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

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April 16, 2024

Abstract

Banxia Baizhu Tianma Decoction (BBTD), a classical compound of traditional Chinese medicine (TCM), has been clinically used for hypertension treatment. There are so many components in this formula that it is necessary to explore whether each component plays a positiverole in anti-hypertension treatment. After hypertension models were established with high-fat diet for 6 weeks, rats in each group were treated with full prescription, no principle prescription, no ministerial prescription and no adjuvant prescription for 6 weeks respectively. It was found that BBTD was effective in reducing body weight gain, decreasing serum TG and T-CHO, as well as lowering diastolic blood pressure (DBP) and systolic blood pressure (SBP). Total 28 metabolites in model rats were identified to be different from that of control group, which focusing on 12 important metabolic pathways: (a) Phenylalanine, Tyrosine and tryptophan biosynthesis, (b) Linoleic acid metabolism, (c) Starch and sucrose metabolism, etc. Eight different metabolites (Talose, Oleic acid, L-Proline, etc) were identified between the full prescription group and the model group, which are mainly involved in 4 metabolic pathways (Arginine and proline metabolism, Biosynthesis of unsaturated fatty acids, Amino sugar and nucleotide sugar metabolism and Aminoacyl-tRNA biosynthesis). Furthermore, it also indicated that the principle drugs ie. Pinellia ternata and Gastrodia elata was better than the other drug treatment. In a word, BBTD has a therapeutic effect on hypertension in rats, and Pinellia ternata and Gastrodia elata play an important role in the treatment. The study provides theoretical basis for further optimization of BBTD formula.

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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

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Key words: Banxia Baizhu Tianma Decoction, hypertension, high-fat diet, compatibility.

Words count:

Table count: 1

Figure count: 5.

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% ($\approx$ 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

61 compatibility of Chinese herbal compounds, BBTD is mainly made up of six

62 herbs, which are monarch drugs: *Pinellia ternata* and *Gastrodia*, ministerial drug:

63 *Atractylodes macrocephala Koidz* and *Poria cocos* and adjuvant drugs: *Citrus*

64 *maxima* and *Glycyrrhiza uralensis Fisch*. BBTD can be used to cure some disease

65 sympotoms, such as irascibility, essential dizziness, palpitations, phlegm, and so on [7].

66 Yue-Hua Jiang with his colleagues found that BBTD has a positive effect on lowering

67 obesity-related blood pressure [8]. Each drug in the prescription possesses different

68 effects. In a word, *Pinellia ternata* and *Gastrodia elata* both can eliminate dampness

69 and phlegm [9]， *Atractylodes macrocephala Koidz* and *Poria cocos* can fortify the

70 spleen and percolate dampness [10-11]， *Citrus maxima* can regulate qi-flowing for

71 eliminating phlegm，and *Glycyrrhiza uralensis Fisch* can coordinate the drug actions

72 of a prescription[12]. But what role each component of BBTD plays in the

73 hypertension treatment remains unknown and it deserves further study in order to

74 optimize the formula.

75 Studies have shown that high-fat-diet can successfully establish obesity disease

76 model in mice, and this model is similar to the pathological symptoms of clinical

77 obesity, along with the advantage of good stablity, simple operation and lower cost

78 [13]. Therefore, this paper adopted hypertension mouse model induced by high fat diet

79 to explore the effect of BBTD on hypertension treatment. Furthermore, we attempted

80 to elucidate the pharmacological effect of each ingredient herb in BBTD on

81 hypertension treatment by studying the efficacy of them and exploring the changes in

82 serum metabolites. It provided a theoretical basis for further optimizing the

83 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 $2.00 \sim 2.50$ 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

110   70% in a dark cycle of 12/12 hours. The rats were fed with qualified standard food

111   and distilled water for one week adaptive feeding.

112   A week later, 50 rats were randomly selected to establish an animal model of

113   hypertension [15]. The rats were fed with a humid environmentroom temperature

114   adjusted at 18~20℃, humidity at 85%~90%) for 4 weeks.

115   The body weight and the blood pressure of rats were measured and recorded after

116   12 h of fasting and banning water. It is recognized that the animal model was

117   successfully established when SBP >140 mmHg and/or DBP >90 mmHg [15],

118   accompanying with anxiety, depressive behavior and significant weight gain, etc [16].

