An Integrative Pharmacology-Based Strategy to Uncover the Mechanism of Xiong-Pi-Fang in Treating Coronary Heart Disease with Depression

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Objectives: This study aimed to explore the mechanism of Xiong-Pi-Fang (XPF) in the treatment of coronary heart disease (CHD) with depression by an integrative strategy combining serum pharmacochemistry, network pharmacology analysis, and experimental validation.

Methods: An ultrahigh performance liquid chromatography-quadrupole-time-of-flight tandem mass spectrometry (UPLC-Q-TOF/MS) method was constructed to identify compounds in rat serum after oral administration of XPF, and a component-target network was established using Cytoscape, between the targets of XPF ingredients and CHD with depression. Furthermore, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were performed to deduce the mechanism of XPF in treating CHD with depression. Finally, in a chronic unpredictable mild stress (CUMS)-and isoproterenol (ISO)-induced rat model, TUNEL was used to detect the apoptosis index of the myocardium and hippocampus, ELISA and western blot were used to detect the predicted hub targets, namely AngII, 5-HT, cAMP, PKA, CREB, BDNF, Bcl-2, Bax, Cyt-c, and caspase-3.

Results: We identified 51 compounds in rat serum after oral administration of XPF, which mainly included phenolic acids, saponins, and flavonoids. Network pharmacology analysis revealed that XPF may regulate targets, such as ACE2, HTR1A, HTR2A, AKT1, PKA, CREB1, BDNF, BCL2, BAX, CASP3, cAMP signaling pathway, and cell apoptosis process in the treatment of CHD with depression. ELISA analysis showed that XPF decreased Ang-II content in the circulation and central nervous system, inhibited 5-HT levels in peripheral circulation, and increased 5-HT content in the myocardia and hippocampus. Meanwhile, western blot analysis indicated that XPF could upregulate the expression levels of PKA, CREB, and BDNF both in the myocardia and hippocampus. TUNEL staining indicated that the apoptosis index of myocardial and hippocampal cells increased in CUMS-and ISO-induced CHD in rats under depression, and XPF could increase the expression of Bcl-2, inhibit the expression of Bax, Cyt-c, and caspase-3, and rectify the injury of the hippocampus and myocardium, which exerted antidepressant and antmyocardial ischemia effects.
**INTRODUCTION**

Coronary heart disease (CHD) is a chronic and complex disease that poses a serious threat to human health (Mozaffarian et al., 2016; Blais et al., 2020). Traditional risk factors are mainly related to hypertension, hyperlipidemia, diabetes, etc (Li C et al., 2020). Moreover, depression is another independent risk factor for CHD, as it has been shown to diminish the quality of life of patients with CHD and also increase the incidence and mortality of major adverse cardiac events (Su et al., 2018; de Heer et al., 2020). Increasing evidence suggests that the incidence of CHD with depression is increasing, ranging from approximately 15–18%, moreover, approximately 31% of these patients develop major depressive disorder (MDD) (Carney and Freedland, 2017). However, there are no effective chemicals to improve the quality of life and survival rate of patients with comorbid CHD and depression (Lee et al., 2018; Magaard et al., 2018).

The ideal medication for CHD with depression would be integrated, improving myocardial blood supply and regulating nervous system function at the same time (Yeung et al., 2014; Chen M et al., 2018; Xue et al., 2019). Xiong-Pi-Fang (XPF), a classical traditional Chinese medicine (TCM) formula, consists of Radix Bupleuri (Bupleurum chinense DC. and Bupleurum scorzonerifolium Willd.) 15 g; Ligusticum wallichii (Carney and Freedland, 2017). However, there are no effective chemicals to improve the quality of life and survival rate of patients with comorbid CHD and depression (Lee et al., 2018; Magaard et al., 2018).

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Integrative pharmacology, combined with conventional pharmacology, network pharmacology, bioinformatics, and other disciplines, is the systematic study of the overall interactions between drugs and humans at the molecular, cell, organ, and network levels (Hart and Xie, 2016; Zhang R. et al., 2018; Zhang R. et al., 2019). Network pharmacology, characterized by integration, systematization, and emphasis on drug interaction (Yuan et al., 2017), predicts the biological molecular mechanism of drug treatment of diseases from a holistic perspective by constructing an interaction network between active ingredients and disease targets. However, most TCM-related databases provide information on chemical ingredients extracted *in vitro* (Zhang W et al., 2019). Previous studies have shown that the drug ingredients absorbed into the blood circulation may be the main active constituents (Chen et al., 2014; Li et al., 2016). Therefore, it is critical to combine serum pharmacochemistry with a network pharmacology strategy to accurately establish the network.

In this study, an integrated pharmacology strategy employing serum pharmacochemistry, network pharmacological analysis, and experimental validation was conducted to illustrate the therapeutic mechanism of XPF in treating CHD with depression (Figure 1). Briefly, an ultrahigh performance liquid chromatography-quadrupole-time-of-flight tandem mass spectrometry (UPLC-Q-TOF/MS) method was established to determine the main active components of XPF in rat serum. Furthermore, a serum pharmacochemistry-based network was constructed and the component–target network between CHD comorbid depression-relevant genes and the targets of active components in XPF was established. Finally, we validated the predicted molecular mechanisms obtained from the network analysis of XPF in treating CHD with depression in a chronic unpredictable mild stress (CUMS)-and isoproterenol (ISO)-induced CHD in a rat model of depression.

