Study on compatibility of *Banxia Baizhu Tianma Decoction* on hypertension treatment in rats

Running title: Monarch drugs of BBTD plays an important role in hypertension treatment

Minhui Li[1,2], Huimin Yang[1,2]#, Yuxin Gu [1,2], Huijuan Cheng[1,2], Xue Bai[1,2], Lianhui Men[1,2], Feifei Yang[1,2], Cheng Chen[1,2], Wenqi Li[1,2], Zhihua Huang[1,2], Boting Liao[1,2], Honghua Zhang[1,2]*, Qingchang Tian[1,2]*, Nan Xu[3]*, Shuling Wang[1,2]*

* Corresponding authors.

E-mail addresses: erin6@163.com (H. Zhang), tianqc@hznu.edu.cn (Q. Tian), 93679706@qq.com (N. Xu), wsling222@163.com (S. Wang).

Co First Author

1. College of Pharmacy, Hangzhou Normal University, Hangzhou, 311121, PR China.

2. Key Laboratory of Elemene Class Anti-Cancer Chinese Medicines; Engineering Laboratory of Development and Application of Traditional Chinese Medicines; Collaborative Innovation Center of Traditional Chinese Medicines of Zhejiang Province, Hangzhou Normal University, Hangzhou, Zhejiang 311121, PR China.

3. Shandong Research Academy of Traditional Chinese Medicine, Jinan 250014, PR China.

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What is already known about this subject:

BBTD has been used for hypertension treatment

BBTD can regulate matabolism of glycerophospholipids

BBTD exhibit endothelial pretection

What this study adds:

There was no difference in the improvement of hypertension symptoms between monarch drugs and BBTD whole prescription

The effect of monarch drugs on improving material imbalance is better than that of the whole prescription

1. Introduction

Hypertension is a significant public health problem around the world. It is estimated that among Chinese adults aged 18 years or older, 23.2% (≈ 244.5 million) suffer from hypertension in 2012-2015 [1]. The prevalence rate of hypertension is high in China, but the rate of treatment and management is low. Over the last few decades, the morbidity rate of hypertension in China has been increased significantly, particularly in countryside [2]. Studies showed that obesity is one of the important factors leading to elevated blood pressure [3], and about 70% hypertension is caused by obesity [4]. High blood pressure is also a major, independent risk factor for cardiovascular disease. Clinical trials have certificated that lowering blood pressure could reduce cardiovascular disease and decline premature death [5]. Many drugs have been approved for hypertension treatment, but the efficacy of many drugs is often limited due to side effects, toxicity, and individual variability. Then it is worth to explore more effective and low toxicity drugs for hypertension treatment.

A large number of published cases and randomized trials have shown the efficacy of TCM on hypertension treatment [4]. BBTD, a classical compound of TCM, has been clinically used for hypertension treatment, especially hypertension with excessive accumulation of phlegm and dampness [6]. Following the principle of

compatibility of Chinese herbal compounds, BBTD is mainly made up of six herbs, which are monarch drugs: *Pinellia ternata* and *Gastrodia*, ministerial drug: *Atractylodes macrocephala Koidz* and *Poria cocos* and adjuvant drugs: *Citrus maxima* and *Glycyrrhiza uralensis Fisch*. BBTD can be used to cure some disease sympotoms, such as irascibility, essential dizziness, palpitations, phlegm, and so on [7]. Yue-Hua Jiang with his colleagues found that BBTD has a positive effect on lowering obesity-related blood pressure [8]. Each drug in the prescription possesses different effects. In a word, *Pinellia ternata* and *Gastrodia elata* both can eliminate dampness and phlegm [9], *Atractylodes macrocephala Koidz* and *Poria cocos* can fortify the spleen and percolate dampness [10-11], *Citrus maxima* can regulate qi-flowing for eliminating phlegm, and *Glycyrrhiza uralensis Fisch* can coordinate the drug actions of a prescription[12]. But what role each component of BBTD plays in the hypertension treatment remains unknown and it deserves further study in order to optimize the formula.

