of PA low, medium, and high concentration peak areas were 106.57%, 111.17%, and 110.46%, respectively. The ratio of low, medium, and high concentration peak areas of HYP were 101.80%, 104.64%, and 95.14%, respectively. The peak area ratios of low, medium, and high concentrations of IR were 105.39%, 99.84%, and 102.77%, respectively. The peak area ratios of AM were 107.89%, 102.24%, and 108.62%, respectively, which were in line with 85–115%, indicating that there was no obvious matrix effect, and the analysis results were accurate and reliable.

Quantitative Test Results of Each Serum Sample

Control samples were purchased and quantified using the UPLC-QTRAP-MS/MS instrument. Finally, five compounds in serum that could be quantified were determined: KF, RU, SY, PA, and HYP (PA was from *P. lactiflora*, and the other four monomers were all from *D. officinale*). Among them, RU was not detected in the DOC group but was detected in the combination group.

The content of five compounds from the DOC (measured with n=3) were as follows: KF, 1.30 ± 0.06 ng/mL; RU, 0.25 ± 0.08 μg/mL; SY, 0.13 ± 0.01 μg/mL, PA, 61.08 ± 5.74 μg/mL, and HYP, 4.68 ± 0.46 ng/mL.

The serum drug contents of the DOC group (experimental animals with n=6) were as follows: KF, 0.04 ± 0.02 ng/mL; SY, 0.56 ± 0.05 ng/mL; PA, 13.20 ± 0.10 ng/mL; HYP, 0.098 ± 0.011 ng/mL. The serum drug contents in the combined group of Chinese and Western medicine were as follows: KF, 0.11 ± 0.05 ng/mL; RU, 0.04 ± 0.01 ng/mL; SY, 0.59 ± 0.07 ng/mL; PA, 15.40 ± 1.83 ng/mL; HYP, 0.096 ± 0.006 ng/mL; IR, 405.97 ± 39.52 ng/mL; AM, 0.78 ± 0.14 ng/mL. The serum drug levels of IR and AM in the Western medicine group were 297.60 ± 42.57 ng/mL and 1.39 ± 0.44 ng/mL, respectively (Figures 2–5).

Results of Drug Effect Experiments

Results of SBP in Part I Experiments

Before treatment, the SBP values of all SHR groups were significantly higher than those of the normal group ($P < 0.01$), suggesting that SHRs had significant hypertension. During the whole treatment process, the SBP values of all the experimental groups were significantly lower than those of the model group ($P < 0.01$). The SBP values of the RU, SY, PA, and MM groups were significantly lower than that of the DOC group ($P < 0.01$ or $P < 0.05$). The efficacy of the HYP group did not differ significantly from that of the DOC group (Table 2 and Figure 6A–C). The decrease in SBP in the MM group was lower than that in the other three groups (except for the HYP group). It was proved that all five components had a certain antihypertensive effect; however, the effect was reduced when the five components were combined, which might be due to the lack of a synergistic antihypertensive effect.

Part II Experimental SBP Results

Before treatment, the SBP values in all SHR groups were significantly higher than those in the normal group ($P < 0.01$), suggesting that SHRs had significant hypertension. The intervention measures in all experimental groups showed good antihypertensive effects ($P < 0.01$), and the antihypertensive effects of two Western medicines combined with DOC solution

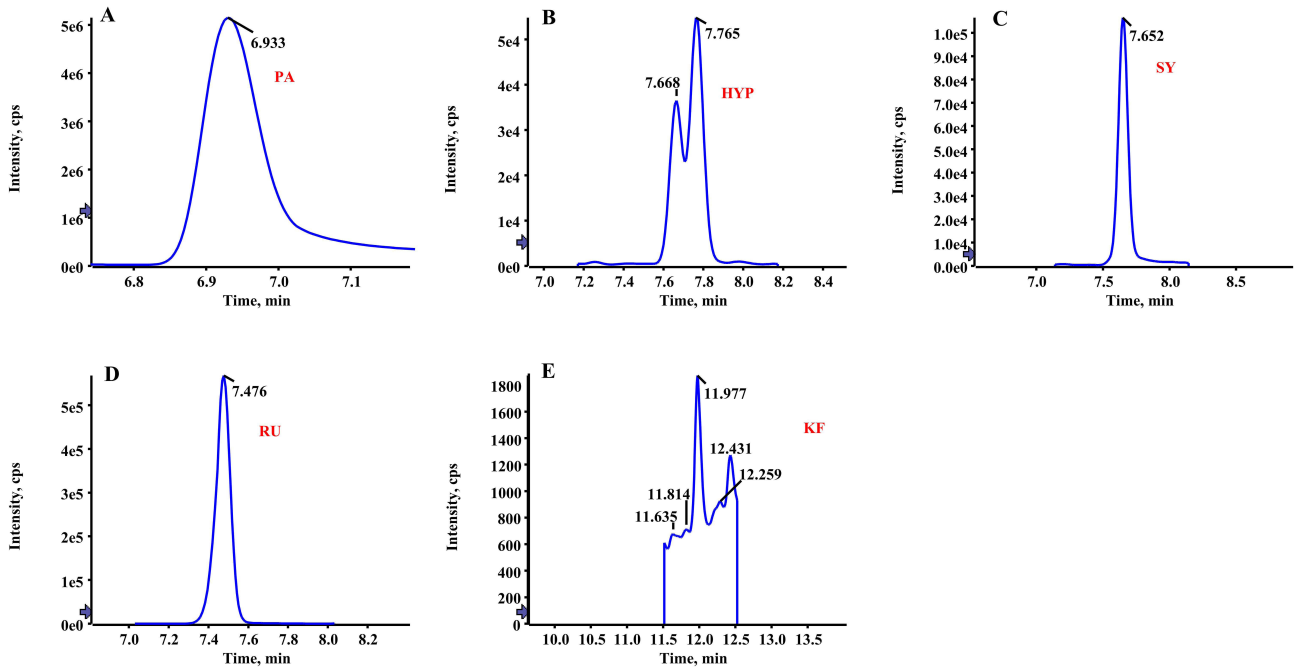

Figure 2 Corresponding mass spectra of components in pilot products. (**A**) PA. (**B**) HYP. (**C**) SY. (**D**) RU. (**E**) KF. When the arrow on the vertical axis is positioned below one or several major peaks, the system can automatically generate retention times for those peaks. Conversely, when the small arrow on the vertical axis is located above one or several major peaks, these peaks will not be able to display retention times automatically.

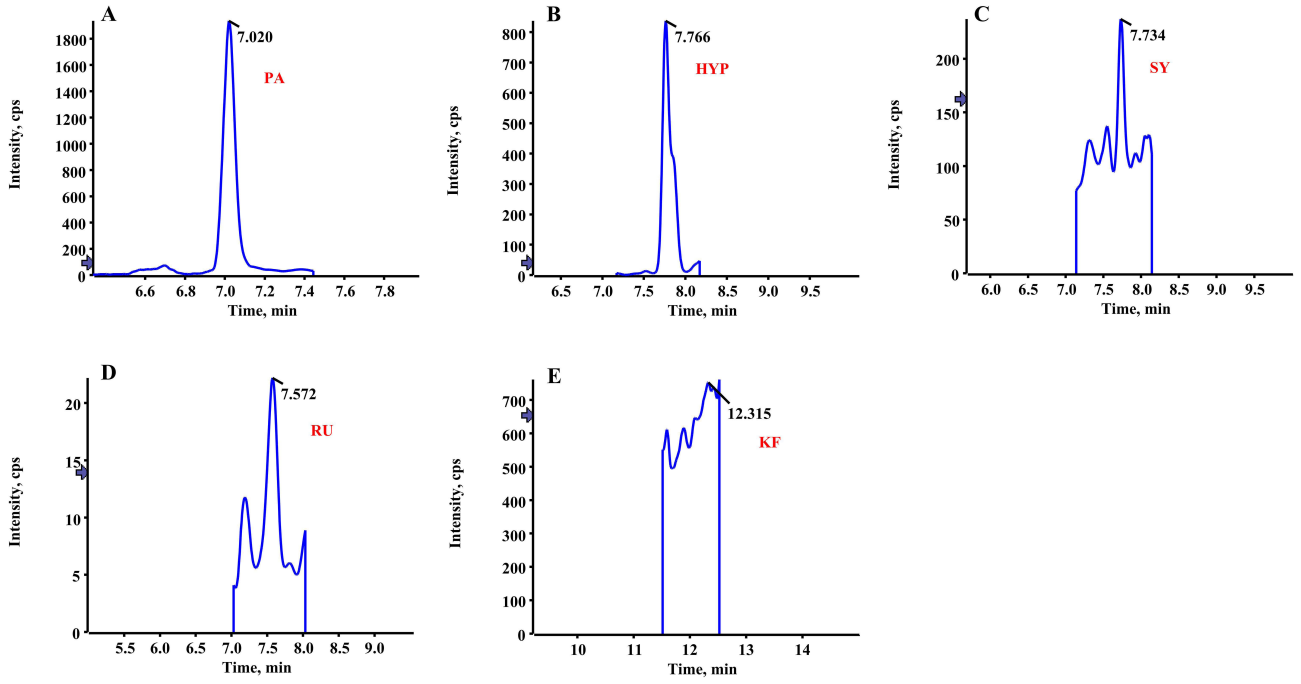

Figure 3 Corresponding mass spectra of the components measured in the serum of the compound group. (**A**) PA. (**B**) HYP. (**C**) SY. (**D**) RU. (**E**) KF. When the arrow on the vertical axis is positioned below one or several major peaks, the system can automatically generate retention times for those peaks. Conversely, when the small arrow on the vertical axis is located above one or several major peaks, these peaks will not be able to display retention times automatically.