**MATERIALS AND METHODS**

**Reagents and Materials**

Sertraline hydrochloride (Pfizer Inc., United States), metoprolol (Asilkan, United Kingdom), Isoproterenol (Sigma Aldrich Co., United States) were purchased from the Shanghai Yuanye Biotechnology Co. Ltd (Shanghai, China). UPLC-Q-TOF/MS grade acetonitrile and HPLC grade acetonitrile, methanol, formic acid, were provided by Fisher Scientific International Inc (Fair Lawn, NJ). The ELISA assay kits for Ang-II (142191202), 5-HT (106191202), cAMP (202004) were purchased from Tianjin Zihan Biotechnology Co., Ltd. (Tianjin, China). TUNEL assay kit (7E310H9) was purchased from Vazyme Biotech Co., Ltd. (Nanjing, China), Rabbit

**Conclusion:** Our study proposed an integrated strategy, combining serum pharmacochemistry and network pharmacology to investigate the mechanisms of XPF in treating CHD with depression. The mechanism of XPF in treating CHD with depression may be related to the activation of the cAMP signaling pathway and the inhibition of the apoptosis.

**Keywords:** integrative pharmacology, serum pharmacochemistry, network pharmacology analysis, Xiong-Pi-Fang, coronary heart disease with depression.
antihuman monoclonal antibodies PKA (5842), CREB (6188), BDNF (3189), Bcl2 (26,599-1-12p), Bax (50,599–2-1 g), Cytochrome c (10993-1-Ap), and mouse antihuman monoclonal antibodies caspase-3 (66,470-E1g) and β-actin were provided by Proteintech Group, Inc. (Wuhan, China).

Animals
Male Sprague-Dawley rats (weighing 200 ± 20 g) were purchased from the Experimental Animal Center of the Academy of Military Medical Sciences (Beijing, China), and kept in an environmentally controlled room (temperature 22 ± 2°C, humidity 50 ± 10%) with food and water available. This study was carried out in accordance with the principles of the Basel Declaration and the guidelines of the National Institutes of Health. All animal experiments were approved by the Ethics Committee of Tianjin University of Traditional Chinese Medicine (Tianjin, China).

UPLC-Q-TOF/MS Method for Serum Pharmacochemistry Analysis
XPF was prepared by the pharmacy of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China). Twelve rats were randomly divided into a control group (n = 6, 0.9% saline IG), and According to the calculation method of equivalent dose coefficient of experimental animals in Methodology of Pharmacological Experiment (Xu et al., 2018), the XPF group (n = 6, 32.7 g/kg XPF IG), equivalent to twice the clinical effective dose, for three days (Xu et al., 1997). All rats fasted for 12 h before the experiment and then the serum samples were collected at 120 min after oral administration, via the postorbital venous plexus. The serum samples were centrifuged at 12,000 rpm for 10 min at 4°C. Then, 300 µL of serum was added to three times the amount of acetonitrile, vortexed for 2 min, followed by ultrasonic extraction for 10 min, and centrifugation (12,000 rpm, 10 min). The supernatant was collected and placed in a N2 blower to blow dry at 40°C, 50% acetonitrile (50 µL) was added to the residue, vortexed for 3 min, ultrasound for 10 min, centrifugation (12,000 rpm, 10 min), and the supernatant was collected for UPLC-Q-TOF/MS analysis.

UPLC-Q-TOF/MS analysis was analyzed on a Waters ACQUITYTM UPLC BEH C18 (100 mm × 2.1 mm, 1.7 µm) system, maintained at 30 °C. The flow rate was set at 0.45 ml/min, and the injection volume was 5 µL. The mobile phase was consisted of water (A) and methanol (B) both containing 0.1% (v/v) formic acid for astragaloside (0–5 min, 2% B; 5–8 min, 2%–20% B, 8–11 min, 20% B, 11–14 min, 20%–48% B, 14–20 min, 48%–70% B, 20–23 min, 70%–90% B, 23–26 min, 90%–100% B, 26–30 min, 100% B). The mass spectrometer analysis was performed with reaction monitoring both in positive and negative ion modes.

Construction of the Compound-Target and Disease-Target Networks
All targets of active components of XPF determined by serum pharmacochemistry analysis were collected from TCM-related databases, including TCMS (http://tcmspw.com/tcmsp.php)
Known therapeutic targets related to CHD and depression were obtained from the DrugBank database (Wishart et al., 2018), Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org) (Amberger and Hamosh, 2017), DisGeNet database

(Ru et al., 2014), DrugBank (https://www.drugbank.ca/) (Wishart et al., 2018), Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Gjöller et al., 2014), and Similarity Ensemble Approach (http://sea.bkslab.org/) (Wang et al., 2016).
The regimen consisted of immobilization for 45° for 12 h; continuous illumination for 24 h; clip inducing depression. The regimen of XPF in the treatment of CHD with depression was established using Cytoscape (Version 3.8.0) (Otasek et al., 2019).

**Topological Analysis and Pathway Enrichment Analysis**

A network analyzer was used to calculate the topological analysis of the nodes of the component-target network. Nodes with degrees higher than the average number 4) were identified as the core targets, and were brought into the STRING database (https://string-db.org/) (Szklarczyk et al., 2017) to obtain the interacting proteins. Then, the PPI network was constructed using Cytoscape for visual analysis and, according to interactive processes and pathways (cutoff at p < 0.05) of XPF in treating CHD with depression.

