Analyzing the pharmacological substances and targets of Xuefu Zhuyu decoction in hypertensive vascular endothelial cells

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Author contributions
Rui-Xue Chen and Li-Guo Chen contributed to study concept; Rui-Xue Chen, Jing Li, Guo-Zhen Dong, Sheng-Yan Qiao, Xiao Hu contributed to study design and performance; Rui-Xue Chen and Jing Li contributed to analysis of data; Rui-Xue Chen contributed to drafting of the paper; Li-Guo Chen contributed to study supervision.

Competing interests
The authors declare no conflicts of interest.

Abstract

BACKGROUND: Xuefu Zhuyu decoction (XFZY) could significantly improve the function of hypertensive vascular endothelial cells, but the targets and mechanism are not clear. This study is to analyze the pharmacological substances and targets of Xuefu Zhuyu decoction in hypertensive vascular endothelial cells. METHODS: This study used Xuefu Zhuyu decoction to intervene human umbilical vein endothelial cells incubated by hypertensive patients’ serum, then detected the function of vascular endothelial cells. The aqueous extract of XFZY was analyzed and validated by liquid chromatography-mass spectrometry technology; Finally, macromolecular docking technology was used to analyze the potential active substances and targets of XFZY in the prevention and treatment of hypertension. RESULTS: Compared with the model group, the XFZY group showed a significant increase in NO expression (P < 0.01) and a significant decrease in ET-1 expression (P < 0.001); and the expression of BIP, P-JNK, CHOP, and BAX in XFZY group cells was significantly decreased (P < 0.001), while the expression of JNK and BCL2 was significantly increased (P < 0.001). 19 main compounds were identified in XFZY and there were 3 pairs of molecular complexes with high affinity for markers of the endoplasmic reticulum stress, including BIP-Hesperidin complex, BIP-HSYA complex and JNK-Naringin complex. Conclusion: This study analyzed the potential pharmacodynamic substance and targets of Xuefu Zhuyu decoction in improving the function of hypertensive vascular endothelial cells, which could provide a scientific basis for the future molecular mechanism of XFZY in treating hypertension.

Keywords: Xuefu Zhuyu decoction; hypertension; vascular endothelial cells; pharmacological substances and targets

References

Hypertension, a chronic systemic disease characterized by elevated blood pressure in the systemic arteries, is the leading cause of cardiovascular disease (CVD) and premature death, affecting the health of 1.39 billion people worldwide [1, 2]. Vascular endothelial dysfunction is one of the major complications of hypertension which could accelerate the development of hypertension and seriously endanger the life and health of patients. According to China's CVD policy model from 2015 to 2025, the implementation of active prevention and treatment strategies during the progression of hypertension could prevent 803,000 cardiovascular events annually and gain an additional 1.2 million quality-adjusted life years (QALY) [3]. There is growing evidence that maintaining vascular health is a critical foundation for preventing CVD. Therefore, the adoption of effective methods to actively prevent and promptly treat hypertensive vascular endothelial dysfunction is of paramount importance for the prognosis of hypertension.

The therapy of promoting blood circulation and removing blood stasis in traditional Chinese medicine has been widely utilized for the treatment of hypertension due to its significant efficacy and favorable prognosis [4]. Xuefu Zhyu decoction (XFZY), a classic formula for promoting blood circulation and removing blood stasis, was originally proposed by Wang Qiwen in the “Correction of Errors in Medical Classics” (Chinese name in pinyin is “Yi Lin Gai Cuo”) during the Qing Dynasty. It is commonly employed in the management of hypertension and other cardiovascular diseases, which is comprised of 11 Chinese herbs, including Persicae Semen, Carthami Flora, Angelicae Sinensis Radix, Chuanxiong Rhizoma, Rehmanniae Radix, Paonieae Radix Rubra, Bupleuri Radix, Platycodonis radix, Auranitii Fructus, Achyranthis Bidentatae Radix, and Glycyrrhizae Radix Et Rhizoma. Modern studies have confirmed that XFZY has the effects on improving vascular endothelial function, anti-platelet aggregation, ameliorating inflammation, promoting angiogenesis, reducing blood lipids and ameliorating myocardial ischemia [5–7]. However, its pharmacological substances and targets for the treatment of hypertension have not been clarified, which will be discussed in this study.

