Effectiveness and mechanism of Huoxin pill on heart failure after percutaneous coronary intervention: Study protocol for a double-blind, randomised, placebo-controlled parallel trial

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ABSTRACT

Background: Coronary heart disease (CHD) is the most common cardiovascular disease facing human beings. Cardiac remodelling is an important pathological factor for the progression of heart failure (HF) after CHD. At present, Chinese medicine is widely used in the treatment of HF, but there are still some drugs lack of evidence-based and mechanism evidence. Multi-omics techniques can deep explore candidate pathogenic factors and construct gene regulatory networks. This trial is intended to evaluate the effect on Huoxin pill (HXP) in the treatment of HF after programmable communication interface (PCI). Meantime, multi-omics analysis technique will be used to target the fundamental pathological links of cardiac remodelling, so as to study the mechanism of HXP in the treatment of HF after PCI.
Methods: This study is a randomized, double-blind, placebo-controlled trial. Sixty patients with HF undergoing PCI are recruited from the First Affiliated Hospital of Henan University of CM. All selected patients will be randomly attributed to receive conventional treatment + HXP or placebo. The packaging, dosage and smell of placebo and heart activating pill were identical. The primary outcome is NYHA cardiac function grade, while the secondary outcomes included Lee's HF score, exercise tolerance test, and quality of life evaluation. Additional indicators include cardiac ultrasound, electrocardiogram, 24-h dynamic electrocardiogram, myocardial injury indicators, and energy metabolism indicators.
Discussion: This study may provide a new treatment option for patients with HF after PCI and provide evidence for the treatment of CHD and HF with HXP.
Trial registration: 2023-10-08 registered in China Clinical Trial Registry, registration number ChiCTR2300076402.

1. Introduction

1.1. Background and rationale

Heart failure (HF) is the final stage of various heart diseases, which seriously affects the health of all human beings owing to its high morbidity, hospitalisation and mortality rates [1]. In China, with the aging of the population and the improvement of medical care, the incidence of chronic diseases such as coronary heart disease (CHD), hypertension, and diabetes mellitus is increasing, which further aggravates the incidence of HF. The results of the 2012–2015 epidemiological survey showed [2] that the prevalence of HF in China continues to grow, and the prevalence of HF in the population aged 35 years and above is about 1.3 %, with an estimated number of up to 13.7 million people, which puts a This has brought a high burden on China's social health-care. CHD has now become the principal primary cause of HF in China,

with an estimated 11.39 million current patients [3]. Although the development of early reperfusion strategies, including coronary stenting and pharmacological treatments, has dramatically reduced the short-term mortality rate after acute infarction, epidemiological studies have shown that the prevalence of HF is increasing year by year [4]. In addition, the incidence of HF was 19.3 % within 7 days after MI, and 13.1 %–37.5 % within 30 days to 6.7 years after myocardial infarction. The incidence of HF after myocardial infarction significantly increased the risk of short-term and long-term death, while the prognosis was poor [5].

Cardiac remodelling is a variety of adaptive responses that occur in the heart under stress, initially compensating to maintain basic cardiac function, but then progressing to the stage of decompensation can induce HF and arrhythmia, and ultimately endanger life. Cardiac remodelling after coronary artery disease includes myocardial remodelling, which directly affects the mechanical function of the heart; electrical remodelling, which induces malignant arrhythmias; and vascular remodelling, which can lead to deterioration of cardiac function, sudden cardiac death, and other cardiovascular events [6,7]. In the process of evolution of myocardial ischemia to cardiac remodelling, the energy metabolism of the myocardium is present throughout the process. In post-infarction myocardium from compensated to decompensated phase, there are varying degrees of damage to the mitochondrial structure, and increased reactive oxygen species (ROS) attack mitochondrial DNA and proteins, disrupting the balance of oxidative stress and creating a vicious cycle. This further aggravates the energy metabolism disorder. In conclusion, cardiomyocyte hypoxia triggers reduced mitochondrial biosynthesis, decreased ATP synthesis, insufficient energy yield, impaired oxidative phosphorylation, altered mitochondrial morphology, and aggravated inflammation and oxidative stress, which is a key factor in causing post-infarction cardiac remodelling, promoting ventricular pathologic remodelling, and ultimately progressing coronary infarction to HF [8,9].

In recent years, Chinese medicine has excelled in the prevention and treatment of post-infarction HF, with its own unique advantages in improving clinical symptoms, delaying disease evolution, and improving prognosis, especially in alleviating recurrent hospitalisations for HF, altering exercise tolerance, and improving quality of life, which is expected to become a new strategy for solving the difficult problem of HF. Therefore, it is of great practical significance to actively study effective Chinese medicines for preventing or delaying cardiac remodelling after coronary heart attack in order to reduce the occurrence of angina pectoris, arrhythmia, and HF after coronary heart attack and to improve the prognosis [10,11].