**Animal Model Preparation and Drug Treatments**

Sixty-four male Sprague-Dawley rats (weighing 200 ± 20 g) were randomly divided into a normal control group (n = 8) and a depressive-like behavioral group (n = 56). The normal control group received 0.9% saline via oral administration. The depressive-like behavioral group (n = 8, 16.35 g/kg, IG), was exposed to chronic unpredictable mild stress (CUMS) to induce depression. The regimen consisted of immobilization for 4 h; cage tilting at 45° for 12 h; continuous illumination for 24 h; clip the distal 1 cm rat tail with tongs for 3 min; deprivation of water for 24 h; noise stimulation for 30 min; damp animal bedding for 24 h; foot shock for 3 min; removal of animal bedding for 24 h; high-speed agitation for 10 min; deprivation of food 24 h; and forced cold swim stress for 6 min at 4 °C. Each animal received two stressors randomly per day for a total of 28 days. The normal control group (n = 8) remained undisturbed. Subsequently, the CUMS-induced rats were injected with ISO (8 mg/kg) for five days to form the CHD model (Aa et al., 2019; Hu et al., 2020). The rat model for CHD with depression induced by CUMS and ISO was evaluated by measuring rat body weight change, open field test, sucrose preference test, and ST-segment of the electrocardiogram. Then, all CHD with depression rats were randomly divided into a model group (n = 15, 0.9% saline, IG), Ser + Met group (n = 8, sertraline 10 mg/kg + metoprolol 5 mg/kg, IG), Ser group (n = 8, sertraline 10 mg/kg, IG), Met group (n = 8, metoprolol 10 mg/kg, IG), high-dose XPF (XPF-H) group (n = 8, 32.7 g/kg, IG), equivalent to CH 3.13 g/kg, CX 2.5 g/kg, XF 2.5 g/kg, TX 1.88 g/kg, ZQ 3.13 g/kg, CP 3.13 g/kg, BX 3.13 g/kg, BH 2.08 g/kg, FL 3.13 g/kg, BZ 3.13 g/kg, ZS 3.13 g/kg, GC 3.13 g/kg, medium-dose XPF (XPF-M) group (n = 8, 16.35 g/kg, IG), equivalent to CH 1.65 g/kg, CX 1.25 g/kg, XF 1.25 g/kg, TX 0.94 g/kg, ZQ 1.65 g/kg, CP 1.65 g/kg, BX 1.65 g/kg, BH 1.04 g/kg, FL 1.65 g/kg, BZ 1.65 g/kg, ZS 1.65 g/kg, GC 0.94 g/kg, and low-dose XPF (XPF-L) group (n = 8, 8.175 g/kg, IG), equivalent to CH 0.78 g/kg, CX 0.62 g/kg, XF 0.63 g/kg, TX 0.47 g/kg, ZQ 0.78 g/kg, CP 0.78 g/kg, BX 0.78 g/kg, BH 0.52 g/kg, FL 0.78 g/kg, BZ 0.78 g/kg, ZS 0.78 g/kg, GC 0.47 g/kg. The control group received 0.9% saline via oral administration. The drugs were administered daily for three weeks, while the animals were exposed to CUMS, except for the control group. At 24 h after the last treatment, all rats were sacrificed, and the brain, hippocampus, and heart tissues were rapidly extracted from each rat, stored at −80 °C until analysis. One part of the brain and heart tissues were placed into a flask containing 4% paraformaldehyde.

**TUNEL Staining**

TUNEL staining was performed according to the manufacturer’s instructions, to determine hippocampus and myocardial apoptosis. The apoptotic cells showed red fluorescence and the nucleus showed blue fluorescence, six high-power fields were selected from each sample. All cells and positive stained cells were counted, the percentage of positively stained cells was apoptotic index (AI) (AI = number of apoptotic cells/total number of nucleated cells).

**ELISA Analysis**

The level of 5-HT, Ang-II, cAMP in hippocampus and myocardium were quantified by ELISA assay kit, according to the manufacturer’s instruction. After color development, the absorbance was measured at 450 nm with fluorescence reader (THERMO USA).

**Western Blot Analysis**

Hippocampus, hearts tissue proteins were mechanically homogenized in lysis buffer, centrifuged at 12,000 rpm for 10 min at 4 °C, collected the supernatant. BCA protein assay was used to determine protein concentrations. Equal concentrations of protein were resolved on 10% SDS-PAGE gels, and were transferred onto PVDF membranes. These membranes PVDF were soaked in TBST buffer with 5% non-fat skim milk for 2 h, and then These membranes PVDF were incubated in the primary antibodies (PKA, CREB, BDNF, Bcl-2, Bax, Cyt-c, caspase-3, and β-actin) overnight at 4 °C. Subsequently, the membrane was washed for 5 times with TBST, followed by secondary antibodies incubated with horseradish peroxidase for 2 h at room temperature. After rewashing with TBST, the membranes were scanned on X-ray film by chemiluminescence reaction, used ImageJ software to analysis the band intensity.

**Statistical Analysis**

Data were expressed as mean ± SD. Differences between groups were analyzed using a one-way analysis of variance (ANOVA), followed by Dunnett’s t test. All data were analyzed statistically.
RESULTS

Serum Pharmacochemistry Analysis of XPF by UPLC-Q-TOF/MS

Based on the established UPLC-Q-TOF/MS method, 51 serum prototypes of the drugs were analyzed and identified (Table 1). These compounds can be roughly divided into three categories: phenolic acids, saponins, and flavonoids. Figure 2 shows the base peak chromatogram (BPC) of each typical sample in the mode of positive and negative ions, and peaks 1–51 are the original components entering the blood. These 51 compounds of XPF detected in the serum were determined to be the main active components and further selected to predict the targets and pathways using network analysis.