Previous studies showed that the high expression genes in hypertension were mainly enriched in the endoplasmic reticulum stress [8–10]. In this study, the aqueous extract of XFZY was used to interfere with vascular endothelial cells cultured from the serum of hypertensive patients, and the effects on markers related to the endoplasmic reticulum function were observed. Secondly, the major chemical components of the aqueous extract of XFZY were analyzed and verified by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry coupled with mass spectrometry (UHPLC-Q-TOF-MS/MS). Finally, the binding energy of several compounds in XFZY and related markers of endoplasmic reticulum function were predicted by molecular docking technique. This research aimed to explore the therapeutic substances and targets of Xuefu Zhyu decoction in the prevention and treatment of hypertension, and to provide a good scientific basis for the prevention and treatment of hypertension.

Materials and methods

Main instruments and reagents

Human venous vascular endothelial cells (ATCC, USA), Fetal bovine serum (Gibco, USA), High glucose DMEM (Gibco, USA), 0.25 % Trypsin (Gibco, SA), PBS (Gibco, USA), TRizol Reagent (Invitrogen, USA), Griess Reagent System (Sigma, USA), Human Endothelin 1 ELISA kit (RayBiotech, CHN), RCA Protein quantification kit (Biyuantian, CHN), UHPLC-Q-TOF-MS/MS (Agilent, USA), C18 chromatographic column (2.1 × 150 mm, 1.8 μm, Agilent, USA), Cell counting kit (CCK-8, Dojindo, JPN), The reference substances (RS) are as follows: Ligustilide, Platycodin D, Liqiuiritin, Isoliquiritin, Amygdalin, Saikosaponin A, Glycyrrhizic acid, ferulic acid, Naringin, Paeoniflorin, Ononin, Benzoyl paeoniflorin (Shanghai Yuanye Biotechnology Co. Ltd), Hydroxysafflor yellow A, Narcoisside, Hesperidin, Nobiletin, Hesperetin, Naringenin, Senkyunolide A (Chengdu Afa Biotechnology Co. Ltd), etc..

Case collection and sample preparation

According to the diagnostic criteria for “hypertension” in the 1999 World Health Organization/International Hypertension Union Guidelines for the Treatment of Hypertension [11]. A total of thirty outpatients and inpatients diagnosed with hypertension were enrolled in the department of cardiology and traditional Chinese medicine, the Second Affiliated Hospital of Guangzhou Medical University. Additionally, thirty healthy volunteers from Jinan University were also enrolled. Fasting blood samples were collected from the vein of each participant and allowed to stand at room temperature for 30 minutes before centrifugation (4°C, 2000 rpm, 15 minutes). The resulting supernatant was transferred to sterile EP tubes, then inactivated at 56°C for 30 minutes and stored at −80°C. Each sample was thoroughly mixed by combining equal volumes before use. All the participants/patient signed informed consent form. The collection of these clinical samples was in accordance with medical ethics guidelines and was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University (2020-KY-141C).

Prepare the XFZY aqueous extract

The Xuefu Zhyu decoction was prepared by weighing the ingredients according to the original prescription in Yi Lin Gai Cuo. All herbs 76 g were soaked in 500 mL dH2O for 1 h and decocted for 30 min. Then cooled the decoction to room temperature and centrifuged (12000 rpm, 5 min). The supernatant was collected and stored at −20°C. Filtered the XFZY aqueous extract sample through a 0.22 μm ultrafiltration membrane before use.

Cell culture

The human umbilical vein endothelial cells (HUVEC) were inoculated at a density of 1×105 cells/mL into culture flasks and cultured for 24 h. Cell morphology was observed under a microscope. The supernatant was removed and the cells were cultured with basal medium for another 24 hours. Cell morphology was observed before any intervention.