Studies have shown that high-fat-diet can successfully establish obesity disease model in mice, and this model is similar to the pathological symptoms of clinical obesity, along with the advantage of good stablity, simple operation and lower cost [13]. Therefore, this paper adopted hypertension mouse model induced by high fat diet to explore the effect of BBTD on hypertension treatment. Furthermore, we attempted to elucidate the pharmacological effect of each ingredient herb in BBTD on hypertension treatment by studying the efficacy of them and exploring the changes in serum metabolites. It provided a theoretical basis for further optimizing the compatibility of the BBTD formula.

2. Methods

2.1. Materials and Reagents.

Triglyceride assay kit (TG, A1101-1-1) and total cholesterol assay kit (TC, A111-1-1) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). N,O-Bis (trimethylsilyl) triluoro-acetamide (Lot No.P1600168) and Methanol (Lot No.WXBC2211V) were purchased from Sigma Aldrich (Amercia); Heptadecanoic acid (Lot. No.K1325026) and O-Methoxyamine-HCl (Lot No.P1602270) were obtained from Aladdin (Shanghai, China). Trichloromethane (Lot No.10006818) and Pyridine (Lot No.20160428) were purchased from Chemical Reagent Co. Ltd (Shanghai, China). The chemcials used in this study were all analytical reagents.

2.2. Preparation of Banxia Baizhu Tianma Decoction

There are 6 kinds of herbs in the BBTD, which are *Pinellia ternata* (9 g), *Gastrodia elata Blume* (6 g), *Atractylodes macrocephala Koidz* (15 g), *Citrus maxima* (6 g), *Poria cocos* (6 g) and *Glycyrrhiza uralensis Fisch* (4 g). These herbs were all purchased from Tongrentang Chinese Medicine (Hangzhou, China). All of the medicinal materials were mixed together and decocted with 500 mL water twice for 30 min each time, then the filtrate were combined together and concentrated under reduced pressure conditions to a final mass concentration of 2.00～2.50 g/mL crude drug. The quality control of BBTD was based on our previous literature [14].

2.3. Experimental Animals and the Modeling Process

Sixty male SPF grade SD rats (weighed 200±20 g) and the standard animal food were commercially obtained from Laboratory Animal Center, Hangzhou Normal University. The high-fat diet (which containing5% egg yolk powder, 10% pig fat, 0.5% pig bile salt, 0.004% methimazole, 25% cane sugar and 8% salt) was homemade. All animals were fed at a temperature from 18 ℃ to 26 ℃ and humidity of 40% to

70% in a dark cycle of 12/12 hours. The rats were fed with qualified standard food and distilled water for one week adaptive feeding.

A week later, 50 rats were randomly selected to establish an animal model of hypertension [15]. The rats were fed with a humid environmentroom temperature adjusted at 18~20℃, humidity at 85%~90%) for 4 weeks.

The body weight and the blood pressure of rats were measured and recorded after 12 h of fasting and banning water. It is recognized that the animal model was successfully established when SBP >140 mmHg and/or DBP >90 mmHg [15], accompanying with anxiety, depressive behavior and significant weight gain, etc [16]. Body weight, SBP and DBP were measured before, during and after the modeling process.

2.4. Drug Treatment and Tissue Collection

The model rats were randomly divided into 5 groups (n=10): B-model group, C-full prescription group, D-no principle drug group, E-no ministerial drug group and F-no adjuvant drug group. Meanwhile, 10 normal rats were used as control (A).

Rats in each drug treatment group were all fed with high-fat diet, while the rats in normal control group were fed with standard diet. As for drug treatment, rats in group C, D, E and F were intragastric administrated with full prescription, no principle drug, no ministerial drug and no adjuvant drug respectively. The drug dose was 1 mL/d.

After anesthetized with 3% pentobarbital sodium (40 mg/kg), blood of rats were harvested from the inferior vena cava and kept in air for 2 h at 4℃, then were centrifuged for 5 min (4℃,4000 rpm). In the end, each upper serum was divided into thirds and stored at -80℃ until further testing.

2.5. Content Detection of TG and T-CHO in Serum

The contents of serum TG and T-CHO were determined by GPO-PAP enzymatic method with Triglyceride assay kit and CHOD-PAP method with total cholesterol assay kit respectively. All operations were strictly performed according to the kit instructions. The absorbance value was detected at 510 nm with microplate

spectrophotometer (Thermo scientific, Multiskan FC). The contents of TG and T-CHO were calculated based on the following formula:

$$\text{T-CHO or TG (mmoL/L)} = (OD_{sample} - OD_{blank}) / (OD_{calibration} - OD_{blank}) * \text{standard concentration (mmol/L)} \quad (1)$$

2.6. Pathological Examination

The general conditions such as diet, water intake, hair, spirit, movement, etc. were observed and recorded every day. The body weight and blood pressure were measured and recorded every week.