or the mixed solution of five monomers were significantly better than those of two Western medicines ($P < 0.01$). Moreover, the antihypertensive effect of Western medicine combined with the mixed solution of five monomers was better than that of DOC solution, which was similar to the results of the first part of the experiment. It was proved that all five monomers

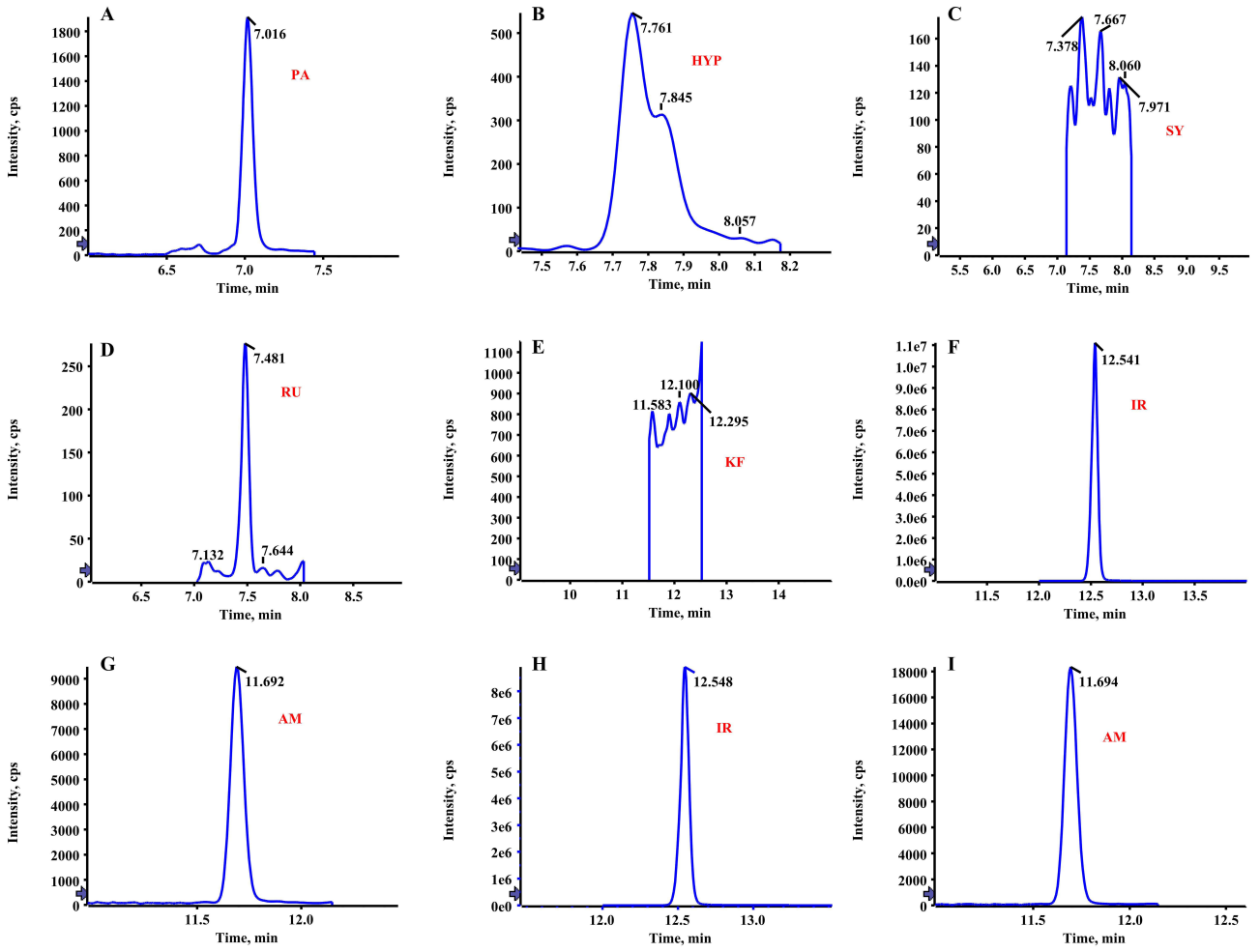

Figure 4 Corresponding mass spectra of components in the combined group of Chinese and Western medicine. (**A**) PA. (**B**) HYP. (**C**) SY. (**D**) RU. (**E**) KF. (**F**) IR. (**G**) AM. The corresponding mass spectrum of components in the serum of the Western medicine group. (**H**) IR. (**I**) AM. When the arrow on the vertical axis is positioned below one or several major peaks, the system can automatically generate retention times for those peaks. Conversely, when the small arrow on the vertical axis is located above one or several major peaks, these peaks will not be able to display retention times automatically.

contributed to the antihypertensive effect of the compound, and the contribution still existed after the combination with Western medicine (Table 3 and Figure 6D–F). If the antihypertensive effect of the monomer solution was better than that of the compound solution 3 hours after the first administration, the drug concentration at this time was used as the drug concentration of the experimental rats as follows: 723 ug/mL (KF), 2 mg/kg (RU), 138 mg/kg (SY), 75 mg/mL (PA), and 2 mg/kg (HYP). However, these concentrations were much higher than the monomer concentrations in the compound solution (12.594-fold, 181.159-fold, 24.038-fold, 27.806-fold, and 9.677-fold, respectively).

Results of Network Pharmacology Analyses

Screening of FC-SH-Related Targets

It′s found that there were 288 FC possible target genes, and 514 SH possible target genes. SH with KF, RU, SY, PA, and HYP may be related to 43 target genes (Figure 7A).

PPI Network Analyses of Potential Target Genes of FC-SH

The number of nodes was 43, the number of edges was 127, the average node degree was 5.91, the average local clustering coefficient was 0.508, the expected number of edges was 35, and the PPI enrichment *p*-value was less than 10^{-16} (Figure 7B).

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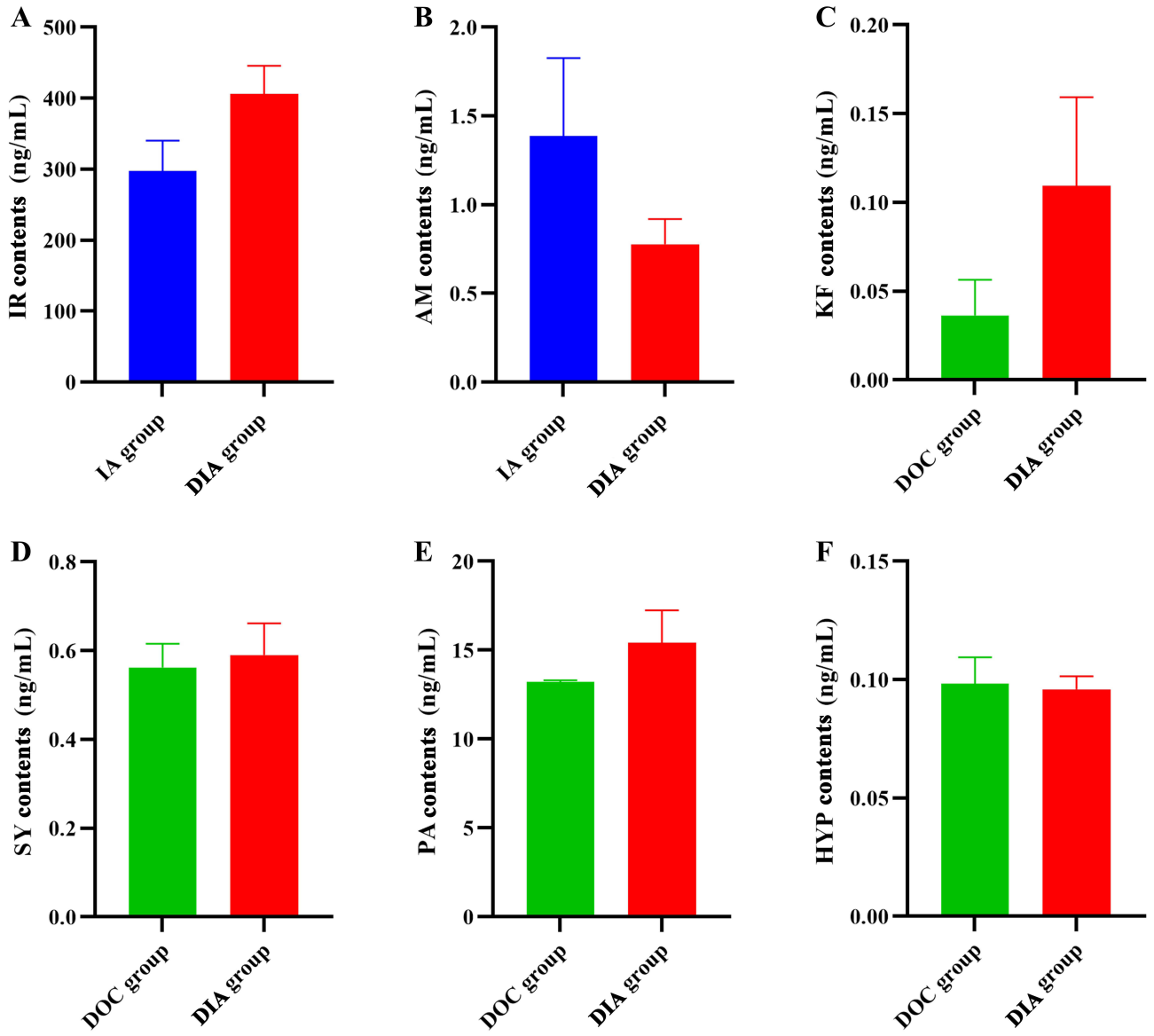