HXP belongs to the national basic medicinal varieties, manufactured by Guangzhou Yuekang Biopharmaceutical Co., Ltd. HXP is composed of Ganoderma lucidum, Ginseng, Safflower, borneol and other herbs, which is suitable for CHD and angina pectoris. In the study of post-infarction HF rats, HXP [12–16] showed antimyocardial fibrosis, reduced apoptosis of cardiomyocytes, prevented acute myocardial ischemic injury, and attenuated inflammatory response, etc. Clinical studies have shown that HXP can significantly improve angina symptoms and duration of attacks, relieve the degree of angina pain, and reduce the daily use of nitroglycerin in patients [17]. At the same time, HXP combined with western medicine can improve the endothelial function of blood vessels in AMI patients, regulate coronary blood circulation, and reduce myocardial blood reperfusion injury [18]. However, the current clinical application of HXP is mostly limited to the treatment of angina pectoris, and its clinical application is not perfect and lacks evidence support. Meanwhile, there are fewer clinical studies on HXP for the treatment of post-infarction HF, with a low level of evidence, and the studies were conducted at an earlier time, lacking the observation of the effects of indicators commonly used in clinical practice at present, especially lacking high-quality clinical trials.

The present study is expected to evaluate the efficacy and safety of HXP in the treatment of Qi deficiency and blood stasis syndrome of HF

after CHD through a randomised, double-blind, placebo and controlled trial. On the other hand, a multi-omics approach will be adopted to interpret the core targets of HXP under the complex TCM syndromes [19], and to focus on the biomarkers of qi deficiency and blood stasis by comparing the healthy and peaceful subjects, and to construct a protein-pathway-metabolite network to elucidate the mechanism of HXP in the treatment of HF in coronary artery disease.

1.2. Methods/design

In this study, a randomized double-blind placebo-controlled trial will be used to determine the clinical efficacy of HXP in the treatment of HF after PCI, and its mechanism of action will be analyzed by multi-omics technology to provide a new strategy for the treatment of HF. HXP (concentrated pill) which produced by Guangzhou Yuekang Biopharmaceutical Co., Ltd. was approved by the State Food and Drug Administration of China (State Drug Permit Z44021835) and registered in the China Clinical Trial Registry (ChiCTR 2300076402) on October 8, 2023. The study was also reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (2023HL-330-01).

1.3. Patient population and setting

In this trial, the diagnosis of CHD will refer to the ACCF/AHA 2014 Guidelines for the Management of Patients with Unstable Angina/Non-ST-Segment Elevation Myocardial, the European Society of Cardiology 2017 Guidelines for the Management of Non-ST-Segment Elevation Acute Coronary Syndromes, the Chinese Society of Cardiovascular Disease Branch of Chinese Medical Association 2017 Guidelines for the Diagnosis and Treatment of Acute ST-Segment Elevation Myocardial Infarction, and the China Society of Chinese Medicine, Cardiovascular Disease Branch, 2018 "Expert Consensus on Chinese Medicine Diagnosis and Treatment of Stable Angina in Coronary Heart Disease", Chinese Medical Association, Cardiovascular Disease Branch, 2018 "Guidelines for Diagnosis and Treatment of Stable Coronary Heart Disease", SIGN 2018 "2018 SIGN National Clinical Guidelines: management of stable angina pectoris (151)", and the 2016 "Chinese Percutaneous Coronary Intervention Guidelines.

Clinical diagnosis was made on the basis of 1) coronary angiography suggesting a stenosis of 50 % or more of the lumen diameter of at least one major branch of the coronary artery, with or without revascularization; 2) history of coronary intervention; 3) history of coronary artery bypass grafting; and 4) history of previous myocardial infarction, with or without evidence of revascularization (PCI or CABG) treatment.

HF will be referred to the Chinese Guidelines for the Diagnosis and Treatment of HF 2018 issued by the Chinese Medical Association, with signs/symptoms of chronic HF (symptoms: orthopnea, nocturnal paroxysmal dyspnoea; signs: lung rales, bilateral lower limb oedema, jugular vein filling, lateral shift or diffusion of apical beats); LVEF <40 % or LVEF >40 % combined with raised natriuretic peptide (BNP >35 ng/L and/or NT-proBNP>125 ng/L), and at least 1 of the following: (i) left ventricular hypertrophy and/or left atrial enlargement; and (ii) abnormal diastolic function of the heart as a diagnostic criterion for HF.

The TCM diagnosis of Qi deficiency and blood stasis evidence will be based on the Expert Consensus on TCM Diagnosis and Treatment of Chronic Heart Failure, the Expert Consensus on Integrative TCM Diagnosis and Treatment of Chronic Heart Failure, and the relevant contents of the Planning Textbook of the National Society for Higher Medical Textbook Construction and Research - Internal Medicine of TCM. Diagnostic criteria include at least two of the main symptoms and minor symptoms, while the tongue and pulse patterns should meet the requirements (Table 1).