2.7. GC-MS Detection

The serum was taken out, thawed and shook at room temperature for 1 h before GC-MS detection. 100 μL of each serum was taken into centrifuge tube (1.5 mL), then 10 μL of 1 mg/mL methanol solution of heptadecanoic acid, 300 μL methanol and chloroform mixed organic solvent (methanol:chloroform=3:1) were added and vortex oscillated for 30s. These mixtures were put in a refrigerator at -20℃ for 10 min to promote protein precipitation. After centrifuged at 10,000 rpm for 10 min, 300 μL of supernatant was dried with nitrogen, then was mixed and reacted under a shaker at 30℃ (200 r/min) with 80 μL of methoxyamine pyridine solution. After 80μl BSFTA (including 1% TMCS) was added in, the mixture were vibrated for 30 s reacted in an oven at 70℃ for 60 min.

The samples were mixed well for 10 s and were left at room temperature for 1 hour for GC-MS analysis. Method for extraction of serum samples was used to prepare Quality control (QC) samples. According to the data processing method of serum metabonomics, the correlation stability of QC samples was analyzed.

Each 1.0 μL aliquot fraction of the exponents was injected into an Agilent 6890N mass spectrometer: Agilent 5975B (Agilent Technologies, Santa Clara, CA, USA). The separation was achieved on a chromatographic column (30m x 250 μm internal diameter, 0.25 μm film thickness, Agilent J&W Scientifc, Folsom, CA, USA). The GC parameters included: inlet temperature (260 ℃), interface temperature (260 ℃),

injection volume (1.0 μL) and the flow rate (1.0 mL/min) of the carrier gas. The split ratio of injection volume was 2:1. The MS parameters were included: the ion source temperature (230 ℃), the quadrupole temperature (150 ℃), the electron impact ionization voltage (70 eV), and the mass spectrum full scan range (30~550 m/z).

2.8. Data Processing

The GC/MS original spectrum file of the obtained sample was converted to NetCDF format by the Databridge program (PerkinElmer Inc, USA) in Turbomass software, then were imported into R2.7 software. After calculation and normalization, a three-dimensional matrix of retention time, mass-to-charge ratio and peak intensity were finally obtained (https://xcmsonline.scripps.edu). This matrix will be used for all subsequent data analysis. Metabolites were identified using commercial metabolite databases (NIST, Wiley Registry) and standard metabolite databases established by our research group.

To perform multi-dimensional statistical analysis, we imported the obtained three-dimensional matrix into SIMCA-P11.0 software (Umetrics, Sweden), and pre-processed the data with mean center and Pareto scale. In the process of establishing mathematical model, we first used the unsupervised principal component analysis (PCA) method to analyze the overall data and observed the natural distribution and group relationship of the experimental samples. In order to further distinguish the differences among different groups, we used supervised analysis methods (partial least squares discriminant analysis, PLS-DA) to find the main difference variables leading to sample aggregation and dispersion.

2.9. Statistics Analysis

Statistical software SPSS 25 (SPSS, Chicago, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation. LSD test was used for comparison between groups. $P< 0.05$ was considered to be statistically different.

3. Results

3.1. BBTD Improves the Hypertension

In general, the mental condition, activity, diet of model rats were became worse over time, but got well after BBTD treatment.

To investigate whether each components of BBTD can treat hypertension in rats fed with high-fat diet, the body weight of rats were measured before and after modeling and administration. There was no significant difference in initial body weight among the 6 groups (Fig.1 a). Compared with the control group, the body weight of the model group increased significantly after 6 weeks of continuous administration with high-fat diet ($P<0.05$). Compared with the model group, the weight of rats in the full prescription group and no adjuvant prescription group decreased significantly ($P<0.05$). and there was no significantly different between them.However, the weight loss of the other two treatment groups were not obvious as that of the full prescription group and no adjuvant prescription group.