Evaluation of cell viability

Cell viability was evaluated using the cell counting kit-8 (CCK-8). The XFZY aqueous extract was filtered through an ultrafiltration membrane and diluted into different concentrations (5, 10, 20, 40, and 60 times) using basic medium. Normal control group (NC) and blank control group (BC) without cells, were included as controls to calculate the cell survival rate after treatment with each concentration of XFZY.

Cell grouping

The cells were incubated as follows: (1) Normal control group (NC): cells were cultured with 10 % normal human serum and 90 % high-glucose DMEM; (2) Hypertensive endothelial cell injury model group (Model): cells were cultured with 10 % hypertensive patient’s serum plus 90 % high-glucose DMEM; (3) Xuefu Zhyu decoction group (XFZY): Cells were cultured with a combination of 10 % hypertensive patients’ serum plus 90 % high-glucose DMEM, and XFZY.

Cytokine and protein assays

The concentration of NO in cell supernatant was detected by Griess method, and the content of ET-1 in cell supernatant was detected by Elisa kit. Western Blot was used to detect the expression of protein in each group. The expression of BIP, CHOP, BCL2 and other markers were detected. Protein expression activity was expressed as the ratio of grayscale value of protein/GAPDH.

UHPLC-Q-TOF-MS/MS detection

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Using the online software TCMSP (https://tcmspw.com/tcmsp.php) to retrieve the chemical composition of each therapeutic formula medicinal material, thereby establishing a database of the chemical composition for this study. Based on the source materials of each compound, the common compounds present in two or more medicinal materials were screened out. Ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry coupled with mass spectrometry (UHPLC-Q-TOF-MS/MS) is employed with a mobile phase eluted with 0.1% formic acid-methanol gradient, and samples were detected in both positive and negative ion modes. By comparing the primary and secondary ion information of mass spectra, UV spectra, and HPLC retention time with the established chemical composition database, the potential compound constituents were determined. Finally, the compounds identified in the XFZY aqueous extract samples were verified by using reference substances with a purity of 98%, according to the retention time and ion information of mass spectra in UHPLC-Q-TOF-MS/MS.

Autodock VINA analysis
The auto dock VINA molecular docking technology was employed to imitate the interaction between multiple components of XFZY and the BIP/CHOP pathway. PDB files of receptor macromolecules were obtained from the PDB database (http://www1.rcsb.org), while ligand small molecules were obtained from the TCMSP database. The software Autodock VINA was used to match the spatial shape of the ligand small molecule and the receptor protein and the binding energy of the forming complex, so as to predict the active components and target proteins of XFZY in the treatment of hypertension.

Data statistics
The experimental data were statistically analyzed using R (version 4.2.1), and the results were visualized using the install packages (ggplot2). One-way ANOVA was employed to compare the data between groups, while Welch’s one-way ANOVA was used when homogeneity of variance assumptions was violated. The results were presented as mean ± standard deviation (X±SD). The statistical analysis yielded a significant result at the 0.05 level.

Results

Screen the concentration of XFZY
XFZY aqueous extract was diluted with basic medium to interfere with normal HUVEC and cultured for 24 h. It showed that, with the increase of dilution ratio of XFZY, the cellular activity increased. At a 20 times dilution, the cellular activity reached a stable level. Therefore, a 20 times dilution of XFZY aqueous extract was selected for the subsequent steps of the experiment (Figure 1A).

Effect of XFZY on the endoplasmic reticulum stress
Blood serum of hypertensive patients was used to induce human vascular endothelial cells to establish a cell injury model (MODEL), and XFZY was used to intervene. The results demonstrated that compared with the MODEL group, the expression of NO in XFZY group was significantly increased (P < 0.01), and the expression of ET-1 was significantly decreased (P < 0.001). Western Blot results showed that compared with MODEL group, the expressions of BIP, P-JNK, CHOP and BAX in NC group and XFZY group were significantly reduced (P < 0.001), while the expressions of JNK and BCL2 were significantly increased (P < 0.001) (Figure 1B-K).