119   Body weight, SBP and DBP were measured before, during and after the modeling

120   process.

121 *2.4. Drug Treatment and Tissue Collection*

122   The model rats were randomly divided into 5 groups (n=10): B-model group,

123   C-full prescription group, D-no principle drug group, E-no ministerial drug group and

124   F-no adjuvant drug group. Meanwhile, 10 normal rats were used as control (A).

125   Rats in each drug treatment group were all fed with high-fat diet, while the rats in

126   normal control group were fed with standard diet. As for drug treatment, rats in group

127   C, D, E and F were intragastric administrated with full prescription, no principle drug,

128   no ministerial drug and no adjuvant drug respectively. The drug dose was 1 mL/d.

129   After anesthetized with 3% pentobarbital sodium (40 mg/kg), blood of rats were

130   harvested from the inferior vena cava and kept in air for 2 h at 4 ℃ , then were

131   centrifuged for 5 min (4℃,4000 rpm). In the end, each upper serum was divided into

132   thirds and stored at -80℃ until further testing.

133 *2.5. Content Detection of TG and T-CHO in Serum*

134   The contents of serum TG and T-CHO were determined by GPO-PAP enzymatic

135   method with Triglyceride assay kit and CHOD-PAP method with total cholesterol

136   assay kit respectively. All operations were strictly performed according to the kit

137   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:

T-CHO or TG (mmoL/L)= (OD_{sample} - OD_{blank}) / ($OD_{calibration}$ - OD_{blank})*

standard concentration (mmol/L)   (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 ℃),

166   injection volume (1.0 µL) and the flow rate (1.0 mL/min) of the carrier gas. The split

167   ratio of injection volume was 2:1. The MS parameters were included: the ion source

168   temperature (230 ℃), the quadrupole temperature (150 ℃), the electron impact

169   ionization voltage (70 eV), and the mass spectrum full scan range (30~550 m/z).

170   *2.8. Data Processing*

171   The GC/MS original spectrum file of the obtained sample was converted to

172   NetCDF format by the Databridge program (PerkinElmer Inc, USA) in Turbomass

173   software, then were imported into R2.7 software. After calculation and normalization,

174   a three-dimensional matrix of retention time, mass-to-charge ratio and peak intensity

175   were finally obtained (https://xcmsonline.scripps.edu). This matrix will be used for all

176   subsequent data analysis. Metabolites were identified using commercial metabolite

177   databases (NIST, Wiley Registry) and standard metabolite databases established by

178   our research group.

179   To perform multi-dimensional statistical analysis, we imported the obtained

180   three-dimensional matrix into SIMCA-P11.0 software (Umetrics, Sweden), and

181   pre-processed the data with mean center and Pareto scale. In the process of

182   establishing mathematical model, we first used the unsupervised principal component

183   analysis (PCA) method to analyze the overall data and observed the natural

184   distribution and group relationship of the experimental samples. In order to further

185   distinguish the differences among different groups, we used supervised analysis

186   methods (partial least squares discriminant analysis, PLS-DA) to find the main

187   difference variables leading to sample aggregation and dispersion.

188   *2.9. Statistics Analysis*

189   Statistical software SPSS 25 (SPSS, Chicago, USA) was used for data analysis.

190   Measurement data were expressed as mean ± standard deviation. LSD test was used

191   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

247   In this study, the status of 28 differential metabolites in different treatment groups

248   was further analyzed by multidimensional statistical method. The statistical results

249   showed that 28 differential metabolites in the full prescription group, no principle

250   drug group, no minister group and no adjuvant drug group were significantly different

251   from that of model group, with an apparent tendency to be approach to the normal

252   group. Above results confirmed that BBTD can regulate the metabolite disorder

253   caused by the modeling process (Fig.4 a-d).

254   In order to more intuitively represent the changes in metabolic patterns of the four

255   drug treatment groups, this study conducted multi-dimensional statistical analysis on

256   the data of the four groups. The results showed that their metabolic spectrum could

257   not be separated. The results (Fig.4 e) showed no difference between the four

258   treatment groups in reversing the metabolite disruption caused by the modeling

259   process.

260   To further observe the influence of BBTD on high-fat diet rats, the changes of 28

261   metabolites in model group and other 5 groups were compared respectively (Table 1).