Component-Target Network Construction

In this work, 820 targets for the 51 (Nine of them had no therapeutic targets) components were explored by using TCMSP, Swiss Target Prediction, Drugbank, and Swiss Target Prediction as shown in Supplementary Table S1, and 1817 candidate targets of CHD and depression were obtained from Drugbank, OMIM, and DisGeNet databases as shown in Supplementary Table S2. Taking the intersection of component targets and candidate targets associated with CHD and depression, 168 consensus targets were generated as potential targets for XPF in treating CHD and depression, which were used to construct a component–target network using Cytoscape. As shown in Figure 3A, the network comprised 42 components, 168 targets, and two diseases, and included 212 nodes and 845 edges.

Topological Analysis and Pathway Enrichment Analysis

Among the 168 consensus targets, 75 major targets with degrees higher than the average number 4) were considered as the major targets (Table 2). Ten components with degrees higher than 20 were identified as the main active components (Kaempferol and quercetin were not included because they are widely distributed and have many targets, but their pharmacological effects are weak) (Table 3). The 75 major targets were brought into the STRING database to obtain protein-protein interaction (PPI) predictions. The PPI network was constructed using Cytoscape, and hide disconnected nodes in the network (5 nodes). A network analyzer was used to calculate topological features (degree) of the 70 major targets (Figure 3B). Among these, 10 target genes (ACE2, HTR1A, HTR2A, AKT1, PKIA, CREB1, BDNF, BCL2, BAX, and CASP3), with higher degree were recognized as the major hub targets of XPF in treating CHD with depression. KEGG pathway enrichment and GO analyses of the 75 major targets were performed using the DAVID database. GO analysis showed 184 enriched processes (Supplementary Table S3), including 126 biological processes, 39 molecular functions, and 19 cellular components. The top 30 according to p-values are shown in Figure 4A. KEGG pathway enrichment showed 117 signaling pathways, as shown in Supplementary Table S4, and the top 20 pathways according to p-values are shown in Figure 4B.
Experimental Validation
Evaluation of Depressive-like Behaviors of Chronic Stress-Depressed Rats

The open field test can reflect the spatial exploration behavior of rats (Chen et al., 2019), and sucrose preference test could be the objective indicator of hedonic behavior (Gross and Pinhasov, 2016b). All rats were evaluated by open field test, sucrose preference test before and after the CUMS and ISO-induced process, then all CHD with depression rats were randomly divided into 7 groups. After three weeks of administration, open field test and sucrose preference test were performed again of all rats. As shown in Table 4, after CUMS and ISO-induced, compared with the control group the open-field test and sucrose preference scores were significantly reduced in the model group and each administration groups (p < 0.005). It was shown that the depression model was successfully established. After treatment for 21 days, compared with the model group, XPF administration significantly improve the open-field test and sucrose preference scores (p < 0.005).

Effects of XPF on Myocardial and Hippocampal Apoptosis

Apoptosis can cause myocardial and hippocampal injury and dysfunction, which are the major mechanisms of CHD and depression. As shown in Figure 5, the myocardial and hippocampus of the CUMS and ISO-induced rats were injured. The effects of XPF on myocardial apoptosis are shown in Figure 5A. And the effects of XPF on hippocampal apoptosis are shown in Figure 5B. Compared with the control group, the myocardial and TUNEL-positive cells were significantly increased in the model group, as shown in Figure 5C (p < 0.005). The myocardial apoptotic index of the XPF-H and XPF-M groups was significantly lower than that of the model group (p < 0.01). Sertraline 10 mg/kg + metoprolol 5 mg/kg and metoprolol 10 mg/kg also significantly inhibited the apoptosis in the myocardia (Figure 5C) (p < 0.005). Sertraline (10 mg/kg) and low-dose XPF treatment did not alter myocardial apoptosis.

There were significant differences in the total apoptotic index in the hippocampus after XPF treatment for 21 days. In the model group, the apoptotic index significantly increased compared with the control group, as shown in Figure 5D. Furthermore, XPF administration significantly decreased the apoptotic index in the hippocampus. Additionally, sertraline and metoprolol also significantly inhibited apoptosis in the hippocampus (p < 0.005).

The results indicated that the brain and myocardial tissues of the rats were injured after CUMS and ISO-induced damage. It was shown that the CHD with depression model was successfully established, while the treatment of XPF can inhibit apoptosis of myocardial and brain tissue simultaneously.

According to key proteins with a higher degree in the PPI network and KEGG pathway enrichment analysis, it is suggested that the mechanism of XPF in the treatment of CHD with depression may be through the regulation of ACE2, HTR1A, HTR2A, AKT1, PKIA, CREB1, BDNF, BCL2, BAX, CASP3, cAMP signaling pathway, and the cell apoptosis process. As the second intracellular messenger, cAMP regulates the expression of downstream proteins and plays an important role in cell growth and apoptosis (Zhou et al., 2012). Bcl-2 and Bax, as
targets downstream of CREB and BDNF, regulate apoptosis through the mitochondrial pathway (Mi et al., 2015; Tang et al., 2016). Recent studies have shown that the cAMP-PKA-CREB-BDNF signaling pathway is closely related to depression (Wang et al., 2017; Ye et al., 2017). Meanwhile, the related proteins in the cAMP-PKA-CREB-BDNF signaling pathway can also improve myocardial microcirculation, improve the ability of myocardial cells to resist ischemia and hypoxia, and effectively prevent the development and deterioration of CHD (Huang et al., 2016). To verify whether XPF works in treating CHD with depression by intervening in the cAMP-PKA-CREB-BDNF signaling pathway and cell apoptosis process, the expression of 5-HT, Ang-II, cAMP, PKA, CREB, BDNF, Bcl-2, Bax, Cyt-c, and caspase-3 in the myocardial and hippocampal tissues were detected.