The study is centered on the First Affiliated Hospital of Henan University of CM (Henan, China). It is expected to recruit 60 patients with HF who have undergone PCI, and it is planned to recruit from 2023 to

Table 1
Diagnostic criteria of Qi deficiency and blood stasis evidence.

Category	Symptoms or signs
Main symptoms	shortness of breath/wheezing, weakness, palpitations
Minor symptoms	tired and easy to fatigue; spontaneous sweating; low voice; dark purple complexion/lips.
Tongue	dark purple (or ecchymosis, petechiae, or tortuosity of sublingual veins)
Pulse condition	sunken, thin or weak pulse

10-08 to 2024-10-31. Fig. 1 shows the study process.

## 2. Inclusion and exclusion criteria

2.1. Inclusion criteria

Patients with CHD and HF who meet the following criteria will be included in this trial:

1) they meet the diagnostic criteria of CHD and HF and the criteria of Chinese medicine identification, and they have undergone PCI;
2) NYHA classification of II to IV;
3) Age 18–80 years old, gender is not limited;
4) Sign informed consent.

2.2. Exclusion criteria

The following patients will be excluded from the study:

1) Patients with shock, severe arrhythmia, acute myocardial infarction, pulmonary infarction, left ventricular outflow tract stenosis, infective endocarditis, pericardial tamponade, constrictive pericarditis;
2) With severe infection, serious liver (ALT $\geq$ 3 times the upper limit of normal), kidney (Cr $\geq$ 3 mg/dL or eGFR $\leq$ 60 ml/min/1.73m2), electrolyte disorders, blood system and mental system and other serious primary diseases;
3) Blood pressure (systolic blood pressure $\geq$ 180 mmHg and/or diastolic blood pressure $\geq$ 110 mmHg) and blood glucose (random blood glucose $\geq$ 10.0 mmol/L, glycosylated hemoglobin $\geq$ 8.0 %) were not effectively controlled;
4) Pregnant and lactating women;
5) Patients with malignant tumors;
6) Allergy, or food allergy to a variety of drugs, or known to be allergic to the ingredients of heart pills;
7) Patients who cannot take care of themselves or take oral drugs;
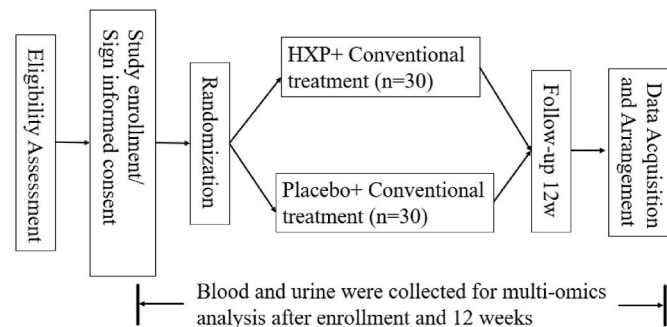
Fig. 1. Study schema: HXP and placebo were all produced by Guangzhou Yuekang Biopharmaceutical Co., Ltd, and their outer packaging, shape, size and smell were guaranteed to be consistent. Conventional treatment refers to conventional western medicine recommended by the guidelines.

8) Participate in other clinical researchers within one month;
9) Suspect or have a history of alcohol or drug abuse;
10) Researchers believe that other reasons are not suitable for inclusion.

2.3. Withdrawal and termination criteria

2.3.1. Shedding criteria

1) Determination of dropout cases: subjects who are eligible for inclusion and have filled out the informed consent form to enter the trial, regardless of when and why they withdraw, as long as the subjects who do not complete the protocol in the specified observation cycle, are treated as dropout cases;
2) Handling of dropout cases: when a subject is dropped out, the investigator should take home visits, telephone calls, letters and other means of Follow-up visits, contact the subject as much as possible, ask for reasons, record the time of the last dose of medication, and complete all the assessment items that can be completed. assessment items that can be completed. In the case of withdrawal from the trial due to allergic reaction, adverse reaction, or ineffective treatment, the investigator should take appropriate measures according to the actual situation of the subject. The investigator should take appropriate treatment measures according to the actual situation of the subject to protect the rights and interests of the subject. All dropout cases should be properly preserved. All the cases should be properly preserved, i.e., for archiving purposes, and also for the purpose of conducting the full set of analyses and statistics.