Figure 5 Comparison of the contents of each component in the serum of each group. (**A**) comparison of the contents of IR in the 2 Western medicine group and the combination group. (**B**) AM content comparison between the two Western medicine groups and the combination group. (**C–F**) The contents of KF, SY, PA, and HYP in the Chinese medicine group and the Chinese medicine combined with Western medicine group, respectively.

"FC-SH-Intersection Targets" Network Map Target Screening

Input PPI network graph information into Cytoscape 3.9.1 visualization analysis, in order to build the network (Figure 8). Ten target nodes with a selection degree value higher than the corresponding median value in the PPI network were used for late molecular docking (Table 4). The network consists of 41 nodes and 127 edges, with an average node degree of 8.195 and an average local clustering coefficient of 0.411. Notably, TNF, ALB, and MMP9 exhibit high connectivity and centrality, suggesting that these three proteins may play a crucial role in the therapeutic effects of five compounds for hypertension (Figure 7). The *AGTR1, MPO, IGF1R*, and *XDH3* genes also showed high betweenness centrality, which may also be important proteins in the treatment of spontaneous hypertension.

GO Function Analysis and KEGG Pathway Enrichment Analysis

The DAVID program was used to perform functional enrichment analyses of 43 candidate targets. Subsequently, GO functional analysis data and KEGG pathway enrichment analysis data were uploaded to the bioinformatics platform for visual analyses. The GO enrichment analysis presents the top ten items ranked in Biological Process (BP), Cellular

Table 2 Part I Experimental SBP Data (Units: mmHg; Number of Experimental Animals per Group: n=6)

Duration of Administration	Normal Group	Model Group	DOC Group	Kaempferin Group (KF)	Rutin Group (RU)	Syringaldehyde Group (SY)	Paeoniflorin Group (PA)	Hyperoside Group (HYP)	Mixed Standard Group (MMG)
Before treatment	112.52 ± 2.80	198.36 ± 1.52**	198.39 ± 1.27	198.14 ± 1.51	198.36 ± 2.15	197.97 ± 2.50	197.97 ± 1.72	198.34 ± 2.44	198.16 ± 2.07
3h after the first dose	113.21 ± 1.72	197.50 ± 3.79**	173.81 ± 2.71$^{\Delta\Delta}$	163.33 ± 1.68$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	162.88 ± 1.33$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	160.93 ± 4.60$^{\Delta\Delta\#\#\blacklozenge\blacklozenge\blacktriangle\blacktriangle}$	162.75 ± 3.18$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	173.54 ± 1.48$^{\Delta\Delta}$	165.64 ± 2.27$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$
24h after the first dose	111.77 ± 1.42	197.62 ± 2.14**	181.74 ± 1.08$^{\Delta\Delta}$	172.71 ± 2.89$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	174.26 ± 2.69$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	174.10 ± 2.77$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	174.71 ± 2.98$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$	183.07 ± 2.24$^{\Delta\Delta}$	174.86 ± 3.47$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$
3 hours after be treated for 1 week	112.95 ± 2.73	198.20 ± 1.82**	171.72 ± 1.65$^{\Delta\Delta}$	161.57 ± 1.33$^{\Delta\Delta\#\#\blacklozenge\blacklozenge\blacktriangle\blacktriangle}$	161.95 ± 1.58$^{\Delta\Delta\#\#\blacklozenge\blacklozenge\blacktriangle\blacktriangle}$	160.78 ± 2.75$^{\Delta\Delta\#\#\blacklozenge\blacklozenge\blacktriangle\blacktriangle}$	160.89 ± 1.44$^{\Delta\Delta\#\#\blacklozenge\blacklozenge\blacktriangle\blacktriangle}$	172.17 ± 2.10$^{\Delta\Delta}$	165.35 ± 2.50$^{\Delta\Delta\#\#\blacklozenge\blacklozenge}$
24h after be treated for 1 week	111.21 ± 2.09	197.67 ± 1.33**	181.21 ± 0.61$^{\Delta\Delta}$	172.14 ± 5.14$^{\Delta\Delta}$	174.26 ± 2.44$^{\Delta\Delta\#\blacklozenge}$	174.00 ± 2.48$^{\Delta\Delta\#\#\blacklozenge}$	173.36 ± 2.64$^{\Delta\Delta\#\#\blacklozenge}$	181.21 ± 2.75$^{\Delta\Delta}$	174.39 ± 3.36$^{\Delta\Delta}$

Note: Comparison between the model group and the normal group: $^{**}P < 0.01$; Compared with the model group: $^{\Delta\Delta}P < 0.05$; Comparison between the treatment groups and the DOC group: $^{\#}P < 0.05$,$^{\#\#}P < 0.01$; Comparison of each treatment group with the HYP group:$^{\blacklozenge}P < 0.05$,$^{\blacklozenge\blacklozenge}P < 0.01$; Comparison between each treatment group and the MMG group: $^{\blacktriangle\blacktriangle}P < 0.01$.

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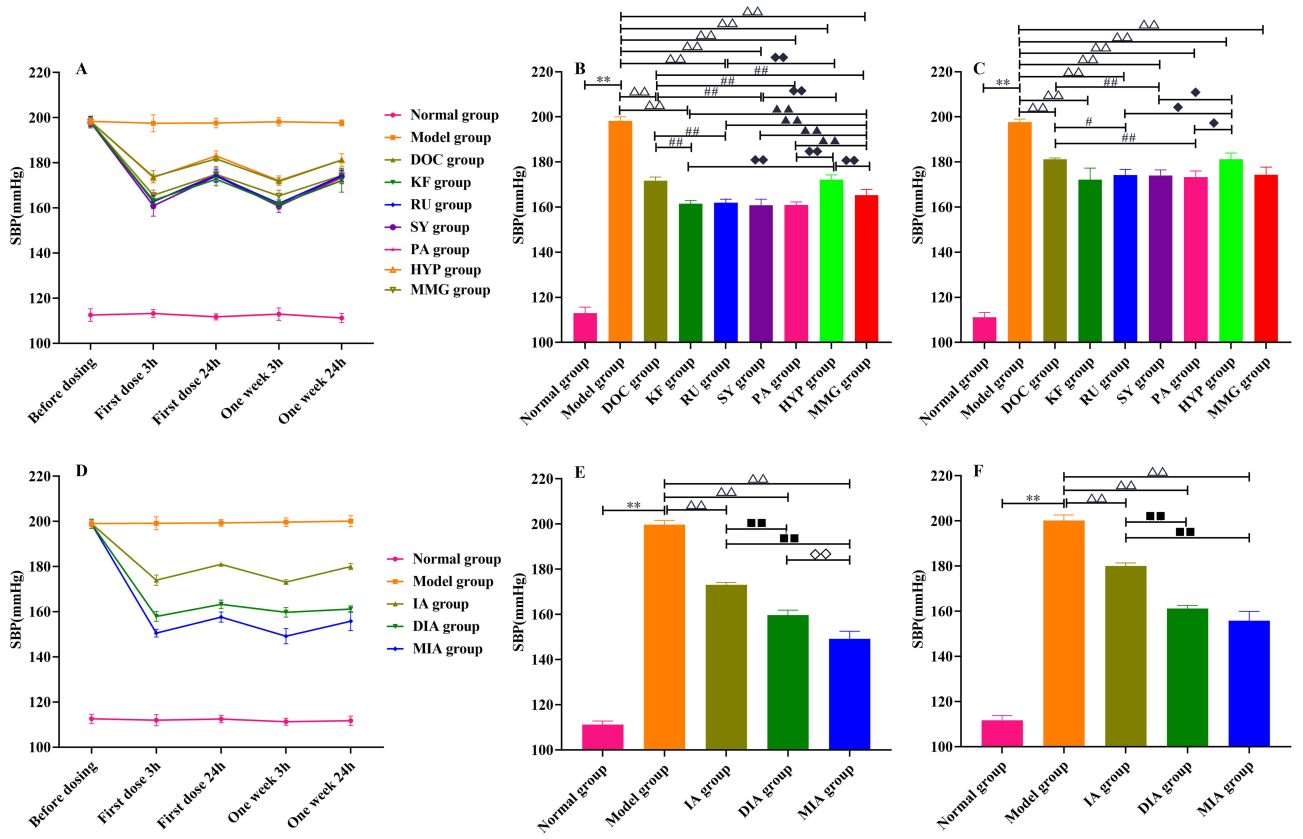