3.4.3 Effects of XPF on the Expression of Ang-II, 5-HT, and cAMP in Myocardial Tissues

As shown in Figures 6A–C, it was noted that compared with the control group, the levels of Ang-II and 5-HT were significantly increased in the myocardial tissues in the model group. Compared with the control group, the expression of cAMP in the myocardial tissues was markedly lower in the model group (p < 0.005). After treatment for 21 days, high-dose XPF decreased the levels of Ang-II and 5-HT in the myocardium (p < 0.01). The treatments with middle-dose XPF solely decreased the levels of Ang-II (p < 0.05), whereas low-dose XPF had no effect on the levels of Ang-II and 5-HT in the myocardium. The levels of cAMP were significantly increased, in a dose-dependent manner, in the myocardium of the rats in the XPF-H, XPF-M, and XPF-L groups (p < 0.005).

3.4.4 Effects of XPF on the Expression of Ang-II and 5-HT cAMP in the Hippocampus

As shown in Figures 6D–F, it was noted that compared with the control group, the levels of Ang-II and 5-HT in the hippocampus were markedly lower in the model group (p < 0.005). After treatment for 21 days, high-dose XPF decreased the levels of Ang-II and 5-HT in the hippocampus (p < 0.01). The treatments with middle-dose XPF solely decreased the levels of Ang-II (p < 0.05), whereas low-dose XPF had no effect on the levels of Ang-II and 5-HT in the hippocampus. The levels of cAMP were significantly increased, in a dose-dependent manner, in the hippocampus of the rats in the XPF-H, XPF-M, and XPF-L groups (p < 0.005).

| TABLE 2 | The topological parameters of 32 major targets. |
| Swiss prot | Genes/proteins | Description | Degree |
| P31749 | AKT1 | RAC-alpha serine/threonine-protein kinase | 74 |
| P05231 | IL6 | Interleukin-6 | 88 |
| P15692 | VEGFA | Vascular endothelial growth factor A | 64 |
| P04637 | TPS3 | Cellular tumor antigen p53 | 61 |
| P01100 | FOS | Proto-oncogene c-Fos | 59 |
| P01375 | TNF | Tumor necrosis factor | 59 |
| P28482 | MAPK1 | Mitogen-activated protein kinase 1 | 58 |
| P45983 | MAPK8 | Mitogen-activated protein kinase 8 | 56 |
| P29474 | NOS3 | Nitric oxide synthase | 55 |
| P35354 | PTGS2 | Prostaglandin G/H synthase 2 | 54 |
| P16220 | CREB1 | Cyclic AMP-responsive element-binding protein 1 | 53 |
| P01133 | EGF | Pro-epidermal growth factor | 53 |
| P06412 | JUN | Transcription factor AP-1 | 53 |
| P23560 | BDNF | Brain-derived neurotrophic factor | 52 |
| P01106 | MYC | Myc proto-oncogene protein | 50 |
| P01584 | IL1B | Interleukin-1 beta | 49 |
| P03372 | ESR1 | Estrogen receptor | 48 |
| P09601 | HMOX1 | Heme oxygenase 1 | 47 |
| P37231 | PPARG | Peroxisome proliferator-activated receptor gamma | 45 |
| P22301 | IL10 | Interleukin-10 | 45 |
| P10790 | ACE | Angiotensin-converting enzyme | 44 |
| P07900 | HSP90AA1 | Heat shock protein HSP 90-alpha | 43 |
| P05362 | ICAM1 | Intercellular adhesion molecule 1 | 42 |
| P60484 | PTEN | Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN | 42 |
| Q04206 | RELA | Transcription factor p65 | 41 |
| P05112 | IL4 | Interleukin-4 | 40 |
| P42224 | STAT1 | Signal transducer and activator of transcription 1-alpha/beta | 38 |
| P01579 | IFNG | Interferon gamma | 38 |
| P60568 | IL2 | Interleukin-2 | 38 |
| P19320 | VCAM1 | Vascular cell adhesion protein 1 | 37 |
| P02741 | CRP | C-reactive protein | 37 |

| TABLE 3 | The topological parameters of 20 main active components. |
| Compounds | Degree |
| Eugenol | 28 |
| Emodin | 27 |
| Isorhamnetin | 25 |
| Nobiletin | 23 |
| Isoliquiritigenin | 22 |
| Rosmarinic acid | 22 |
| 4-Hydroxy-3 -butylphthalide | 21 |
| Acacetin | 20 |

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high-dose XPF and middle-dose XPF significantly decreased the levels of Ang-II (p < 0.005) and increased the levels of 5-HT and cAMP in the hippocampus (p < 0.05). Similarly, treatment with metoprolol markedly decreased the levels of Ang-II, and treatment with sertraline increased the levels of 5-HT and cAMP in the hippocampus (p < 0.05). However, low-dose XPF had no effect on the levels of Ang-II, 5-HT, and cAMP in the hippocampus.