2.3.2. Eliminate criteria

1) Violation of legality: case selection violates the inclusion/exclusion criteria and randomisation should not have been carried out;
2) Subjects do not cooperate with randomisation, or after randomisation, they do not take the medication in accordance with the method of administration or the course of treatment required by the protocol, the amount of medication taken is less than 80 % of the prescribed dose, or the amount of medication taken is more than 120 % of the dose required by the protocol;
3) The use of other treatments or medications that are prohibited for use in combination, etc., which affects the determination of efficacy and safety.
4) Patients enrolled in the clinical study who have been diagnosed with other diseases during the treatment period and need to stop taking the drug or receive other treatment.

2.3.3. Withdrawal criteria

1) Patients with aggravated conditions, those with possible or already occurred serious AEs and those with deteriorated conditions, i.e. the case should be withdrawn from this clinical study.
2) Special physiological changes or complications that make it inadvisable to continue taking the study medication, the patient should be withdrawn from the study through the judgement of the doctor.
3) Poor compliance, violation of the treatment plan, or simultaneous combination of other treatments affecting the judgement of efficacy.
4) Subjects who are unwilling to continue the clinical study may withdraw from the clinical study after requesting withdrawal from the supervising physician.

2.3.4. Suspension criteria

1) Serious safety problems in the course of the trial.
2) Serious deviations in implementation, making it difficult to evaluate the efficacy of the drug.

3) It is found in the trial that the therapeutic effect of the drug is poor, and it does not have clinical value, and the trial should be discontinued at the request of the trial sponsor.
4) The administrative authority withdraws the trial.

2.4. Randomisation and blinding

2.4.1. Randomised grouping

This study used a random number table for grouping: 60 patients were numbered 1–60 according to the order of their visits to the clinic, 60 random three-digit numbers were produced using SPSS statistical software and recorded as random numbers under the original number, and the 60 random numbers were numbered from smallest to largest, with numbers 1–30 designated as the experimental group of the study, and numbers 31–60 as the control group of the study.

2.4.2. Design and implementation of the blinding method

This study adopted a double-blind method, blinding the participating physicians, clinical followers, data entry administrators, statistical analysts, and enrolled patients. Placebo setting The placebo was provided by the pharmacy of the First Affiliated Hospital of Henan University of CM, and the placebo appearance, packaging, smell, colour and taste needed to be the same as HXP. The control group was treated with western medicine plus placebo; the treatment group was treated with HXP plus western medicine. Randomisation scheme concealment The trial was conducted using a secondary blind bottoming system, i.e., the patient randomisation number was different from the drug number, and the process was handled by a dedicated person. The doctor who clinically collected the cases for enrolment did not know the random grouping scheme, but only the total sequential number of the enrolled cases, the subjects' sequential number and the corresponding random numbers and grouping results (i.e., subjects were assigned to group A or B) for the first level of blinding bottom; and then set up a blinded code for the medication used in group A and B (i.e., which group in group A and B was using HXP or placebo), and then randomly compiled a medication number for each subject according to the sequential order. Follow-up physicians had only the enrolment number and drug number of the enrolled patient when completing the case report form; they did not know the first level of blinding bottom and the second level of blinding bottom. The clinical inspector was in charge of the Hospital Clinical Evaluation Centre. The pharmacy of Henan University of Traditional Chinese Medicine was commissioned as the packer, and only the drug packer knew the first and second level blinding; the blinding was given to the investigator at the end of the study. In an emergency situation where a serious AEs occurs and the investigator needs to clarify the drug used by the patient, the investigator can ask the drug packer, which is called emergency blinding.

2.4.3. Interventions

All patients will be randomly assigned to either the experimental group or the control group. All patients in the experimental group will receive HXP + conventional treatment, and the control group will receive placebo + conventional treatment, 20 mg/pill, 2 pills/dose, 3 times a day for 3 consecutive months. Conventional treatment: in accordance with the China HF Diagnostic and Treatment Guidelines 2018 drug therapy programme was implemented, and drug therapy was selected according to the severity of the patient's condition and indications, and indications, dosage, and duration of treatment were formulated in strict accordance with the recommendations of the Guidelines: diuretics + ACEI/ARB/ARNI + β-receptor blockers + aldosterone receptor antagonists + sodium-glucose cotransporter protein 2 + if necessary, ivabradine and digitalis analogues.

2.4.4. Concomitant treatments and forbidden medication

The use of medications or other treatments that are necessary for the continuation of co-morbidities and symptoms (other than symptoms of the disease) that existed prior to the start of the study is permitted. Any comorbidities or symptoms that develop during the study will be treated as clinically indicated. Comorbid medications and therapies that need to be used as a result of AEs during the study should be recorded in detail on the CRF form. All medications used should be documented in the original study medical record, including the name of the drug (or other therapy), usage, dosage, mode of administration, and duration of treatment. All subjects are prohibited from using traditional Chinese medicines (Chinese patent medicines, Tang medicines, injections), except for the use of medications regulated by the course of treatment study.