Figure 6 See the experimental blood pressure diagram (**A**–**C**) The first results. (**A**) The whole blood pressure data. (**B**) Blood pressure data of each group at 3h after one week of administration. (**C**) Blood pressure data of each group at 24h after one week of administration. (**D**–**F**) The second batch of experiment results. (**D**) The whole blood pressure data. (**E**) Blood pressure data of each group at 3h after one week of administration. (**F**) Blood pressure data of each group at 24h after one week of administration. There was a significant difference compared with the Model group: $^{\Delta\Delta}p < 0.01$; There was a significant difference compared with the HYP group: $^{\blacklozenge}p< 0.05$, $^{\blacklozenge\blacklozenge}p< 0.01$; **There was a significant difference between the Model group and the Normal group, $p < 0.01$; There was a significant difference compared with the DOC group: $^{\#}p< 0.05$, $^{\#\#}p < 0.01$; ▲▲: There was a significant difference compared with the MMG group, $p < 0.01$; ◇◇: There was a significant difference compared with the DIA group, $p < 0.01$; ■■: There was a significant difference compared with the IA group, $p < 0.01$.

Component (CC), and Molecular Function (MF) (Figure 9). Figure 9 shows that these genes are closely related to response to hypoxia, response to exogenin stimulation, negative regulation of the apoptotic process, smooth muscle contraction, plasma membrane and plasma membrane components, presynaptic membrane and its integral components, iron ion binding, protein binding, endopeptidase activity, and aromatase activity. The KEGG pathway analysis selected pathways with $P < 0.05$ to make a bubble map (Figure 10). Figure 10 shows that the target genes are mainly related to the cGMP-PKG signaling pathway, vascular smooth muscle contraction, the calcium signaling pathway, the neuroactive

Table 3 Part II Experimental SBP Data (Units: mmHg; Number of Experimental Animals per Group: n=6)

Duration of Administration	Normal Group	Model Group	2 Western Medicine Group (IA)	2 Western Medicine + Compound Group (DIA)	2 Western Medicine + Mixed Standard Group (MIA)
Before treatment	112.62 ± 2.04	199.05 ± 1.32**	198.98 ± 2.10	198.96 ± 2.09	198.88 ± 1.98
3h after the first dose	111.98 ± 2.48	199.19 ± 2.87**	173.99 ± 2.31$^{\Delta\Delta}$	157.90 ± 2.19$^{\Delta\Delta\blacksquare\blacksquare}$	150.54 ± 1.72$^{\Delta\Delta\blacksquare\blacksquare\diamond\diamond}$
24h after the first dose	112.48 ± 1.68	199.37 ± 1.54**	181.00 ± 0.81$^{\Delta\Delta}$	163.26 ± 1.93$^{\Delta\Delta\blacksquare\blacksquare}$	157.64 ± 2.22$^{\Delta\Delta\blacksquare\blacksquare\diamond\diamond}$
3h after 1 week of administration	111.28 ± 1.55	199.67 ± 1.89**	173.10 ± 1.07$^{\Delta\Delta}$	159.77 ± 2.17$^{\Delta\Delta\blacksquare\blacksquare}$	149.15 ± 3.40$^{\Delta\Delta\blacksquare\blacksquare\diamond\diamond}$
24h after 1 week of administration	111.74 ± 2.05	200.15 ± 2.49**	180.00 ± 1.41$^{\Delta\Delta}$	161.17 ± 1.44$^{\Delta\Delta\blacksquare\blacksquare}$	155.80 ± 4.15$^{\Delta\Delta\blacksquare\blacksquare}$

Note: Comparison between the model group and the normal group: $^{**}P < 0.01$; Compared with model group: $^{\Delta\Delta}P < 0.01$; Each treatment group was compared with IA group: $^{\blacksquare\blacksquare}P < 0.01$. Each treatment group was compared with DIA group: $^{\diamond\diamond}P < 0.01$.

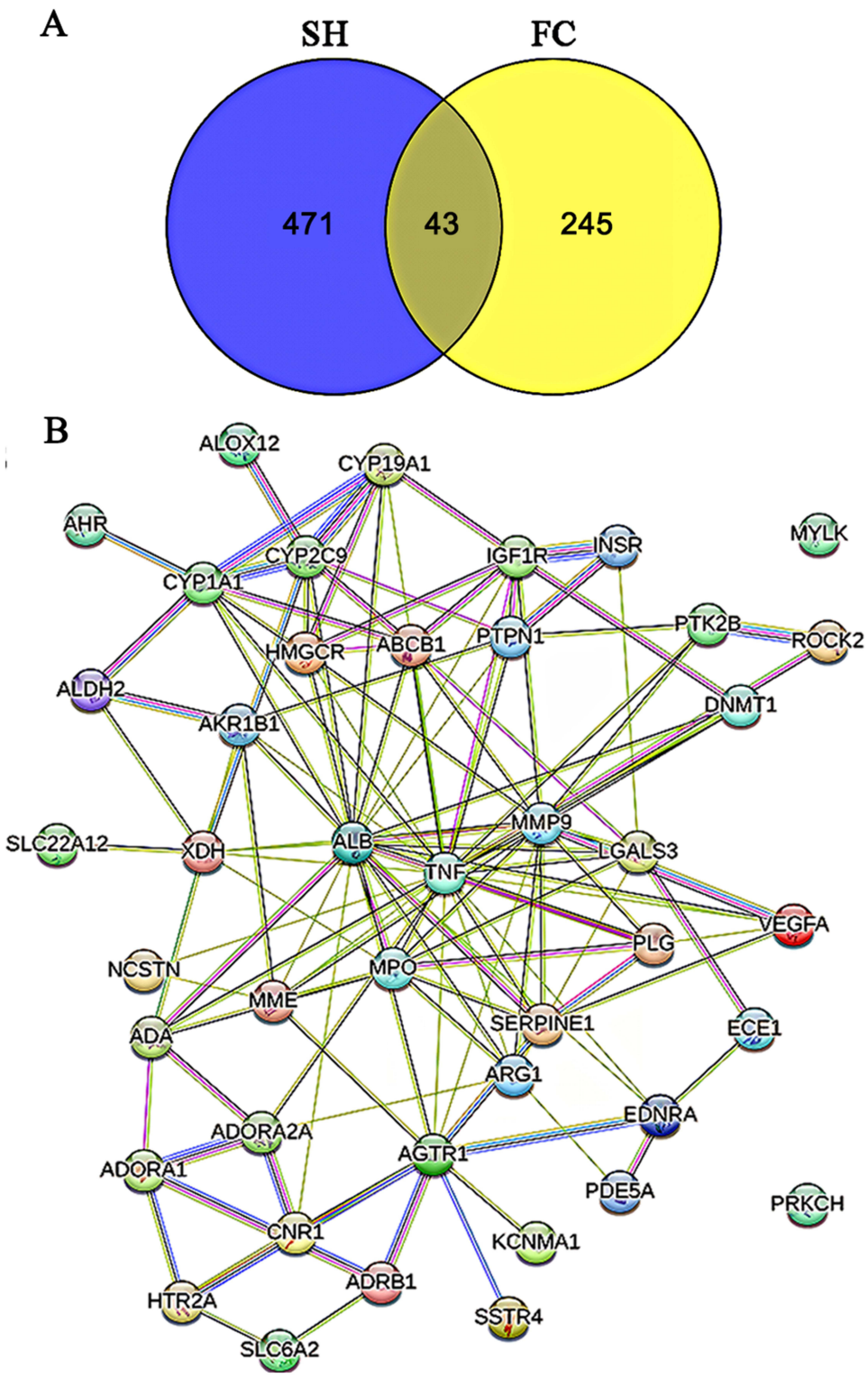

Figure 7 (**A**) Wayne diagram of possible targets of FC-SH. (**B**) PPI network diagram of possible targets of FC-SH.

ligand-receptor interaction, renin secretion, ovarian steroidogenesis, the AGE-RAGE signaling pathway in diabetic complications, the HIF-1 signaling pathway, and the proteoglycan in cancer Rap1 signaling pathway. Lipids and atherosclerosis are related to other pathways.