Effects of XPF on the Expression of PKA, CREB, and BDNF in the Hippocampus

As shown in Figures 7D–F, the PKA, CREB, and BDNF protein levels of the model group were significantly lower than those of the control group. High-dose XPF and middle-dose XPF were able to upregulate the expression of PKA, CREB, and BDNF compared with the model group (p < 0.05). However, low-dose XPF had no effect on the levels of CREB and BDNF in the hippocampus.
Effects of XPF on the Expression of Bcl-2, Bax, Cyt-C, and Caspase-3 in the Myocardium

As shown in Figure 8 and Table 5, compared with the control group, the levels of Bax, Cyt-c, and caspase-3 were significantly increased in the model group, although Bcl-2 levels significantly decreased. Treatment with XPF significantly increased the levels of Bcl-2 and decreased the levels of Bax, Cyt-c, and caspase-3 \((p < 0.005)\), in a dose-dependent manner.

Effects of XPF on the Expression of Bcl-2, Bax, Cyt-C, and Caspase-3 in the Hippocampus

As shown in Figure 9 and Table 6, the results of the WB analysis indicated that the expression of Bax, Cyt-c, and caspase-3 was significantly increased in the model group, while Bcl-2 levels were significantly decreased. After treatment for 21 days, high-dose XPF and middle-dose XPF significantly decreased the levels of Bax, Cyt-c, and caspase-3 \((p < 0.005)\). High-dose, and middle-dose XPF also increased the levels of Bcl-2 \((p < 0.05)\), in a dose-dependent manner. In contrast, low-dose XPF only inhibited the expression of Bax and Cyt-c \((p < 0.005)\), but had no effect on the expression of Bcl-2 and caspase-3.

DISCUSSION

XPF, characterized by multiple components and targets, has been shown to have a relatively satisfactory therapeutic effect in treating CHD with depression. XPF can improve myocardial blood supply and mental state of patients at the same time, which reflects the overall concept of traditional Chinese medicine (Hou et al., 2017). Through this study, we found that XPF could protect the myocardium and hippocampus in CUMS and ISO-induced CHD in depression rats, it shows that XPF has the advantages and characteristics of treating the heart and brain at the same time. However, it is difficult to accurately identify and understand the active compounds and mechanisms of XPF in treating CHD with depression solely by using conventional pharmacological methods. Therefore, new research approaches to reveal the interaction between components of TCM and biological system networks are urgently required. Here, an integrated strategy was established by combining serum pharmacochemistry and network pharmacology to comprehensively and systematically investigate the components and mechanisms of XPF in treating CHD with depression, and validated in experiments.

In this study, 51 compounds were detected in rat serum after oral administration of XPF using UPLC-Q-TOF/MS technology. The identified constituents, mainly phenolic acids, saponins, and flavonoids, were determined to be the main active components of XPF. Among them, phenolic acids, such as rosmarinic acid and eugenol, can inhibit vascular smooth muscle cell proliferation and have a cardioprotective effect (Liu Y et al., 2018). Rosmarinic acid can treat neurodegenerative diseases via anti-neuroinflammatory activity (Wei et al., 2018). Furthermore, saponins, such as saikosaponin A, are the major bioactive component extracted from Radix Bupleuri (Chen et al., 2018; Liu R et al., 2018) and...
have anti-inflammatory and antioxidant pharmacological activities, as well as a good therapeutic effect on many diseases of the central nervous system and cardiovascular system (Guo et al., 2020). Flavonoids, such as hesperidin and hesperetin, can inhibit inflammatory reactions and oxidative stress, and protect nerve cells and vascular endothelium (Hwang et al., 2012; Van Rysmenant et al., 2017).

Moreover, the component–target network between the targets of absorbed components in the serum of XPF and CHD with depression was built using Cytoscape, followed by topological parameters and PPI analysis. As a result, 8 components (eugenol, emodin, isorhamnetin, nobiletin, isoliquiritigenin, rosinarinic acid, 4-Hydroxy-3-butyphthalide, and acacetin), with higher degree centrality were determined to be the active ingredients of XPF, and 10 hub targets, including (ACE2, HTR1A, HTR2A, AKT1, PKIA, CREB1, BDNF, BCL2, BAX, CASP3, cAMP) were determined as hub targets of XPF in treating CHD with depression. GO analysis and KEGG pathway enrichment analysis showed that XPF may regulate the cAMP signaling and apoptosis pathways by interfering with the expression of Ang-II, 5-HT, cAMP, PKA, CREB, BDNF, Bcl-2, Bax, Cyt-c, and caspase-3.

In this study, CUMS and ISO-induced CHD in a rat model of depression was used to explore the mechanism of XPF in treating CHD with depression. These rats showed a large number of apoptotic cells in myocardial and hippocampal tissues compared with the control group, which indicated that the CHD with depression model was successfully established. Sertraline and metoprolol were used as positive control drugs to treat CHD in rats with depression. Sertraline can increase the content of 5-hydroxytryptamine (5-HT) in the brain and exert antidepressant-like effects (Sun et al., 2020). Metoprolol is a beta-blocker commonly used in the clinic (Podlesnikar et al., 2019; Qin et al., 2020). Studies have shown that metoprolol could reduce the oxygen consumption of the myocardium and reduce the risk of cardiovascular events (Assimon et al., 2018). However, co-administration of sertraline and metoprolol increases the blood concentration of metoprolol. Therefore, we adjusted the dose of metoprolol in the sertraline and metoprolol groups in this study (Bahar et al., 2018).