Similar to body weight change in each group, the contents of TG and T-CHO in serum of drug-treated rats were significantly reduced ($P<0.05$) after 6 weeks of full prescription administration, indicating the effect of BBTD on lowering TG and T-CHO. (Fig.1 d, e). The lowering TG and T-CHO effects of other two treatment groups were not as good as that of the full prescription and no adjuvant prescription group.

The SBP and DBP of each group were measured before, during and after modeling. Compared with the control group, the SBP and DBP of the model group increased significantly while decreased in the treatment groups, especially in the full prescription and no adjuvant prescription group. In short, BBTD could reduce blood pressure in rats (Fig.1.b,c). The lowering blood pressure effect of the full prescription group was the most obvious, followed by the no adjuvant prescription group.

3.2. GC/MS Spectra of the Six Groups

To account for the metabolic differences, GC/MS spectra was further pretreated and analyzed by pattern recognition. A typical GC/MS total ion current (TIC) chromatogram of the urine samples from the control, model, and drug treatment groups was illustrated in Figure 2. There are some notable differences in the macro view.

3.3. *Metabolic Spectrum Analysis of Normal Group and Model Group*

To clarify whether there were differences in metabolites between the model group and the normal group, the further experimental analysis was conducted. First of all, it was found that the metabolic characteristics of the normal group and the model group could not be well separated under an unsupervised analysis (PCA analysis) (Fig. 3 a), but could be well separated under the PLS-DA loading plot (Fig. 3 b) with a high interpretation rate and prediction rate in model rats ($R^2X=0.714$, $Q^2Y=0.845$).

3.4. Different Metabolites between Model Group and Normal Group

In order to find the metabolites highly associated with the hypertension, a cross-validation partial least squares method was used in this study to establish a PLS-DA model between the model group and the normal group. According to VIP >1 [17], 88 differential variables in model rats were found, of which 28 metabolites were identified. Among them, 5 metabolites (D-Glucose, L-Tyrosine, Propionamide, Morpholine, Arachidonic acid) were up-regulated ($P<0.05$), whereas the other 23 metabolites (Ethanedioic acid, Butanoic acid, phosphate, L-Isoleucine, L-Proline, Glycine, L-threonine, Glutamine, L-phenylalanine, Xylitol, Phosphoric acid, Formic acid, L-Ornithine, benzoic acid, D-Mannitol, D-Fructose, Sedoheptulose, L-Tryptophan, 9,12-Octadecadienoic acid, Phenol, Talose, Oleic acid, trans-9-Octadecenoic acid) were down-regulated ($P<0.05$)_in model mice than that of mice in normal_group (Table 1). These results indicated that there was obvious substance metabolism disorder in rats treated with high fat diet.

3.5 The Influence of BBTD on Differential Metabolites Induced by High-fat diet

In this study, the status of 28 differential metabolites in different treatment groups was further analyzed by multidimensional statistical method. The statistical results showed that 28 differential metabolites in the full prescription group, no principle drug group, no minister group and no adjuvant drug group were significantly different from that of model group, with an apparent tendency to be approach to the normal group. Above results confirmed that BBTD can regulate the metabolite disorder caused by the modeling process (Fig.4 a-d).

In order to more intuitively represent the changes in metabolic patterns of the four drug treatment groups, this study conducted multi-dimensional statistical analysis on the data of the four groups. The results showed that their metabolic spectrum could not be separated. The results (Fig.4 e) showed no difference between the four treatment groups in reversing the metabolite disruption caused by the modeling process.

To further observe the influence of BBTD on high-fat diet rats, the changes of 28 metabolites in model group and other 5 groups were compared respectively (Table 1).