The results of UHPLC-Q-TOF-MS/MS
The online software TCMSP was utilized to retrieve the constituents in XFZY. After the intersection calculation of all components, 252 compounds from two or more herbs were obtained, which were used as the constituents database for this study. The XFZY aqueous extract samples were analyzed using ultra-high performance liquid chromatography (UHPLC). The C18 column (2.1*150 mm, 1.8 μm) was run with 100% methanol, and the mobile phase was pre-equilibrated for 2 minutes before injection, with an initial concentration. The injection volume was set at 2 μL. A gradient elution method used a mobile phase consisting of a 0.1% formic acid aqueous solution (phase A) and methanol (phase B). Ensure that all components in the samples are elution under one chromatographic condition. The specific elution conditions were as follows: 1-2 min(95 % A: 5 % B); 2-15 min(50 % A: 50 % B); 15-30 min(5 % A: 95 % B); 30-35 min(5 % A: 95 % B); 35-37 min(0 % A:100 % B); 37-47 min(0 % A: 100 % B). Flow rate: 0.4 mL/min, column temperature: 40 °C, DAD detection wavelength: 254 nm.

Figure 1 The effect of XFZY aqueous extract on vascular endothelial cells induced by hypertensive patients’ serum. The impact of varying dilutions of XFZY on cellular viability (A). The levels of NO and ET-1 in the cell supernatant (B, C). Western Blot results of BIP, JNK, P-JNK, P-JNK/JNK, CHOP, BCL2, BAX (D-K). *P < 0.05, **P < 0.01, ***P < 0.001
Ultra-high performance liquid chromatography-mass spectrometry technology was used to analyze the UV chromatographic peak of the XFZY aqueous extract sample. Combined with electrospray ionization (ESI) and quadrupole-Time of Flight (Q-TOF) mass analyzer. The MS conditions were as follows: Gas Temp: 320 °C; Gas Flow: 8 L/min; Nebulizer: 45 psig. Sheath Gas Temp: 350 °C; Sheath Gas Flow: 11 L/min; VCap: 3500 V; Nozzle Voltage: 500 V; Fragmentor: 120 V. The UHPLC-Q-TOF-MS/MS detection results showed that the ion response in positive ion mode is higher than in negative ion mode, so the mass spectrometry data is mainly analyzed in positive ion mode (Figure 2A-B).

According to the chemical composition database and the detection results obtained from UHPLC-Q-TOF-MS/MS, the ion chromatographic peaks in the total ion chromatogram (TIC) of the XFZY aqueous extract were analyzed and identified. These peaks were subsequently identified in both the ultraviolet spectrum and TIC. It showed that the UV absorption of compound 11–19 is weak (Figure 2C). The main identification results for chromatographic peaks in the XFZY aqueous extract samples are presented as follows (Table 1).

Figure 2 Identification of the main chromatographic peaks of the XFZY aqueous extract. A: TIC positive ion mode; B: TIC negative ion mode; C: UV chromatogram of UHPLC.

Table 1 Identification of multiple chromatographic peaks of the XFZY aqueous extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Rt (min)</th>
<th>Species</th>
<th>m/z</th>
<th>MW (Da)</th>
<th>Compound</th>
<th>Formula</th>
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<tr>
<td>1</td>
<td>6.646</td>
<td>[M+H]^+</td>
<td>475.1923</td>
<td>457.1584</td>
<td>Amygdalin</td>
<td>C_{24}H_{25}NO_{11}</td>
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<td>2</td>
<td>6.646</td>
<td>[M+H]^+</td>
<td>613.1765</td>
<td>612.169</td>
<td>Hydroxysafflor yellow A</td>
<td>C_{24}H_{26}O_{14}</td>
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<tr>
<td>3</td>
<td>9.230</td>
<td>[M+NH4]^+</td>
<td>498.1980</td>
<td>480.1634</td>
<td>Paoniflorin</td>
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<td>4</td>
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<td>[M+H]^+</td>
<td>193.0510</td>
<td>194.0579</td>
<td>Ferulic acid</td>
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<tr>
<td>5</td>
<td>11.052</td>
<td>[M+H]^+</td>
<td>417.1327</td>
<td>418.126</td>
<td>Liquiritin</td>
<td>C_{14}H_{10}O_{7}</td>
</tr>
<tr>
<td>6</td>
<td>13.655</td>
<td>[M+H]^+</td>
<td>581.1865</td>
<td>580.1793</td>
<td>Naringin</td>
<td>C_{19}H_{14}O_{9}</td>
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<td>7</td>
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<td>Hesperidin</td>
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<td>8</td>
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<td>11</td>
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<td>273.0752</td>
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<td>13</td>
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<td>190.0992</td>
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</tr>
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<td>780.466</td>
<td>Saikosaponin A</td>
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</table>
Results of validation of reference substances