2.4.5. Intervention adherence and compliance

In order to improve subjects' adherence to the intervention programme, the researcher will take the following measures:

(1) When the subjects are enrolled in the group, the subjects' contact information (email, wechat, mobile phone number, telephone number, etc.) will be recorded in detail to ensure timely and smooth communication.
(2) Before enrolment, the content of the informed consent form was explained in detail to the subjects and they were informed of the potential risks and benefits, so that the patients fully understood the study protocol and were aware of the importance of taking the medication carefully.
(3) The researchers used timekeeping tools such as calendars or mobile phone software to record the date of follow-up visits in advance. Subjects were reminded of the date the day before the follow-up visit through an effective means of communication.
(4) At the end of the study, subjects are asked to bring back the remaining medication that was dispensed, and a detailed record is kept of whether the subject took the medication on time and in the correct amount, and whether there was any under or overdose of the medication. All information should be promptly recorded in the original study medical record.

2.5. Outcome measures and efficacy judgement criteria

2.5.1. Primary outcome:NYHA cardiac function classification

Grade I: Patients with heart disease have unrestricted physical activity, and ordinary activities do not cause fatigue, palpitations, dyspnoea or angina pectoris.Grade II: patients with heart disease are mildly restricted in physical activity, with no conscious symptoms at rest, but fatigue, palpitations, dyspnoea or angina pectoris may occur with ordinary activities.Grade III: the physical activity of heart disease patients is obviously limited, less than the usual general activities that cause the above symptoms.Grade IV: The patient with heart disease cannot engage in any physical activity. Symptoms of HF also appear in the resting state and worsen after physical activity. Significant effect: HF is basically controlled or heart function is improved by 2 or more grades; Effective: heart function is improved by 1 or more grades, but less than 2 grades; Ineffective: heart function is improved by less than 1 grade, and there is no improvement in symptoms and signs; Deterioration: heart function is deteriorated by 1 grade or more than 1 grade.

2.5.2. Secondary outcomes

1) Lee's HF Score

Lee's HF Score includes 6 aspects of lung image changes, dyspnoea, hepatomegaly, lung rales, and oedema, with 0–4 points for each item, and the higher the score, the more severe the patient's HF. Refer to the Guidelines for Clinical Research of New Chinese Medicines (Trial). Significant effect: Lee's heart failure score decreased by $\geq 75\%$; Effective: Lee's heart failure score decreased by 50 %–75 %; Ineffective: Lee's heart failure score decreased by <50 %; Exacerbation: Lee's heart failure

score higher than the pre-treatment score.

2) Evaluation of the efficacy of Chinese medicine evidence

Referring to the Guiding Principles for Clinical Research of New Chinese Medicines (Trial Implementation) and the Diagnostic Efficacy Criteria for Internal Medicine Evidence of Traditional Chinese Medicine, the primary symptoms were divided into four grades of none, mild, moderate, and severe, with scores of 0, 2, 4, and 6, respectively, and the secondary symptoms were divided into four grades of none, mild, moderate, and severe, with scores of 0, 1, 2, and 3, respectively.

Significant effect: basic or complete disappearance of clinical symptoms and secondary symptoms, and reduction of evidence points by >70 %; Effective: obvious improvement of clinical symptoms, and reduction of evidence points by 30 %–70 %; Ineffective: no obvious improvement of clinical symptoms, and reduction of evidence points by <30 %; Exacerbation: aggravation of clinical symptoms, and the points of the post-treatment period exceeded that of the pre-treatment period.

3) Exercise tolerance test

Before and after treatment, patients were assessed by 6-min walking distance, patients walked back and forth in a straight corridor of 40 m in length for 6 min, and their walking distance was measured accurately.

4) Quality of life evaluation

The Minnesota Heart Failure Quality of Life Questionnaire (MLHFQ) will be used to measure the patients' quality of life, with 21 items, 5 points for each question, totalling 105 points, with higher scores indicating poorer quality of life. It will be evaluated once before and once after.

5) Additional indicators

The following indicators will also be collected and analyzed: Cardiac color ultrasound was used to evaluate cardiac remodelling (Left ventricular ejection fraction, left ventricular end-systolic internal diameters, left ventricular end-diastolic internal diameters, left ventricular end-systolic volume, and left ventricular myocardial mass index); electrocardiogram and 24-h ambulatory electrocardiogram to evaluate electrical remodelling; other indicators of myocardial injury including CK, CK-MB, Myo, cTNI, NT-proBNP.

2.5.3. Data collection and management

After patients were enrolled in the clinical trial, basic data were collected and filled in NYHA cardiac function classification, Lee's Heart Failure Score, TCM evidence efficacy evaluation, exercise tolerance test, quality of life evaluation, cardiac ultrasound, electrocardiogram, 24-h ambulatory electrocardiogram, and biochemical indexes (CK, CK-MB, Myo, cTNI, and NT-proBNP) in order to make comparison of baseline data. Various rating scales were recorded after 12 weeks of treatment initiation for efficacy evaluation. Follow-up, loss and withdrawal of observed cases, adverse events and adverse reactions to drugs were recorded. Table 2 shows the details of the study procedures.