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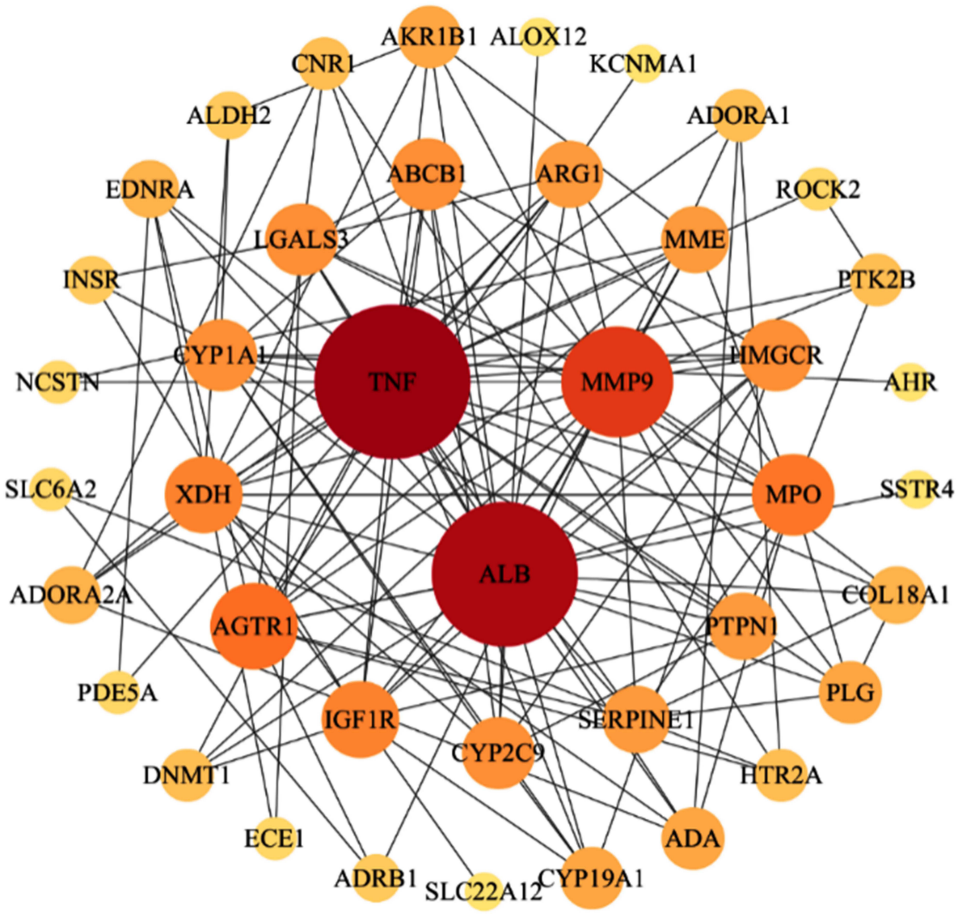

Figure 8 Network of potential target proteins for FC-SH. Each circle represents a protein, color shades, and circle size matching with link degree of discretion. The deeper the color link the higher the degree.

The "Spontaneously Hypertension-Pathway-Target-Component" Network Diagram Was Constructed and Analyzed

Cytoscape 3.9.1 was used to construct the "SH-pathway-target-FC" network diagram (Figure 11, Table 5 and Supplementary Table 1). Figure 11 shows that among the FC, RU had the highest connectivity degree and proximity centrality with target

Table 4 Data of the Possible Target Protein Network of "FC-EH"

Name	The Name of the Protein	Connection Degree (degree, D)	Medium Degree of Centricity (BC)	Close to Central (CC)	Average Shortest Path Length (ASPL)
TNF	Tumor necrosis factor	25	0.300	0.702	1.425
ALB	Albumin	23	0.242	0.690	1.450
MMP9	Matrix metalloproteinase 9	16	0.070	0.588	1.700
AGTR1	Angiotensin II receptor type 1	11	0.213	0.541	1.850
MPO	Myeloperoxidase	10	0.013	0.513	1.950
IGF1R	Insulin-like growth factor 1 receptor	9	0.018	0.476	2.100
XDH	Xanthine dehydrogenase	9	0.077	0.494	2.025
ABCB1	Atp-binding cassette subfamily B member 1	8	0.009	0.494	2.025
LGALS3	Galectin 3	8	0.043	0.482	2.075
CYP2C9	Cytochrome P450 family 2 subfamily C member 9	8	0.057	0.476	2.100

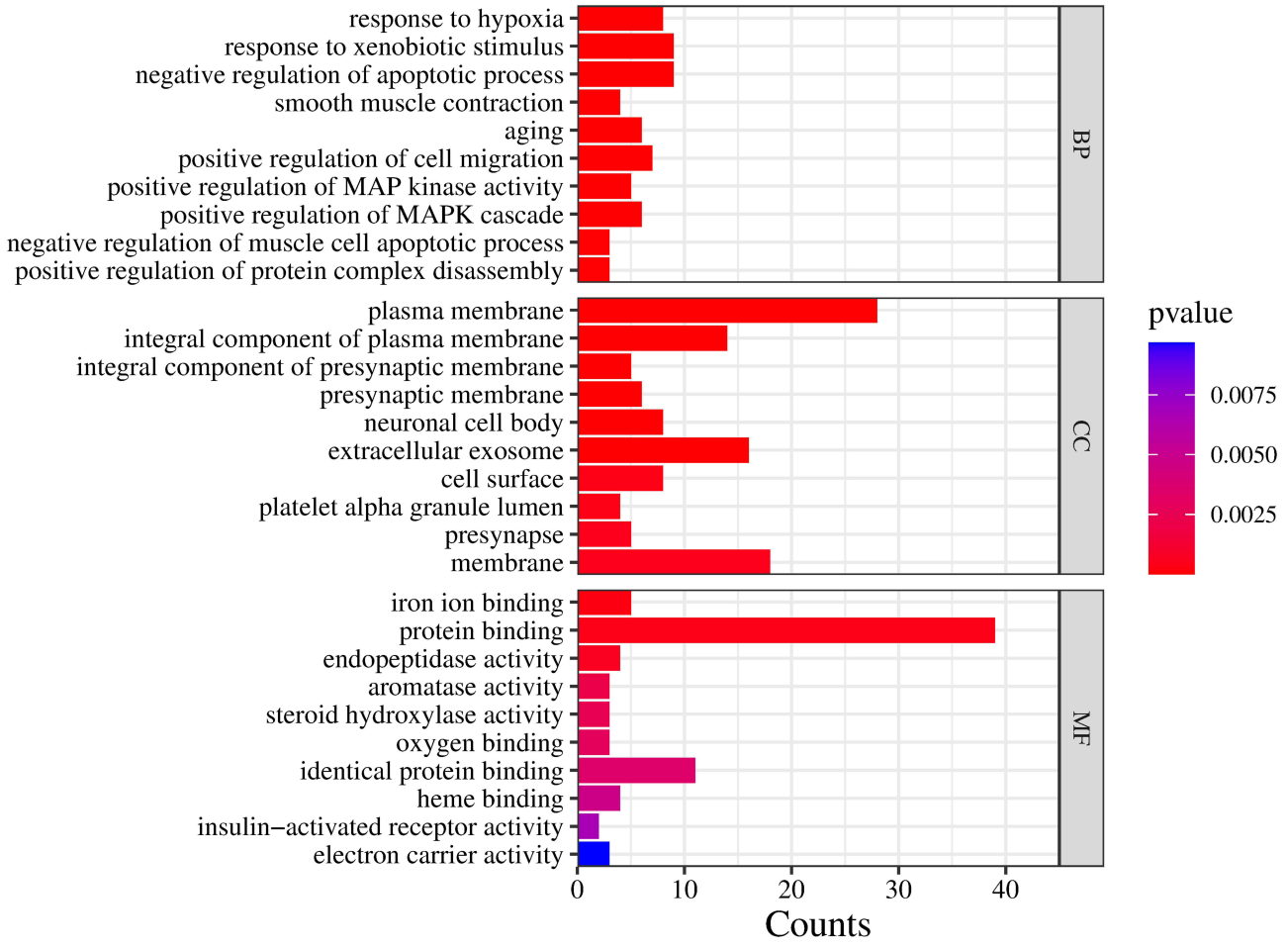

Figure 9 Bar graph of the GO enrichment analysis. The color of the bar represents its p-value size, the darker the red, the smaller the p-value; the darker the purple, the larger the p-value. The number of target genes included in the pathway is written on the X-axis, while the target gene enrichment items are shown on the Y-axis.

genes and pathways, while paeoniflorin had the highest connectivity degree, betweenness centrality, and proximity centrality, indicating that these two compounds were closely related to SH target genes and pathways. The connectivity degree of the cGMP-PKG signaling pathway, the calcium signaling pathway, and the vascular smooth muscle contraction pathway closely related to spontaneous hypertension ranked 3rd, 4th, and 5th among the nine pathways. Among the 10 possible target genes, *VEGFA* was the most highly connected, followed by *IGF1R* and *INSR*. There were also some target genes that were strongly related to SH, including *EDNRA, AGTR1, ADRB1*, and *ADORA1*.