In total 8 metabolites among the 28 different metabolites in the full prescription group, which are Talose, Oleic acid, L-proline, D-fructose, phosphate, trans-9-octadecenoic acid, Morpholine and Propionamide, were significantly different from that of model group ($P<0.05$). While, the contents of Xylitol, Phosphoric acid, Formic acid, L-Ornithine, benzoic acid, D-Mannitol, D-Glucose, 9,12-Octadecadienoic acid and Phenol were slightly different from that of model group. As for no adjuvant drug treatment, the contents of Xylitol, Phosphoric acid, Formic acid, L-Ornithine, benzoic acid, D-Mannitol, D-Fructose , Propionamide, Morpholine,Talose,Oleic acid and Arachidonic acid were significantly different from that of model group ($P<0.05$), along with sightly increase of Glycine, L-threonine, D-Glucose, L-Tyrosine, L-Tryptophan, 9,12-Octadecadienoic acid, Phenol and trans-9-Octadecenoic acid. Treatment with no ministerial drug , there were 8 metabolites significantly different from that of model group (L-threonine, Xylitol,

benzoic acid, D-Mannitol, D-Fructose, Propionamide, Morpholine and Arachidonic acid, $P<0.05$). Compared with model group, there were only two disturbance metabolites (D-Fructose and trans-9-Octadecenoic acid) were dramatically up-regulated ($P<0.05$), there other 24 differential metabolites all were sightly different from that of model group. There data showed that BBTD and each component drug play an important role in regulating metabolite disturbance caused by HFD-diet., especially the two monarch drugs: *Pinellia ternata* and *Gastrodia elata.*

3.6. Analysis of the Differential Metabolites Involved Metabolic Pathways

In order to further explore the mechanism of the high-fat diet induced hypertension, 28 differential metabolites were introduced into the online system - MetaboAnalyst for the analysis of metabolic pathways. It is generally believed that changes in key positions in the network have a serious impact on the occurrence of events. The results showed that 28 differential metabolites perhaps mainly were related to 12 important metabolic pathways, which are (a) Phenylalanine, Tyrosine and tryptophan biosynthesis, (b) Linoleic acid metabolism, (c) Starch and sucrose metabolism, (d) Phenylalanine Metabolism, (e) Arachidonic acid metabolism, (f) Glycine Serine and threonine metabolism, (g) Arginine and proline metabolism, (h) Pentose and glucuronate interconversions, (i) Tryptophan metabolism, (j) Tyrosine metabolism, aspartate and glutamate metabolism, (k) Alanine, aspartate and glutamate metabolism and (l) Glyoxylate and dicarboxylate metabolism. The results are shown in Figure 5 A.

In the same way, significant differences in metabolites between the four treatment groups and the model group were also introduced for online systematic analysis. Differential metabolites in the full prescription group mainly involved in four pathways: (g) Arginine and proline metabolism, (m) Biosynthesis of fatty acids, (n) Amino sugar and nucleotide sugar metabolism, and (do) aminoacyl-tRNA biosynthesis, which were shown in Figure 5 B. The no principle drug group had only one nonessential metabolic pathway: Amino sugar and nucleotide sugar metabolism. The no ministerial drug group and adjuvant drug group have three identical metabolic

pathways: (g) Arachidonic acid metabolism, (h) Pentose and glucuronate interconversions, and (e) Arginine and proline metabolism. The results showed that Arginine and proline metabolism appeared in the model group and the other three treatment groups except for the no principle drug group, suggesting that this metabolic pathway may be influenced by principle drugs.

4. Discussion

In this study, an animal model with hypertension induced by high-fat diet was established to study the efficacy and mechanism of BBTD on model rats, and to explore the effects of each component of the formula in order to provide a theoretical basis for further optimization of the prescription compatibility.

The results showed that BBTD formula can effectively reduce the weight increase of model rats, improve the histological characteristics, reduce the levels of TG and T-CHO in serum, and significantly reduce SBP and DBP. In addition, BBTD can also reverse the substance metabolism disorders caused by high-fat diet in rats, and its possible pathways are arginine and proline metabolism, linoleic acid metabolism and arachidonic acid metabolism, etc. In other administration groups, the overall effect of the no principle drug group was worse than that of the full prescription group, and the efficacy of the no adjuvant drug group was similar to that of the full prescription group, suggesting that the principle drugs (*Pinellia pinellia* and *gastrodia elata*) in the drug formulation were the key act factor in anti-hypertension and anti-hyperlipidemia process.

Animal weight gain, high blood pressure (SBP>140 mmHg or DBP>90 mmHg) and high lipid content are important markers of high-fat diet induced hypertension. The body weight increase, high blood pressure, high serum TG and T-CHO content of the model rats were found decreased after intragastric administration of BBTD. Interestingly, the results of the present study are consistent with those reported in the literature [18]. In the other administration groups, the indexes mentioned above of the no principle drug group were not significantly reduced. Although the no minister drug

group and the no adjuvant drug group were not as effective as the full prescription group, but both of them improved the obesity symptoms of rats to a certain extent, which further indicated that the principle medicines played an important role in the treatment.