The study medical record is the original document of this trial and should be kept properly by the investigator. The following plan is made to ensure the completeness and accuracy of the study data. The following plans were made to ensure the subjects' smooth cooperation in completing the study: 1) Let the subjects fully know and understand the importance of participating in the study; 2) Record the subjects' contact information in detail (e.g., email, WeChat, mobile phone number, phone number, etc.) in order to ensure timely and smooth communication; 3) Apply the timekeeping means such as mobile phone software and record the date of the required follow up visit in advance. Subjects were reminded of this via an effective means of communication the day before

Table 2
Schedule of trial measures.

Study period	Screening	Baseline	Treament	Follow-up
Visit	Visit 1	Visit 2	Visit 3	Visit 4
Time point	Day 0	Day 1	Day 2	Day 90
Baseline information	X			
Eligibility Assessment		X		
Sign informed consent		X		
Randomization		X		
Treatment group			◆————————◆	
Control group			◆————————◆	
NYHA Cardiac Function Classification		X		X
Lee's HF Score		X		X
Evaluation of the efficacy of Chinese medicine evidence		X		X
Exercise tolerance test		X		X
Life quality evaluation		X		X
Additional indicators		X		X
Blood		X		X
Urine		X		X

the follow-up visit. If the visit time is missed, try to re-contact or re-schedule.

The following requirements are made for the researcher to fill in the data: 1) All subjects must record the items in the CRF form in detail after entering the trial; 2) All the data in the CRF form should be guaranteed to be accurate; 3) The original data in the CRF form, if need to be modified, need to be crossed out and then annotated with the altered data beside the line, and signed.

Ombudsman monitoring data to do the following requirements: 1) Ombudsman should be regularly screened for the inclusion of subjects, informed consent monitoring; 2) Ombudsman should confirm that the CRF form is filled out correctly; 3) Ombudsman should confirm that any errors or omissions have been standardised corrections and notes to confirm that there is a researcher's signature with the date of the date; 4) Ombudsman should confirm that the inclusion of subjects withdrawn from the study and lost. 5) The Ombudsman should confirm that any changes in the treatment regimen of subjects in this study should be recorded in detail; 6) The Ombudsman should confirm that adverse events have been recorded accurately.

3. Multi-omics analysis: proteomics and metabolomics

3.1. Proteomics analysis

3.1.1. Blood sample processing and detection

Take whole blood and leave it at room temperature for 2h, centrifuge it at 3000 rpm for 10min, collect the supernatant, freeze it in liquid nitrogen, and store it in the refrigerator at −80 °C; transport it in sufficient quantity of dry ice, 200μl/example, and hand it over to a professional company for processing.

3.1.2. Screening of differentially expressed proteins

Statistical methods were used to screen differentially expressed proteins, in which the screening criteria for differential proteins were $P < 0.05$ and Fold Change < 0.83 or Fold Change > 1.2. Combined with MALDI-TOF-MS differential protein spot analysis and the OmicsBean cloud platform for histological data integration and analysis, credible

proteins and differential proteins were further screened at the large-scale level of different evidence-based protein change characteristics. The differentially expressed proteins were analyzed by hierarchical clustering and visualised by heat map. Differential protein analyses were performed for each group in the stage of HF unable to ejection fraction separately from healthy subjects to obtain the relevant proteins in different stages of HF.

3.1.3. Bioinformatics analysis

Through the GO database (geneontology.org), we obtained three aspects of functional information, namely, biological processes, cellular location and molecular functions of the differentially expressed proteins, and organised the functional concepts with different conceptual coarseness into the structure of DAG (Directed Acyclic Graph); the Pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG), we analyzed the metabolic pathways that were significantly enriched by the differentially expressed proteins, which were enriched in the different experiments. and Genomes (KEGG) Pathway database (www.kegg.jp/kegg/pathway.html), metabolic pathways significantly enriched for differentially expressed proteins were analyzed, and pathways that underwent significant systematic changes under different experimental conditions were enriched. Analysis of differential protein interaction network construction: using STRING database (V11, string-db.org), differentially expressed proteins queried the STRING database Homo sapiens for protein interactions, constructed a network diagram of differentially expressed proteins, and searched for key nodes in differentially expressed proteins.