According to the 19 chemical components identified in the chromatographic peaks, the corresponding reference substances (RS) were selected, and the molecular structure formula of each RS was as follows (Figure 3). 5 mg of each of the 19 RS was dissolved in anhydrous methanol and the solution was fixed to 1 mL. Equal volumes of the standard solutions were thoroughly mixed to create a control solution. The solution was filtered using a 0.22 µm ultrafiltration membrane prior to injection.

Based on the UHPLC chromatographic conditions of the XFZY aqueous extract, the UHPLC detection of the mixed RS sample was carried out. The UV chromatographic peaks of the XFZY sample and the mixed RS sample were observed, which showed that the retention times of the target compounds and RS were similar. Notably, the RS14 and RS19 showed no significant UV absorption (Figure 4). According to the ion mass-to-charge ratio of the target compound in the sample, the ion signal is extracted so as to verify whether the ESI mass spectrometry data (mass and peak time of ion fragments) of the compound and the RS were consistent. It showed that the ESI data of compounds 1–19 were basically consistent with those of the 19 reference substances.

Results of Autodock VINA analysis

The AutoDock VINA molecular docking technology was employed to predict the binding interactions between the 19 compounds of XFZY and the endoplasmic reticulum stress. The PDB files of the markers, including BIP, JNK, and CHOP, were obtained from the PDB database, and molecular docking was performed with the ligand small molecules to simulate the formation of the complexes and predict the binding energy of the complexes. The results showed that the CHOP protein molecule has a special structure and can not form a complex with any molecule; the other protein receptor molecules can form complexes with several compounds ligand molecules, and examples of pairs of molecules with higher affinity are as follows: The binding energy of BIP-Hesperidin complex is –10.64 kcal/mol; the binding energy of BIP-HSYA complex is –8.47 kcal/mol; and the binding energy of JNK-Naringin complex is –9.37 kcal/mol (Figure 5).

Figure 3 The molecular structures of the 19 reference substances.
**Conclusions and discussion**

Clinical studies have confirmed that Xuefu Zhuyu decoction has a significant effect on hypertension, which may be related to improving vascular endothelial function, anti-platelet aggregation, and improving inflammation, etc. However, the pharmacological substances and targets of its action in hypertension have not been elucidated. In this study, Xuefu Zhuyu decoction was used to intervene in the vascular endothelial cells induced by the hypertensive patients’ serum, and then the expression levels of NO and ET-1 were evaluated in the cell supernatant. It showed that Xuefu Zhuyu decoction could significantly improve the function of vascular endothelial cells induced by the hypertensive patients’ serum. It also showed that XFZY could inhibit the endoplasmic reticulum stress and apoptosis of endothelial cells induced by the hypertensive patients’ serum. In addition, UHPLC-Q-TOF-MS/MS was used to analyze the main compounds in the XFZY aqueous extract and the 19 chemical substances were verified, including: Amygaldin, Hydroxysafflor yellow A (HSYA), Paeoniflorin, ferulic acid, Liquiritin, Naringin, Hesperidin, Ononin, Isoliquiritin, Narcissoside, Naringenin, Hesperetin, Benzoyl paeoniflorin, Platycodin D, Senkyunolide A, Ligustilide, Nobiletin, Glycyrrhizic acid, Saikosaponin A. Finally, the AutoDock VINA molecular docking technique was used to examine the affinity of several molecule pairs. It showed that the following pairs of molecules have obvious affinity: BIP-Hesperidin, BIP-HSYA, and JNK-Naringin. The study suggested that there may be several compounds in Xuefu Zhuyu decoction that directly target the endoplasmic reticulum stress.