Previous studies have shown that the serum endogenous metabolites of hypertension rats induced by high-fat diet are significantly different from those of normal rats [19]. In the present study, a total of 28 different metabolites were found between the normal group and the model group. We further analyzed chemical properties, 28 differential metabolites were mainly belonged into amino acids (L-Isoleucine, L-Proline, Glycine, L-threonine, L-phenylalanine, L-Ornithine, L-Tryptophan, L-Tyrosine), sugars (Xylitol, D-Mannitol, D-Fructose, Sedoheptulose, Talose and D-glucose), fatty acids (Ethanedioic acid, Butanoic acid, Formic acid, benzoic acid, 9,12-Octadecadienoic acid, Oleic acid, trans-9-Octadecenoic acid and Arachidonic acid), salts (phosphate, Phosphoric acid) and so on. It is shown that metabolites imbalance caused by high-fat diet is mainly concentrated in amino acids and fatty acids. Among which, disterbance of Formic acid, L-Ornithine, benzoic acid, L-Trytophan, Oleic acid, 9,12-Octadecadienoic acid and trans-9-Octadecenoic acid was regulated to varying degrees after drug treatment. As for the other disturbance metabolites, elevated blood D-glucose is one of the main causes of hypertension [20], Xylitol, L-Ornithine, benzoic acid, Olieic acid, Short-chain fatty acids (Butanoic acid, Formic acid,etc) and amino acids(L-Proline, Glutamine) all exhibt anti-hypertension effect with different manner [21-27]. In the present study, D-glucose was up-regulated in model group while down-regulted in full presciption group and no adjuvant group, the other anti-hypertension factors firstly decreased after modeling finally increased in each drug treated group. Interestingly, there was significant difference between the no adjuvant group and model group. In general, most metabolites disturbance caused by high-fat diet were corrected to normal in varying degrees after each drug treatment. In terms of the amount and degree of correcting the matabolites disorder, the intervention effect of no adjuvant presciption was the best, followed by BBTD, no ministeral drug group and no priciple drug drug group.

In order to explore the mechanism of BBTD on hypertension, we further carried out pathway analysis of 28 differential metabolites. It showed that the 28 markers in the model group were mainly involved in 12 important metabolic pathways. The possibile metabolic pathways of BBTD mainly lie in Arginine and proline metabolism, Biosynthesis of fatty acids, Amino sugar and nucleotide sugar metabolism, and aminoacyl-tRNA biosynthesis. The first three of which were all metabolic pathways altered in the hypertension model group of rats. The main metabolic pathways of no ministerial drug group and the no adjuvant drug group were the same, focusing on three pathways: Arachidonic acid metabolism, Pentose and glucuronate interconversions, Arginine and proline metabolism. There was no important metabolic pathway in the no principle drug group, which indicated that the therapeutic effect could only be achieved when principle drugs exist in the BBTD prescription. It further demonstrated that minister medicine and adjuvant medicine play a similar auxiliary role in the treatment.

This study confirmed that BBTD could effectively alleviate the symptoms of hypertension in model rats and modulate the metabolites imbalance caused by the high-fat diet, and predicted the possible pathway of BBTD through bio-informatics technology. However, due to limited experimental conditions, this study did not further verify the metabolic pathways in which BBTD may participate in. Traditional Chinese medicine compound has many compatible components and complex pharmacodynamic substances. In order to further optimize the ratio of drugs, this study further studied the role of each prescription drugs in the formulation. The study found that the pharmacological effect of the group without principle drugs (*Pinellia pinellia* and *Rhizoma elata*) was the worst, suggesting that the two kinds of herbs were the main pharmacological components in the compound. Therefore, it was planned to further select *Pinellia pinellia* and *Rhizoma elata* to study their anti-hypertension and anti-hyperlipidemia effect.