262   In total 8 metabolites among the 28 different metabolites in the full prescription

263   group, which are Talose, Oleic acid, L-proline, D-fructose, phosphate,

264   trans-9-octadecenoic acid, Morpholine and Propionamide, were significantly different

265   from that of model group ($P<0.05$).   While, the contents of Xylitol, Phosphoric acid,

266   Formic   acid,   L-Ornithine,   benzoic   acid,   D-Mannitol,   D-Glucose,

267   9,12-Octadecadienoic acid and Phenol were slightly different from   that of model

268   group. As for no adjuvant drug treatment,   the contents of Xylitol, Phosphoric acid,

269   Formic acid, L-Ornithine, benzoic acid, D-Mannitol, D-Fructose , Propionamide,

270   Morpholine,Talose,Oleic acid and Arachidonic acid were significantly different from

271   that of model group ($P<0.05$), along with sightly increase of Glycine, L-threonine,

272   D-Glucose, L-Tyrosine, L-Tryptophan, 9,12-Octadecadienoic acid, Phenol and

273   trans-9-Octadecenoic acid. Treatment with no ministerial drug , there were 8

274   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

332 group and the no adjuvant drug group were not as effective as the full prescription

333 group, but both of them improved the obesity symptoms of rats to a certain extent,

334 which further indicated that the principle medicines played an important role in the

335 treatment.

336   Previous studies have shown that the serum endogenous metabolites of

337 hypertension rats induced by high-fat diet are significantly different from those of

338 normal rats [19]. In the present study, a total of 28 different metabolites were found

339 between the normal group and the model group. We further analyzed chemical

340 properties, 28 differential metabolites were mainly belonged into amino acids

341 (L-Isoleucine, L-Proline, Glycine, L-threonine, L-phenylalanine, L-Ornithine,

342 L-Tryptophan, L-Tyrosine), sugars (Xylitol, D-Mannitol, D-Fructose, Sedoheptulose,

343 Talose and D-glucose), fatty acids (Ethanedioic acid, Butanoic acid, Formic acid,

344 benzoic acid, 9,12-Octadecadienoic acid, Oleic acid, trans-9-Octadecenoic acid and

345 Arachidonic acid), salts (phosphate, Phosphoric acid) and so on. It is shown that

346 metabolites imbalance caused by high-fat diet is mainly concentrated in amino acids

347 and fatty acids. Among which, disterbance of Formic acid, L-Ornithine, benzoic acid,

348 L-Trytophan, Oleic acid, 9,12-Octadecadienoic acid and trans-9-Octadecenoic acid

349 was regulated to varying degrees after drug treatment. As for the other disturbance

350 metabolites, elevated blood D-glucose is one of the main causes of hypertension [20],

351 Xylitol, L-Ornithine, benzoic acid, Olieic acid, Short-chain fatty acids (Butanoic acid,

352 Formic acid,etc) and amino acids(L-Proline, Glutamine) all exhibt anti-hypertension

353 effect with different manner [21-27]. In the present study, D-glucose was up-regulated in

354 model group while down-reglted in full presciption group and no adjuvant group, the

355 other anti-hypertension factors firstly decreased after modeling finally increased in

356 each drug treated group. Interestingly, there was significant difference between the no

357 adjuvant group and model group. In general, most metabolites disturbance caused by

358 high-fat diet were corrected to normal in varying degrees after each drug treatment. In

359 terms of the amount and degree of correcting the matabolites disorder, the

360 intervention effect of no adjuvant presciption was the best, followed by BBTD, no

361 ministeral drug group and no priciple drug drug group.

362   In order to explore the mechanism of BBTD on hypertension, we further carried

363   out pathway analysis of 28 differential metabolites. It showed that the 28 markers in

364   the model group were mainly involved in 12 important metabolic pathways. The

365   possibile metabolic pathways of BBTD mainly lie in Arginine and proline metabolism,

366   Biosynthesis of fatty acids, Amino sugar and nucleotide sugar metabolism, and

367   aminoacyl-tRNA biosynthesis. The first three of which were all metabolic pathways

368   altered in the hypertension model group of rats. The main metabolic pathways of no

369   ministerial drug group and the no adjuvant drug group were the same, focusing on

370   three pathways: Arachidonic acid metabolism, Pentose and glucuronate

371   interconversions, Arginine and proline metabolism. There was no important metabolic

372   pathway in the no principle drug group, which indicated that the therapeutic effect

373   could only be achieved when principle drugs exist in the BBTD prescription. It further

374   demonstrated that minister medicine and adjuvant medicine play a similar auxiliary

375   role in the treatment.