Core Targets Were Further Screened

When the top 10 connectivity genes analyzed in Figure 11 were intersected with the top 10 connectivity genes analyzed in Figure 8 above, it was found that *AGTR1, MMP9*, and *IGF1R* had repeated occurrence. Figure 11 shows that *AGTR1* is the target gene of PA and participates in six pathways: hsa04924, hsa04080, hsa04270, hsa04022, hsa04020, and hsa04933. *MMP9* was the common target gene of the RU, PA, HYP, and KF compounds, and was involved in four pathways (hsa05417, hsa04670, hsa05200, and hsa05205). *IGF1R* was a common target gene of RU, HYP, KF, and SY compounds, and it was involved in seven pathways (hsa05205, hsa05200, hsa04066, hsa04015, hsa04520, hsa04510 and hsa04913). To predict the strength of the bonds between proteins produced by three core target genes (*AGTR1, MMP9*, and *IGF1R*) and FC to identify potentially active components in the treatment of SH, molecular docking was performed with the use of CB-DOCK (Figure 12). The best binding scores of *AGTR1* with PA and RU were −8.7 and −8.8 (kcal/mol), *MMP9* with KF and PA were −8.4 and −8.1 (kcal/mol), while *IGF1R* with RU and PA were stronger. The docking scores were −9.8 (kcal/mol) and

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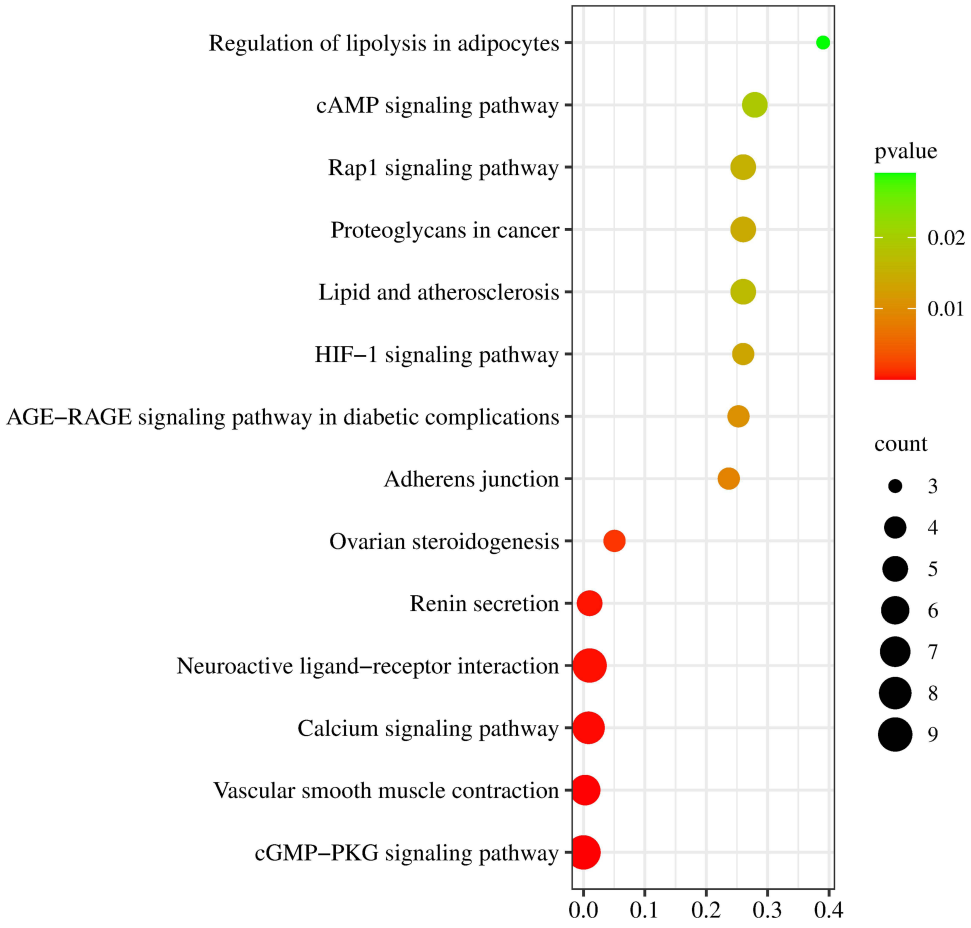

Figure 10 Bubble map of the KEGG enrichment analysis. Bubble colors represent p-value coloring. Enrichment scores are listed on the X-axis and target gene enrichment items are written on the Y-axis. The bubble size represents the number of genes contained in the pathway. The larger the shape, the greater the number of genes contained.

−8.4 (kcal/mol). PA, KF, and RU all have the ability to bind to the proteins of the three core target genes relatively strongly, suggesting that the above three compounds may play a key role in the treatment of SH.

Molecular Docking Analysis

The molecular docking of three repeatedly identified core target proteins, AGTR1, MMP9, and IGF1R, with FC was conducted using CB-Dock2 (see Table 6 for results and Figure 12 for docking diagrams). It is generally accepted that a binding energy (Vina score) below 0 kcal/mol indicates some degree of binding activity between the protein and the compound, while a score lower than −5 kcal/mol suggests strong binding activity.[17] The smaller the binding energy, the higher the stability of the interaction between the protein and compound.[16] Additionally, if the cavity size is close to or larger than that of the ligand, it often enhances docking accuracy. As shown in Table 6, IGF1R and AGTR1 exhibited optimal binding affinity with rutin molecules, achieving docking scores of −9.8 and −8.8 kcal/mol respectively. This was followed by paeoniflorin (−8.4 and −8.7 kcal/mol) and hyperoside (−8.7 and −8.5 kcal/mol). In contrast, MMP9 demonstrated its strongest binding affinity with quercetin at a docking score of −8.4 kcal/mol. It can be inferred that IGF1R and AGTR1 may serve as key genes through which rutin, paeoniflorin, and hyperoside exert their effects; whereas MMP9 appears to be a critical gene mediating the action of quercetin.

Discussion

SH is currently one of the most prevalent cardiovascular diseases worldwide. Its primary clinical characteristic is persistent systemic arterial pressure elevation. The sustained high levels of arterial blood pressure can lead to damage in major vessels, including those supplying the heart and kidneys, as well as other target organs, resulting in a series of

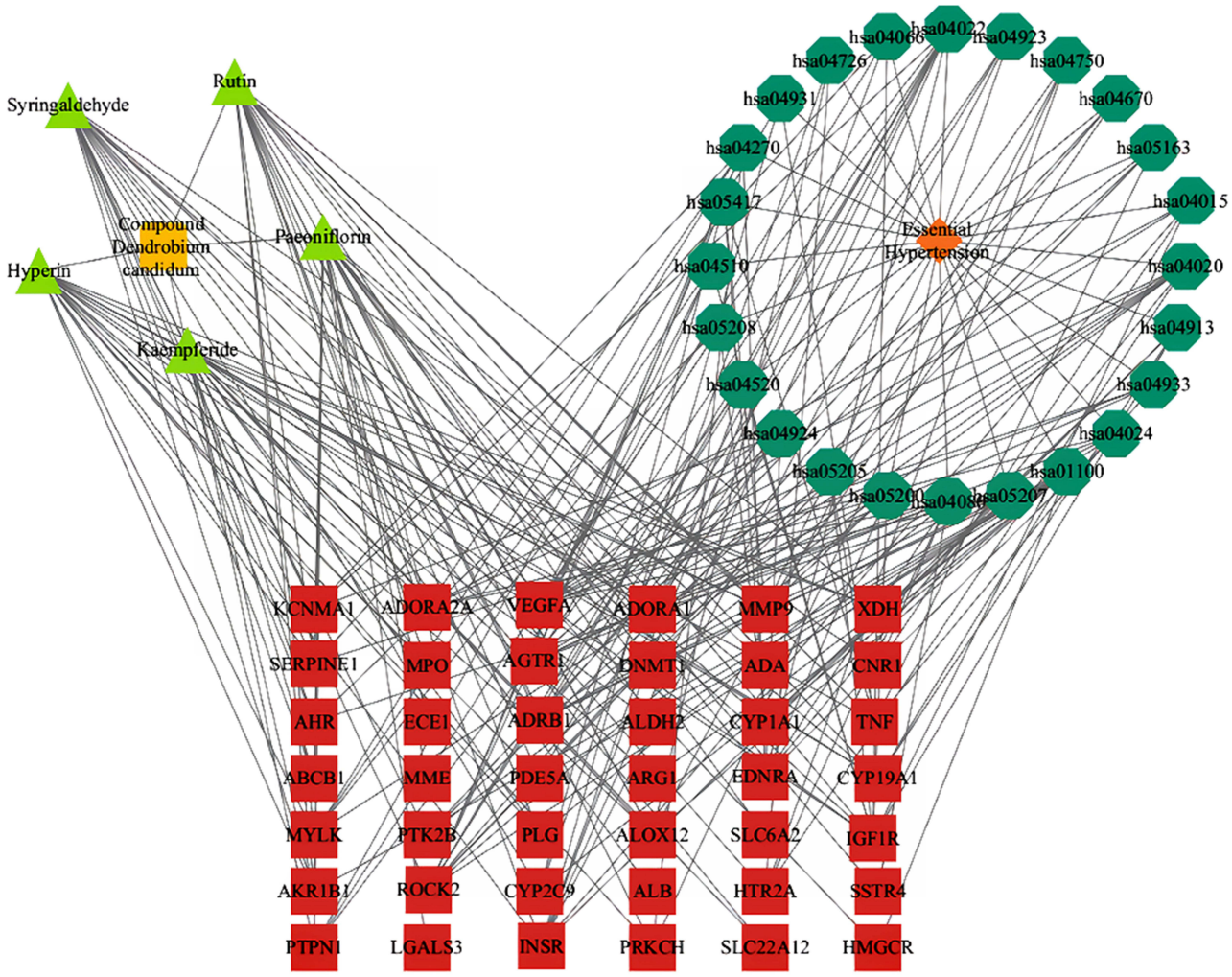

Figure 11 "SH-pathway-target-FC" network diagram. The light-green triangles represent the five compounds, the Orange squares represent compound Dendrobium officinale, the yellow diamonds represent spontaneous hypertension, the dark-green octagons represent pathways, and the red squares represent genes.