3.2. Metabolomics analysis

3.2.1. Sample processing and detection

Urine Pre-configured sodium azide working solution (0.5 mg/L), stored at $-20\ ^{\circ}\mathrm{C}$; morning mid-stream urine was directly dispensed into centrifuge tubes, 1 ml per tube and 10 μl of sodium azide working solution was added; stored at $-80\ ^{\circ}\mathrm{C}$ and transported on dry ice, 1 ml/case. Serum Collect venous blood with blood collection tubes without pre-added anticoagulant, leave it at $37\ ^{\circ}\mathrm{C}$ (or room temperature) for 1h for coagulation and stratification; centrifuge at 3000 rpm at room temperature for 10 min, take the supernatant and transfer it to clean centrifuge tubes, centrifuge at 12000 rpm at $4\ ^{\circ}\mathrm{C}$ for 10 min, and take the supernatant and divide it into 1.5 ml centrifuge tubes, 0.2 ml for each tube; Store it at $-80\ ^{\circ}\mathrm{C}$, transport it by dry ice, 200μl/cases. Then the treated samples will be analyzed for metabolomics.

3.2.2. Differential metabolite screening

1) PCA analysis (principal component analysis): after the data were standardised, the samples were subjected to principal component analysis (PCA), which responds to the overall metabolic differences between the samples in each group and the magnitude of variability between the samples within a group. The software was SIMCA15 from Umetrics, Sweden, and the data were processed using UV formatting (Unit Variance Scaling) and Mean-Centered to obtain more reliable and more intuitive results. The software performs automated model fitting analysis to obtain the number of principal components of the most reliable mathematical model.
2) Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA): Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was used to filter the signals that were not related to the classification of the model, i.e., orthogonal signals, and eliminate the noise information that was not related to the classification, so as to obtain the information of the relevant metabolites that led to the significant differences between the two groups, and to establish the OPLS-DA model.
3) Differential metabolite screening

The VIP (Variable Importance in the Projection) value (threshold >1) of the first principal component of the OPLS-DA model was used and combined with the *t*-test (*t*-test) p-value (threshold <0.05) to find differentially expressed metabolites. Differential metabolite analyses were performed in each group with different ejection fractions in HF separately from healthy subjects to obtain relevant metabolites in different stages of HF.

3.3. Bioinformatics analysis

3.3.1. KEGG enrichment analysis of differential metabolites

1) Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database, the cellular physiological and biochemical processes are shown graphically based on the functional information of genes and genomes, and metabolic reactions as clues to link the possible metabolic pathways and corresponding regulatory proteins.
2) Further metabolic pathway enrichment analysis of differential metabolites through enrichment analysis and topology analysis, focusing on the key pathways with the highest relevance to metabolite differences.

3.4. Proteomics and metabolomics association analysis

3.4.1. O2PLS association analysis

Differential proteins and metabolites are divided into three parts: the part that jointly determines the trend of model differences (overlap), the part that alone determines the trend of model differences (unique) and the part that does not determine the trend of model differences. Through O2PLS association analysis, we can clarify whether differential proteins and differential metabolites have the tendency to jointly determine the model difference, and focus on the key variables that determine the model difference trend.

3.5. Correlation analysis of different proteins and different metabolites

The "spearman" algorithm was used to analyze the correlation heat map of the differential proteins and metabolites. The correlation coefficient Q-value <0.05 was used as the condition for significant correlation, and the content of all differentially expressed proteins and differential metabolites was calculated. The correlation between the two was compared, and the results were constructed. The correlation heat map example, p value reflects the significant level of correlation, p value <0.05, indicating that the gene is significantly related to metabolites.

3.6. Differential protein and differential metabolite pathway analysis

Differential proteins and differential metabolites were simultaneously mapped to the KEGG pathway database via R's Pathview package to obtain their common pathway information. The nodes enriched for significant metabolic pathways of differential metabolites and differential proteins were shown in different colours.

3.7. Metabolome-proteome correlation network analysis

By using Cytoscape_v3.3.0 software, we screened the nodes with "| correlation coefficient|" >0.9 and correlation P value <0.01, and construct a correlation network diagram. Network analysis and construct the correlation network diagram.Based on the correlation analysis of metabolomics and proteomics, the network chain of key proteins, pathways and metabolites of HXP in cardiac remodelling was constructed.

3.8. Adverse events reporting

Adverse events (AES) include clinical signs or symptoms and abnormal laboratory results that are not related to patients with coronary artery disease after PCI. All adverse reactions during administration should be recorded by the physician in the AES report of the CRF table. If a serious AES occurs, the date of occurrence, duration, treatment method, and possible relationship to treatment should be documented in detail, and the treatment plan should be reported to the study leader and clinical ethics committee within 24 h.

3.9. Safety assessment

During the course of the study, the subject should promptly notify the doctor of any discomfort, and the doctor will make a judgment and give appropriate treatment. The ombudsman will monitor and record any adverse reactions in all patients in the study.