376   This study confirmed that BBTD could effectively alleviate the symptoms of

377   hypertension in model rats and modulate the metabolites imbalance caused by the

378   high-fat diet, and predicted the possible pathway of BBTD through bio-informatics

379   technology. However, due to limited experimental conditions, this study did not

380   further verify the metabolic pathways in which BBTD may participate in. Traditional

381   Chinese medicine compound has many compatible components and complex

382   pharmacodynamic substances. In order to further optimize the ratio of drugs, this

383   study further studied the role of each prescription drugs in the formulation. The study

384   found that the pharmacological effect of the group without principle drugs (*Pinellia*

385   *pinellia* and *Rhizoma elata*) was the worst, suggesting that the two kinds of herbs

386   were the main pharmacological components in the compound. Therefore, it was

387   planned to further select *Pinellia pinellia* and *Rhizoma elata* to study their

388   anti-hypertension and anti-hyperlipidemia effect.

389   In conclusion, BBTD can effectively improve the symptoms of hypertension

390   induced by high-fat diet, which may be related to the increase of serum linoleic acid

391   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.

Acknowledgements

Conflict of interest statement

There was no conflict of interst.

Fundings

References

[1] Wang Z, Chen Z, Zhang L et al., "China Hypertension Survey Investigators. Status of Hypertension in China: Results From the China Hypertension Survey, 2012-2015," *Circulation,* vol. 137(22), no. 2344-2356, PMID: 29449338, 2018.

[2] Bundy JD, Li C, Stuchlik P et al., "Systolic Blood Pressure Reduction and Risk of Cardiovascular Disease and Mortality: A Systematic Review and Network Meta-analysis," *JAMA Cardiol,* vol. 2(7), no.775-781, PMID: 28564682; PMCID: PMC5710614, 2017.

[3] Seravalle G, Grassi G. Obesity and hypertension. Pharmacol Res. 2017 Aug;122:1-7. doi: 10.1016/j.phrs.2017.05.013. Epub 2017 May 19. PMID: 28532816.

[4] Chrysant SG. Pathophysiology and treatment of obesity-related hypertension. J Clin Hypertens (Greenwich). 2019 May;21(5):555-559. doi: 10.1111/jch.13518. Epub 2019 Mar 24. PMID: 30907058.

[5] Desai AN, "High Blood Pressure," *JAMA,* vol. 324(12), no. 1254-1255, PMID: 32960243, 2020.

[6] Li H, Liu LT, Zhao WM et al., "Effect of traditional and integrative regimens on quality of life and early renal impairment in elderly patients with isolated systolic hypertension," *Chin J Integr Med,* vol. Jun;16(3), no. 216-21, PMID: 20694775, 2010.

[7] Li J, Wu H, Liu Y,et al., High fat diet induced obesity model using four strainsof mice: Kunming, C57BL/6, BALB/c and ICR. Exp Anim. 2020 Aug 5;69(3):326-335. doi: 10.1538/expanim.19-0148. Epub 2020 Mar 19. PMID: 32188837; PMCID: PMC7445062.

[8] Yang J Y, " Recent research progress on the real medicinal material of Citri tangerine," Guangming Chinese Medicine, vol. 35.04, no. 626-628, 2020.

[9] Fu Y, Shan M, Hu M et al., "Chemical profiling of *Banxia Baizhu Tianma Decoction* by ultra-fast liquid chromatography with tandem mass spectrometry," *J Pharm Biomed Anal,* vol. 174, no. 595-607, PMID: 31261041, 2019.

[10] TAO Ning, ZHANG Jie, "Zhang Jieji's Experience in Treating Rheumatoid Arthritis with Spleen Deficiency and Generating Damp," Journal of liaoning university of traditional Chinese medicine,Vol. 1-7, 2021.

[11] Zuo Jun, Zhang Jinlong, Hu Xiaoyang, "The chemical composition of atractylodes and modern pharmacology research progress," Journal of liaoning university of traditional Chinese medicine*,* vol. 1-8, 2021.