severe complications.[18,19] Therefore, the effective and smooth management of blood pressure in patients with hypertension has emerged as a significant public health concern that directly impacts human life and health safety. In recent years, antihypertensive medications have increasingly become the primary blood pressure control method due to their rapid efficacy and the wide range of available drugs that have benefited many patients. However, in this context, studies conducted across various countries indicate that only approximately 30–40% of hypertensive patients derive benefits from Western medicine. About 70% of these patients require a combination of at least two different medications to achieve normal blood pressure levels.[20,21] In addition to the challenges associated with improving blood pressure control rates, the safety and side effects of these medications have garnered increasing attention from researchers. For instance, calcium channel blockers (CCBs) may lead to headaches and edema, while ACEIs can cause dry cough in patients. Furthermore, some studies have demonstrated that patients taking ACEIs experience a significantly increased risk of certain adverse effects.[22–24] In addition, Western medicine has the following limitations: It usually only acts on a single target, and its regulatory effect on the overall physiological function of patients is slightly insufficient. Secondly, the lack of sensitivity of some patients to drugs leads to prolonged medication cycles and increased drug doses.[25–27] As a traditional form of natural medicine in China, TCM possesses unique advantages that have increasingly garnered the attention of scholars regarding its significance in the prevention and treatment of hypertension. Due to its diverse and complex components, TCM can comprehensively regulate blood pressure through multiple targets, thereby

Table 5 Network Analysis Data on "Spontaneously Hypertension-Pathway-Target-Component"

Name	Connection Degree (degree, D)	Betweenness Centrality (BC)	Close to Central (PC)	Average Shortest Path Length (ASPL)
Rutin	19	0.092	0.474	2.111
Paeoniflorin	18	0.184	0.462	2.167
Hyperoside	18	0.078	0.462	2.167
Syringaldehyde	17	0.131	0.456	2.194
Kaempferide	16	0.081	0.450	2.222
hsa01100	13	0.079	0.444	2.250
hsa04080	10	0.039	0.429	2.333
hsa04022	10	0.029	0.429	2.333
hsa04020	9	0.026	0.424	2.361
hsa04270	8	0.020	0.419	2.389
hsa05200	7	0.012	0.414	2.417
hsa05417	6	0.013	0.409	2.444
hsa05205	6	0.008	0.409	2.444
hsa04924	6	0.009	0.409	2.444
VEGFA	11	0.040	0.402	2.486
ROCK2	11	0.032	0.407	2.458
IGF1R	11	0.042	0.436	2.292
INSR	9	0.021	0.385	2.597
ADORA2A	9	0.036	0.442	2.264
MMP9	8	0.034	0.431	2.319
EDNRA	8	0.020	0.398	2.514
AGTR1	8	0.020	0.402	2.486
ADRB1	8	0.016	0.393	2.542
ADORA1	8	0.022	0.398	2.514

demonstrating certain distinct benefits.[28,29] A meta-analysis indicated that, compared with the use of Western medicine alone, the efficacy of the combination of TCM and Western medicine for the treatment of hypertension was significantly enhanced. Furthermore, this combination demonstrated superior antihypertensive safety.[30]

Our study confirmed that the combination of DOC with IR and AM is more effective in lowering blood pressure and has a longer-lasting effect compared with Western medicine alone. To investigate the primary components and potential targets of this compound formulation, we conducted a pharmacodynamic analysis of the key active ingredients from DOC combined with Western medicine in SHRs serum, building upon previous experimental findings. Additionally, we explored related targets through network pharmacology. The results indicated that KF, RU, SY, PA, and HYP were present in the serum of SHRs within the DIA group. However, RU was not detected in the serum of SHRs in the DOC group. Additionally, the concentration of serum monomer components in SHRs from the DIA group was slightly higher than that observed in the DOC group. We speculated that IR and AM could increase the serum concentrations of RU in DOC, which was lower than the detectable range. It has been reported that PA in combination with metoprolol was not only effective in lowering blood pressure in SHRs but that metoprolol increased the blood concentration of PA.[31] The combination of DOC and two Western medicines was identified as the optimal antihypertensive regimen in this study. While the integration of the FC of DOC with two Western medications yielded the most significant antihypertensive effect—observed both at initial administration and one week post-administration—the concentrations of these individual monomers within the combination were considerably higher than those found in the compound formulation of DOC. Therefore, we concluded that the antihypertensive effect of the DOC was superior to that of the mixed solution containing five monomers. This phenomenon may be attributed to the synergistic interactions between certain trace components in DOC and the monomeric constituents, which enhance the pharmacological effects of the individual monomers within the compound formulation. Network pharmacology analyses indicated that *AGTR1, MMP9*, and *IGF1R* may play a crucial role in the antihypertensive effects of DOC. Furthermore, these targets demonstrated strong binding