Under these experimental conditions, XPF could decrease Ang-II content in the circulation and central system, inhibit 5-HT level in peripheral circulation, and increase 5-HT content in the central nervous system. Meanwhile, XPF could upregulate the expression levels of cAMP, PKA, CREB, and BDNF both in the myocardium and hippocampus, which matched the predicted results of the network pharmacological analysis. Interestingly, sertraline regulated the cAMP, PKA, CREB, and BDNF levels in the hippocampus, but had a limited effect on the myocardium. Metoprolol can regulate the cAMP, PKA, CREB, and BDNF expression in myocardial, but it has a limited effect on the central nervous system. XPF treatment not only activated the cAMP signal pathway, but also further inhibited apoptosis and protected...
the myocardium and hippocampus. The cAMP signaling pathway can increase the expression of Bcl-2 and inhibit the activation of caspase-3 by regulating the expression of CREB and BDNF proteins (Mo et al., 2020; Yang et al., 2020). In this study, XPF rectified the injury of the hippocampus and myocardium caused by CHD in depressed rats, significantly decreasing the hippocampal and myocardial levels of Bax, Cyt-c, caspase-3, and increased Bcl-2 expression, which suggests that XPF may inhibit hippocampal and myocardial apoptosis, improve heart function and nerve function, and exert antidepressant and anti-myocardial ischemia effects.

Chronic stressful life events can stimulate the renin-angiotensin-aldosterone system (RAAS). Evidence has shown that Ang-II levels are significantly higher in patients with CHD and depression (Amare et al., 2017; Wang and Mao, 2019; Sandrini et al., 2020). In this study, XPF rectified the injury of the hippocampus and myocardium caused by CHD in depressed rats, significantly decreasing the hippocampal and myocardial levels of Bax, Cyt-c, caspase-3, and increased Bcl-2 expression, which suggests that XPF may inhibit hippocampal and myocardial apoptosis, improve heart function and nerve function, and exert antidepressant and anti-myocardial ischemia effects.

Chronic stressful life events can stimulate the renin-angiotensin-aldosterone system (RAAS). Evidence has shown that Ang-II levels are significantly higher in patients with CHD and depression. Ang-II is metabolized into Ang-(1–7), which directly interferes with the intestinal uptake of tryptophan and affects the metabolism of 5-HT (Klempin et al., 2018; W et al., 2020). The decrease of 5-HT levels in the central nervous system leads to depression or other mental disorders (Jiao et al., 2019; Wenxiu et al., 2020). 5-HT metabolic disorder inhibits the cAMP signaling pathway (Louiset et al., 2017). The cAMP signaling pathway was observed in both CHD and depression patients (Amare et al., 2017; Wang and Mao, 2019; Sandrini et al., 2020).

Activated cAMP and PKA can phosphorylate the Ca$^{2+}$ channel on the cell membrane, promote calcium flow in cardiomyocytes, promote excitation contraction coupling, enhance myocardial contractility, improve the anti-ischemic effects of cardiomyocytes, and produce protective effects on cardiomyocytes (Inserte et al., 2004; Zhang et al., 2010). At the same time, cAMP and PKA are also widely distributed in the nervous system, which can guide the regeneration of neurons, improve the plasticity of synapses, and play a role in protecting the nervous system (Zhang M et al., 2018). CREB is involved in a wide range of neural plasticity processes, including neuronal survival, neuroprotection, neurogenesis, synaptic plasticity, and regulation of emotional expression (Liu et al., 2016; Tang et al., 2020). BDNF is a classical downstream target gene of CREB, which can nourish nerves and promote the differentiation, increment, and regeneration of neurons (Wang et al., 2019). BDNF also has a modulatory role in cardiovascular function, is involved in angiogenesis maintenance of endothelial integrity (Kaess et al., 2015; Jin et al., 2018), repairing myocardial
microcirculation, and maintaining myocardial cell function after myocardial injury (Lee et al., 2017). Low expression of CREB and BDNF causes Bax overexpression, Bcl-2 expression is inhibited, and the promotion of the release of apoptosis factors such as cytochrome c (Cyt-c) into the cytoplasm and increased caspase-3. Caspase-3, the final executive factor of apoptosis, leads to apoptosis (Xu et al., 2018). Bcl-2 can form a heterodimer with Bax to inhibit its function, Bcl-2 can also inhibit the activation of caspase-3, and inhibit apoptosis (Abu et al., 2018). Meng (Meng and Wang, 2013) have found that Baishile (BSL) capsule could significantly improve the CUMS rats with depression-like behavior; K252a could block the cAMP signaling pathway to antagonize the antidepressant effect of BSL capsule. Jiang (Jiang et al., 2017) have found that acupuncture could improve depressive-like behaviors via PKA/CREB signaling pathway, H89 could block the PKA/CREB signaling pathway to antagonize the antidepressant effect of acupuncture. Mao (Mao and Zhang, 2014) have found H89 could block the PKA/CREB signaling pathway to antagonize the myocardial protective effect of Danlou tablets. Therefore, enhanced expression of related proteins in the cAMP signaling pathway and inhibition of the cell apoptosis process contributes to the alleviation of angina pectoris, chest pain, and depression symptoms. Essentially, it promotes protection of the cardiovascular and nervous system against coronary heart disease with depression.

The 8 active components (eugenol, emodin, isorhamnetin, nobiletin, isoliquiritigenin, rosmarinic acid, 4-Hydroxy-3-butylyphthalide, and more) could significantly regulate the expression of Bcl-2, Bax, Cyt-c, and caspase-3 in rat myocardium. These findings provide a potential pharmacological basis for the clinical treatment of coronary heart disease with depression.