3.10. Sample size calculation

Based on the relevant literature and our team's previous treatment experience, the estimated effective rate of HXP is about 85 %. For the therapeutic effect produced by the placebo of Chinese patent medicine, there is no exact data on the effectiveness rate after searching the literature. Based on clinical experience as well as consultation with experts, we will set the effective rate of placebo at 35 %. Setting the test level $\alpha = 0.05$, $\beta = 0.1$, the sample size of the experimental group and the control group as 1:1, and assuming a 25 % failure rate, it is calculated that a minimum of 20 patients need to be enrolled in each group (n= $(2\sigma^2[Z_{1-\alpha/2}+Z_{1-\beta}]^2)/([\mu_1-\mu_2]^2))$ [20]. In order to achieve a better quality of research, combined with the experience of previous clinical trials, we expanded the number of patients enrolled in each group to 30, and a total of 60 patients were collected in the control group and the experimental group.

3.11. Statistical analysis

SPSS Statistics (version 25.0, IBM, Armonk, NY) will be used for the statistical analyses. All statistical tests will be two-sided, and a $P < 0.05$ will be considered statistically significant for the differences tested. Quantitative indicators will be described by calculating the mean, standard deviation, median, minimum, maximum, lower quartile (Q1), upper quartile (Q3), and categorical indicators will be described by calculating the number of cases and percentage of each category. According to the type of indicators, the general situation between the two groups will be compared and analyzed by corresponding methods. For the comparison between the groups of quantitative data, the inter-group t-test or Wilcoxon rank sum test were used according to the distribution of the data; Chi-square test or exact probability method were used for classified data, and Wilcoxon rank sum test was used for stratified data.

3.12. Ethical considerations and dissemination

The trial followed the Declaration of Helsinki (2013), the Measures for Ethical Review of Biomedical Research Involving Human Beings (2016), and relevant Chinese clinical trial research norms and regulations. The trial passed the ethical review by the Ethics Committee of the First Affiliated Hospital of Henan University of CM, and the ethical review board approval document number 2023HL-330-01 and was registered in the China Clinical Trial Registr.

The HXP applied in the intervention group of this study have been used in the clinic for more than 30 years, and there are no reports of increased clinical risks associated with HXP. Blood and urine samples were collected at the same time as routine clinical tests, which did not add additional clinical burden.The remaining samples from the subjects will be destroyed after completion of the test. Participants will be briefed

on the study protocol and its advantages and disadvantages. All subjects may refuse to participate in the trial and withdraw from the trial at any time. Pay attention to the confidentiality principle of identity information in data processing, and the subject will use the code to identify.The trial results will be peer-reviewed and published in an international conference or journal.

4. Discussion

4.1. Contributions and problems of previous research by others in this field

At present, there have been some preliminary explorations on the basic and clinical studies of HXP in protecting the heart. HXP can effectively alleviate the clinical symptoms of angina pectoris patients, protect the cardiac function, and control the development and prognosis of the disease by combining with the Chinese patent medicine HXP on the basis of conventional treatment in Western medicine. HXP related clinical studies of a single disease, expanding the clinical scope of HXP lack of evidence to support, mostly limited to angina pectoris, the treatment of HF only one trial, and clinical research evidence level is not high, the sample size is small, the lack of high-quality large-sample, multi-centre, randomized, double-blind and prospective clinical trials. Moreover, most of the current basic experiments of Chinese patent medicine research are mostly carried out in rats and mice, and lack of exploring the mechanism of action from the clinical point of view.

4.2. Limitations and follow-up reflections

Due to the limitations of research design and funding, the participants were all from the same hospital, and the sample size and study duration were relatively small. We also face limitations in patient adherence and shedding similar to those seen in most clinical studies. In addition, it was difficult for the study to identify whether the emergency in the participants stemmed from the HXP or something else. We will record the facts and submit feedback to the ethics committee and the pharmaceutical company for analysis. Then,we plan to cooperate with a number of hospitals to conduct a multi-center, large sample and long-term study to provide more experimental evidence for the clinical application of HXP.

4.3. Characteristics of the trial programme

A prospective, double-blind, placebo-controlled, randomized controlled clinical trial was conducted to observe the efficacy and adverse reactions of HXP in the treatment of HF after PCI. At the same time, multi-omics technology is used to solve the characteristics of multi-target in the process of TCM intervention. In this study, we will construct a gene-protein-metabolite regulatory network to elucidate the mechanism of HXP treatment for heart failure.

Trial status

All participants are expected to be enrolled in 2024 and results will be available in 2025.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Bo-yong Qiu: Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Funding acquisition, Data curation, Conceptualization. **Bai-rong Xu:** Writing – original draft, Software, Project administration, Investigation. **Yan-kun Song:** Formal analysis, Data curation. **Yu-cai Hu:** Resources, Data curation. **Hong-jie Ren:** Software, Data curation. **Jia Zheng:** Software, Resources. **Peng Chen:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition. **Yong-xia Wang:** Writing – review & editing, Validation, Supervision, Project administration, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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