The immunoglobulin heavy-chain binding protein BIP acts as a sensor for maintaining endoplasmic reticulum homeostasis and interacts with three endoplasmic reticulum transmembrane sensing proteins under normal physiological conditions. When endoplasmic reticulum stress occurs, BIP dissociates from transmembrane sensing proteins and instead binds to a substantial quantity of unfolded proteins, thereby facilitating proper protein folding. Upon dissociation from BIP, the transmembrane sensing proteins become activated to initiate the unfolded protein response and restore endoplasmic reticulum homeostasis to promote cell survival [12, 13]. CHOP is an important signaling molecule in the transition from anti-apoptotic to...
pro-apoptotic. When endoplasmic reticulum stress occurs continuously, CHOP is heavily activated by the unfolded protein response and regulates multiple downstream apoptotic mechanisms [14–16]. In this study, Xuefu Zhuyu decoction could inhibit the unfolded protein response and apoptosis in vascular endothelial cells induced by hypertensive patients’ serum, which may be the mechanism of its action in improving hypertension. Modern medical treatment of hypertension is based on antihypertensive drugs and a reasonable diet. Although there is a certain effect, long-term or irreligious use of antihypertensive drugs will lead to a variety of adverse reactions, which seriously affect the prognosis of patients [17]. Traditional Chinese medicine (TCM) has achieved significant and safe therapeutic effects in patients with hypertension, among which XFZY is widely used in hypertension and other cardiovascular diseases [18, 19]. Pharmacological studies of XFZY are very important to understand the basis of its efficacy and mechanism of action. Researchers have extracted XFZY in various ways and the extracted compounds are relatively complicated. However, the aqueous decoction of XFZY is commonly used in clinical treatments, so the XFZY aqueous extract was used for chemical analysis in this study. In addition, UHPLC-Q-TOF/MS/MS technique was used for the analysis of XFZY, for the reason that it is one of the effective means to identify the complex components of natural products due to its rapid analysis, high sensitivity and resolution, and good reproducibility. In this study, 19 compounds were screened in XFZY aqueous extract. Pharmacological studies have confirmed that several of these compounds play important roles in cardiovascular diseases. For example, the flavonoid HSYA can exert a multifaceted protective role in the cardiovascular system by inhibiting inflammatory responses, suppressing cardiac cell apoptosis, regulating autophagy, modulating vascular function and angiogenesis [20–22]. The molecular docking results suggested that there are several potential pharmacodynamic substances in Xuefu Zhuyu decoction that target endoplasmic reticulum stress, which may be the underlying mechanism of Xuefu Zhuyu decoction on hypertension.

In conclusion, this study combined experimental research and molecular docking techniques to analyze the underlying mechanism of XFZY in the prevention and treatment of hypertension through improving endoplasmic reticulum function. It was found that Xuefu Zhuyu decoction can improve vascular endothelial function in hypertension, which might be related to its improvement of endoplasmic reticulum function. Multiple compounds in Xuefu Zhuyu decoction were verified, and their binding energy with endoplasmic reticulum was predicted by molecular docking technology. Several pairs of potential therapeutic substances and targets were screened, which provided important reference value for further elucidating the therapeutic mechanism of Xuefu Zhuyu decoction. However, there are limitations to this study; for example, it only predicted the therapeutic substances and targets of Xuefu Zhuyu decoction in the treatment of hypertension, which still needs to be verified by experimental research in the future. It is hoped that with the research progress of the modernization of TCM, its mechanism and clinical application will be widely recognized at home and abroad, and the prospect of TCM in the prevention and treatment of diseases will be broader.

References


17. GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural,