**TABLE 5** | Comparison of gray values of Bcl-2, Bax, Cyt-C and Caspase-3 in myocardium of rats in different groups.

| Group         | Bal-2/β-actin (Mean ± SD) | Bax/β-actin (Mean ± SD) | Cyt-c/β-actin (Mean ± SD) | Caspase/β-actin (Mean ± SD) |
|---------------|---------------------------|-------------------------|---------------------------|-----------------------------|
| Control       | 0.9080 ± 0.0083           | 0.1728 ± 0.0084         | 0.1437 ± 0.0077           | 0.1139 ± 0.0071             |
| Model         | 0.2864 ± 0.0076***        | 0.8701 ± 0.0143***      | 0.8300 ± 0.0159***        | 0.6454 ± 0.0072***          |
| Ser + Met     | 0.6814 ± 0.0072***        | 0.3348 ± 0.0142***      | 0.3945 ± 0.0075***        | 0.278 ± 0.0067***           |
| Ser           | 0.6505 ± 0.0065***        | 0.3850 ± 0.0065***      | 0.2534 ± 0.0065***        | 0.2743 ± 0.0044***          |
| Met           | 0.6419 ± 0.0067***        | 0.1408 ± 0.0070***      | 0.1615 ± 0.0020***        | 0.1328 ± 0.0029***          |
| XPF-H         | 0.9032 ± 0.0055***        | 0.5394 ± 0.0084***      | 0.3657 ± 0.0113***        | 0.4830 ± 0.0104***          |
| XPF-M         | 0.7402 ± 0.0084***        | 0.7331 ± 0.0173***      | 0.5366 ± 0.0082***        | 0.5879 ± 0.0126***          |
| XPF-L         | 0.5665 ± 0.0130***        | 0.8143 ± 0.0053***      | 0.6337 ± 0.0096***        | 0.6024 ± 0.0069***          |

Values are expressed as the mean ± SD; n = 3 in each group. Compared with control, ***P < 0.005. Compared with model, ###P < 0.005.
and acacetin) in XPF have been reported to activate the cAMP signaling pathway of CHD and depression. Eugenol can reverse oxidative stress, inhibit caspase-3 activity, and it has an anti-myocardial ischemia effect. Emodin opposes CUMS-induced depressive-like behavior in rats by upregulating the levels of hippocampal BDNF (Choudhary et al., 2006; Liu et al., 2017). Isorhamnetin has a positive effect on H/R-induced injury by reducing caspase-3 and attenuating oxidative stress in H9c2 cardiomyocytes (Zhao et al., 2018). Nobiletin and acacetin exerted antidepressant-like effects by increasing the level of 5-HT in the hippocampus (Yi et al., 2011). Isoliquiritigenin has been shown to have neuroprotective effects by increasing the level of cAMP in peripheral nerves (Shindo et al., 1992). Rosmarinic acid can regulate the expression of BDNF in the hippocampus (Makhathini et al., 2018), and inhibit angiotensin-converting enzyme activity (Ferreira et al., 2018).

Increasing evidence has shown a reciprocal causation relationship between CHD and depression. Regulating psychopathy can significantly improve the quality of life of patients with CHD and reduce the incidence of acute cardiovascular events (Kuhlmann et al., 2019). Recently, TCM has attracted increasing attention in treating cardiovascular and psychological diseases because of its holistic approach and satisfactory clinical efficacy. Through this study, we found that XPF could protect the myocardium and hippocampus in CUMS and ISO-induced CHD in depression rats by regulating the cAMP signal cascade. Furthermore, XPF can inhibit cell apoptosis, improve heart function, reduce neuropathy, improve nerve function, and exert an antidepressant and antimyocardial ischemia effect. These results preliminarily show the material basis and mechanism of XPF in the treatment of CHD with depression.
CONCLUSION

In this study, we proposed an integrative systems pharmacology strategy to illustrate the mechanism of XPF in treating CHD with depression by combining serum pharmacochemistry, network analysis, and experimental validation. First, we identified 51 ingredients in rat serum after oral administration of XPF, which was used for the construction and analysis of the component–target network between the targets of absorbed components in serum and CHD with depression. As a result, 10 components with higher degree centrality were determined to be the active ingredients of XPF, and 10 hub genes, including ACE2, HTR1A, HTR2A, AKT1, PKIA, CREB1, BDNF, BCL2, BAX, CASP3, and cAMP were determined as targets of XPF in treating CHD with depression. Furthermore, the hub targets (AngII, 5-HT, cAMP, PKA, CREB, BDNF, Bcl-2, Bax, Cyt-c, and caspase-3) predicted by network pharmacology analysis were validated. We confirmed the myocardial protective and neuroprotective effects of XPF in treating CHD with depression, which was associated with its activation of the cAMP signaling pathway and inhibition of myocardium and hippocampus apoptosis. In conclusion, these results indicate that the integrated pharmacology strategy provides an efficient approach for exploring the pharmacological mechanisms of TCM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by and the guidelines of the National Institutes of Health. All animal experiments were approved by the Ethics Committee of Tianjin University of Traditional Chinese Medicine. Written informed consent was obtained from the owners for the participation of their animals in this study.

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AUTHOR CONTRIBUTIONS

LZ is the first author and performed all the experiments and drafted the manuscript. YZ contributed toward study design and revised the manuscript. LP, FD, and JC helped the first author, prepared the materials of this paper. ZC, SZ, and MZ provided fund support. WD and XX contributed toward study design, experimental setup, results supervision, and manuscript correction.

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SUPPLEMENTARY MATERIAL

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