In conclusion, BBTD can effectively improve the symptoms of hypertension induced by high-fat diet, which may be related to the increase of serum linoleic acid and proline content, which can improve NO utilization rate. The experimental results

showed that the two kinds of principle medicines were the main components of the medicinal effect, which were consistent with the principle of the compatibility of the Chinese herbal compound, providing theoretical basis for the further rational optimization of the compatibility of the prescription.

Data Availability

The raw data used to support the findings of this study was made available from the corresponding author upon reasonable request.

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Conflict of interest statement

There was no conflict of interst.

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Table 1 Changes of 28 differential disease-related metabolites of each group

metabolites	variation tendency				
	B vs A	C vs B	D vs B	E vs B	F vs B
Ethanedioic acid	↓ *	↓	↑	↓	↓
Butanoic acid	↓ *	↓	↑	↑	↓
phosphate	↓ *	↑ *	↑	↓	↓
L-Isoleucine	↓ *	↓	↑	↓	↓
L-Proline	↓ *	↑ *	↑	↓	↓
Glycine	↓ *	↓	↑	↑	↑
L-threonine	↓ *	↓	↑	↑ *	↑
Glutamine	↓ *	↓	↑	↓	↓
L-phenylalanine	↓ *	↓	↑	↑	↓
Xylitol	↓ *	↑	↑	↑ *	↑ *
Phosphoric acid	↓ *	↑	↑	↑	↑ *
Formic acid	↓ *	↑	↑	↑	↑ *
L-Ornithine	↓ *	↑	↑	↑	↑ *
benzoic acid	↓ *	↑	↑	↑ *	↑ *
D-Mannitol	↓ *	↑	↑	↑ *	↑ *
D-Fructose	↓ *	↑ *	↑ *	↑ *	↑ *
Sedoheptulose	↓ *	↓	↑	↑	↓
D-Glucose	↑ *	↓	↑	↑	↓
L-Tyrosine	↑ *	↑	↑	↑	↓
Propionamide	↑ *	↓ *	↓	↓ *	↓ *
Morpholine	↑ *	↓ *	↓	↓ *	↓ *
L-Tryptophan	↓ *	↓	↑	↓	↑
9,12-Octadecadienoic acid	↓ *	↑	↑	↓	↑
Phenol	↓ *	↑	↑	↓	↑
Talose	↓ *	↑ *	↑	↑	↑ *
Oleic acid	↓ *	↑ *	↑	↑	↑ *
Arachidonic acid	↑ *	↑	↑	↓ *	↓ *
trans-9-Octadecenoic acid	↓ *	↑ *	↑ *	↑	↑

*p<0.05

FIGURE CAPTIONS

Fig. 1. BBTD improves the hypertension. a.Body weight from animals of different groups; b. DBP from animals of different groups ; c. SBP from animals of different groups; d. TG from animals of different groups; e. T-CHO from animals of different groups. A: Normal control group, B: Model group, C: Full prescription group, D: No principle drug group, E: No ministerial drug group, F. No adjuvant drug group (The following illustration is the same.), *comperad with the group B, $p<0.05$. # comperad with the group A, $p<0.05$.

Fig. 2. Total ion current (TIC) chromatograms of six typical GC/MS sera. A. Normal control group, B. Model group, C. Full prescription group, D. No principle drug group, E. No ministerial drug group, F. No adjuvant drug group.

Fig. 3. Analysis of metabolic differences between the moel and normal groups. a. Plots of principle component analysis (PCA, normal vs model mice, n = 9). b. Plots of orthogonal partial least-squares-discriminant analysis (PLS-DA analysis, normal vs model mice, n = 9). ■Samples from normal group ●Samples from model group.

Fig. 4. PLS-DA three-dimensional metabolic map of each group. a. ●A *vs* ●B *vs* ●C; b. ●A *vs* ●B vs ●D; c. ●A *vs* ●B *vs* ●E; d. ●A vs ●B vs ●F; e. ●C vs ●D vs ●E vs ●F.

Fig. 5. Analysis of metabolic pathways identified by MetPA software. A. Metabolic pathways in model rats, B. Metabolic pathways of BBTD treatment group, C. Metabolic pathways of no ministerial drug group, D. Metabolic pathways of no adjuvant drug group. The abscissa axis represents the pathway impact, the vertical axis represents the P value of enrichment analysis. The bubble is more larger and the bubble color is more deeper , the enrichment degree.is more significant.

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