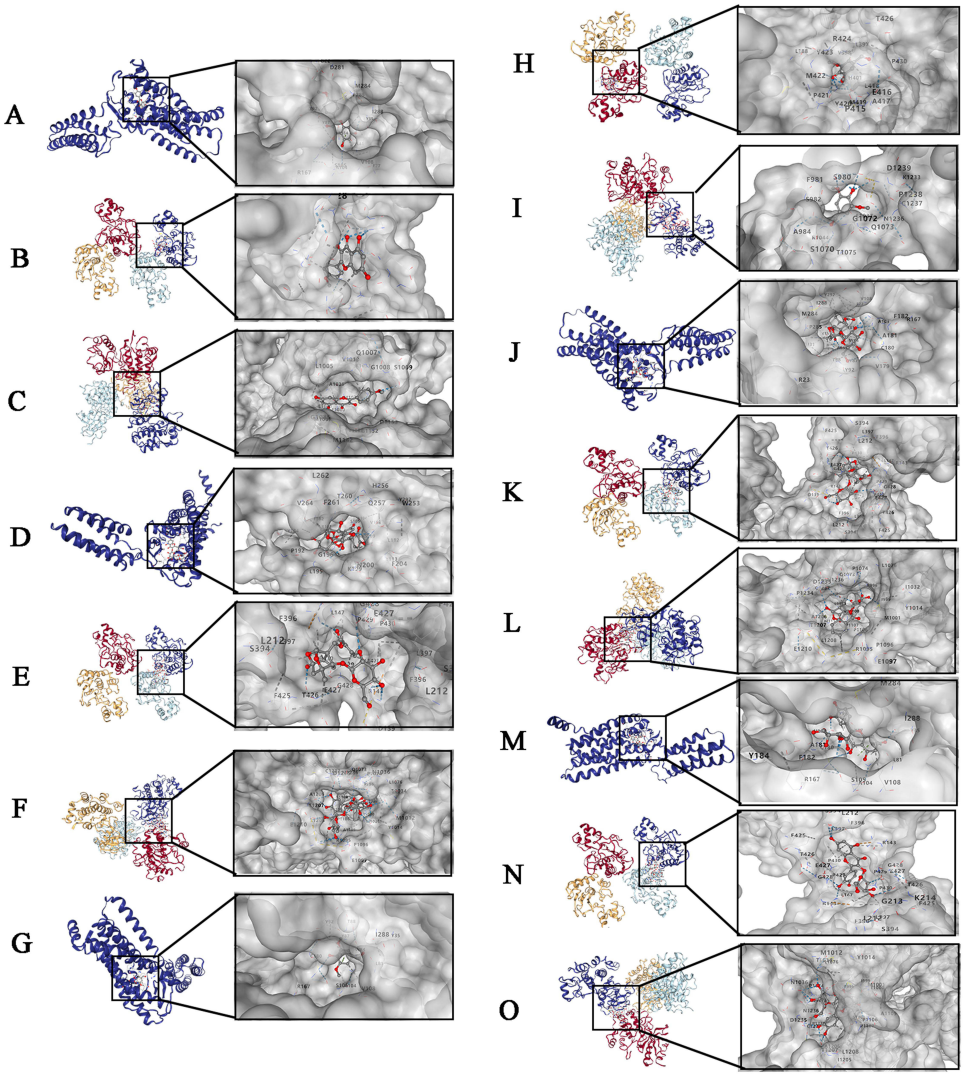

Figure 12 Molecular docking simulation of proteins and compounds. (**A**) Kaempferide-AGTR1 protein molecular docking. (**B**) Kaempferide-MMP9 protein molecular docking. (**C**) Kaempferide-IGF1R protein molecular docking. (**D**) Rutin-AGTR1 protein molecular docking. (**E**) Rutin-MMP9 protein molecular docking. (**F**) Rutin-IGF1R protein molecular docking. (**G**) Syringaldehyde-AGTR1 protein molecular docking. (**H**) Syringaldehyde-MMP9 protein molecular docking. (**I**) Syringaldehyde-IGF1R protein molecular docking. (**J**) Paeoniflorin-AGTR1 protein molecular docking. (**K**) Paeoniflorin-MMP9 protein molecular docking. (**L**) Paeoniflorin-IGF1R protein molecular docking. (**M**) Hyperoside-AGTR1 protein molecular docking. (**N**) Hyperoside-MMP9 protein molecular docking. (**O**) Hyperoside-IGF1R protein molecular docking.

affinity with the three monomers: PA, KF, and RU. Therefore, we propose that PA, KF, and RU are likely to be significant contributors to the therapeutic efficacy of DOC.

KF and RU both belong to the family of polyphenolic bioflavonoids. Studies have found that KF has a certain antihypertensive effect but its specific mechanism is still unclear. Some researchers have found that there is a 4-methoxy group in the B ring of KF chemical structure, which may be related.[32] Through network pharmacology experiments, we found that KF binds strongly to *MMP9*, which may be one of the pharmacological mechanisms by which KF reduces blood pressure. An epidemiological study found that *MMP9* levels were abnormally increased in hypertensive patients, and the increased levels were significantly correlated with blood pressure increments.[33,34] These results suggest that KF can reduce blood pressure by regulating the level of *MMP9* and also prevent the development of atherosclerosis in hypertensive patients.

RU is commonly used as an antifungal and anti-allergic agent.[35] It has been confirmed that RU can lower blood pressure by promoting the relaxation of blood vessel smooth muscles in various animal models of hypertension. Although the precise underlying mechanism remains unclear, some researchers have suggested that this effect may be

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Table 6 Prediction of the Minimum Binding Fraction and Binding Site Coordinates of Core Target Proteins to 5 Blood Components

Ligand	Protein	Vina Score (kcal/mol)	Cavity Volume (Å3)	Center (x, y, z)	Docking Size (x, y, z)
Kaempferide	AGTR1	−8.2	659	−20, 13, 35	21, 21, 21
Kaempferide	MMP9	−8.4	2672	24, 33, 3	29, 21, 21
Kaempferide	IGF1R	−7.7	1650	1, 99, 63	35, 21, 21
Rutin	AGTR1	−8.8	559	−19, 2, 48	22, 22, 22
Rutin	MMP9	−8.0	471	0, 48, 2	22, 22, 22
Rutin	IGF1R	−9.8	3631	12, 79, 57	35, 22, 33
Syringaldehyde	AGTR1	−6.1	659	−20, 13, 35	18, 18, 25
Syringaldehyde	MMP9	−6.5	996	20, 16, 46	18, 18, 18
Syringaldehyde	IGF1R	−5.4	3631	12, 79, 57	35, 26, 33
Paeoniflorin	AGTR1	−8.7	659	−20, 13, 35	22, 22, 22
Paeoniflorin	MMP9	−8.1	2672	24, 33, 3	29, 22, 22
Paeoniflorin	IGF1R	−8.4	3631	12, 79, 57	35, 22, 33
Hyperoside	AGTR1	−8.5	659	−20, 13, 35	22, 22, 22
Hyperoside	MMP9	−7.7	2672	24, 33, 3	29, 22, 22
Hyperoside	IGF1R	−8.7	3631	12, 79, 57	35, 22, 33

attributed to RU's potential action on the angiotensin-converting enzyme (ACE). In an in vitro study, a sustained antihypertensive effect of orally administered RU was observed. The inhibitory effect of RU on ACE was assessed by measuring the IC_{50} of RU (which indicates the concentration of a compound that inhibits ACE activity by 50%). The results showed that ACE was inhibited by different concentrations of RU, and the inhibitory effect of 500 μM RU was about 87%.[36] A cross-sectional study conducted on American adults has revealed an inverse correlation between the intake of flavonoids, including RU, and the occurrence of hypertension events. This finding provides valuable insights for dietary adjustments aimed at preventing high blood pressure.[37] From this study, it can be concluded that RU has good antihypertensive activity. We found that DOC was effective in controlling blood pressure when combined with Western medicine, and RU was one of the most important components of the combination. Network pharmacology analyses revealed that *AGTR1*, a gene encoding the renin-angiotensin-aldosterone system, was the target gene of RU. Based on these results, it may be hypothesized that acting like an ACEI might be one of the pharmacological mechanisms of DOC in lowering blood pressure.

Cardiac remodeling is a compensatory hyperplasia of the myocardium caused by hypertension-induced excessive cardiac load.[38] Studies have shown that hypertension is a chronic inflammatory disease, and inflammation is also a key pathological feature of cardiac remodeling in hypertension, which may lead to left ventricular dysfunction and myocardial fibrosis.[39] PA, the main component of *P. lactiflora*, is an important component of many TCM compound preparations for the treatment of hypertension. Studies have shown that PA has a variety of pharmacological effects such as anti-inflammation, anti-oxidation, and body immunity enhancement, making it an important molecule.[40] PA can reduce blood pressure by regulating the levels of serum NO and endothelin. At the same time, it can activate the eNOS/NO pathway to improve myocardial injury, thereby protecting cardiovascular function.[41] The cardioprotective effect of PA is also reflected in the improvement of cardiac hypertrophy and ventricular remodeling in SHRs. Studies have shown that PA reverses left ventricular dysfunction and left ventricular remodeling in SHRs by regulating the MAPK signaling pathway, mainly manifested as an increase in the left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS), and the levels of some inflammatory factors such as IL-6 and MCP-1 were also significantly decreased.[42] In conclusion, the benefits of PA in hypertensive patients lie not only in blood pressure reduction but also in the protection of cardiovascular endothelial function and the recovery of cardiac remodeling.

In recent years, the combined treatment strategy of integrating TCM with Western medicine for hypertension has gradually gained attention. Numerous studies have demonstrated that the co-administration of traditional herbal remedies

and antihypertensive Western medications can benefit a greater number of hypertensive patients compared to treatment with Western medication alone, while also reducing the risk of adverse events associated with hypertension.[43–45] Our previous research confirmed that a compound formulation containing Dendrobium officinale in conjunction with antihypertensive Western drugs effectively lowers blood pressure in spontaneously hypertensive rats (SHR). In this study, we further identified five key chemical monomers responsible for this effect and predicted their potential targets. Our experimental results not only provide a new reference model for the integrated treatment of hypertension using TCM and Western medicine but also elucidate possible mechanisms by which TCM may exert its therapeutic effects on hypertension. However, certain limitations remain. Firstly, we lack experimental data to validate these targets; this is an area we are currently addressing through ongoing work. We will conduct both in vivo and in vitro experiments to obtain activity data on these targets to substantiate our conclusions. Secondly, the dose-response relationship between the compound formulation containing Dendrobium officinale and antihypertensive Western medications warrants further investigation, particularly concerning its application in treating human hypertension.

Conclusion

In conclusion, our study confirmed that DOC could enhance the antihypertensive efficacy when combined with Western medicine. Western medicine could not only synergistically reduce blood pressure but also increase the concentration of the hypotensive monomers in DOC. In SHRs treated with DOC combined with Western medicine, we found high levels of five monomers, among which RU, PA, and KF were considered to play the major roles. Combined with the above research evidence, we believe that the advantage of DOC combined with Western medicine is not only to improve the antihypertensive effect but also to protect cardiovascular function, prevent myocardial remodeling, and improve chronic inflammation. It also proves that TCM has its unique advantages in the treatment of hypertension. Therefore, it is worthy of further research and application.

Abbreviations

SHRs, spontaneously hypertensive rats; TCM, Traditional Chinese medicine; MRM, Multiple Reaction Monitoring; KF, Kaempferide; RU, Rutin; SY, Syringaldehyde; PA, Paeoniflorin; HYP, Hyperoside; IR, Irbesartan; AM, Amlodipine; PPI, protein-protein interaction; ACEIs, angiotensin-converting enzyme inhibitors; ACE, angiotensin-converting enzyme.

Ethics Statement

This study was approved by the Experimental Animal Welfare Ethics Committee of Zhejiang Academy of Traditional Chinese Medicine (approval number: [2022]025 and [2022]026).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest.

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