[12] Cheng Yue, Ding Zexian, Zhang Yu, et al., "Progress in chemical structure and pharmacological action of poria cocos polysaccharide and its derivatives," China Journal of Chinese Materia Medica*,* vol. 45(18), no. 4332-4340, 2020.

[13] Wang J, Xiong X, "Control strategy on hypertension in chinese medicine," *Evid Based Complement Alternat Med*. 2012:284847. PMID: 22194771; PMCID: PMC3239016, 2012.

[14] Jiang YH, Zhang P, Tao Y, "*Banxia Baizhu Tianma decoction* attenuates obesity-related hypertension," *J Ethnopharmacol,* vol. 266, no. 113453, PMID: 33039628, 2021.

[15] Chen T J, Xu N. Construction, analysis and evaluation of the rat model of spontaneous hypertension with excessive phlegm-dampness obstruction [J]. Pharmaceutical research,2015,34(09):503-506.

458   [16]   Y. Zhu, Z. Guo, L. Zhang et al.,"System-wide assembly of pathways and modules
459   hierarchically reveal metabolic mechanism of cerebral ischemia," *Scientifific Reports*, vol. 5,
460   no. 1, Article ID 17068, 2015.

461   [17]   Xu Nan, Sun Rong, Zhu Xiaoming et al., "Identification of Q-marker based on multivariate
462   statistical method and component difference analysis for *Pinellia Atractylodes Tianma*
463   *Decoction* [J]," *Chinese herbal medicine,* vol. 50 (19), no. 4595-4602, 2019.

464   [18]   Xie K, Bao L, Jiang X et al., "The association of metabolic syndrome components and chronic
465   kidney disease in patients with hypertension," *Lipids Health Dis,* vol. 18(1), no. 229, PMID:
466   31881889; PMCID: PMC6935087, 2019.

467   [19]   Jiang YH, Zhang P, Tao Y et al., "Banxia Baizhu Tianma decoction attenuates
468   obesity-related hypertension," J Ethnopharmacol, vol. 266, no. 113453, PMID: 33039628,
469   2020.

470   [20]   Corry DB, Tuck ML. Glucose and insulin metabolism in hypertension. Am J Nephrol.
471   1996;16(3):223-36. doi: 10.1159/000169002. PMID: 8739882.

472   [21]   Wölnerhanssen BK, Meyer-Gerspach AC. Effekte von Zuckerkonsum auf die Gesundheit und
473   mögliche Alternativen [Health effects of sugar consumption and possible alternatives]. Ther
474   Umsch. 2019 Sep;76(3):111-116. German. doi: 10.1024/0040-5930/a001070. PMID:
475   31498044.

476   [22]   Ytrebø LM, Kristiansen RG, Maehre H, Fuskevåg OM, Kalstad T, Revhaug A, Cobos MJ,
477   Jalan R, Rose CF. L-ornithine phenylacetate attenuates increased arterial and extracellular
478   brain ammonia and prevents intracranial hypertension in pigs with acute liver failure.
479   Hepatology. 2009 Jul;50(1):165-74. doi: 10.1002/hep.22917. PMID: 19554542.

480   [23]   Luo LJ, Nguyen DD, Lai JY. Benzoic acid derivative-modified
481   chitosan-g-poly(N-isopropylacrylamide): Methoxylation effects and pharmacological
482   treatments of Glaucoma-related neurodegeneration. J Control Release. 2020 Jan
483   10;317:246-258. doi: 10.1016/j.jconrel.2019.11.038. Epub 2019 Nov 29. PMID: 31790728.

484   [24]   Terés S, Barceló-Coblijn G, Benet M, Alvarez R, Bressani R, Halver JE, Escribá PV. Oleic
485   acid content is responsible for the reduction in blood pressure induced by olive oil. Proc Natl
486   Acad Sci U S A. 2008 Sep 16;105(37):13811-6. doi: 10.1073/pnas.0807500105. Epub 2008
487   Sep 4. PMID: 18772370; PMCID: PMC2544536.

488   [25]   Bier A, Braun T, Khasbab R, et al., A High Salt Diet Modulates the Gut Microbiota and Short
489   Chain Fatty Acids Production in a Salt-Sensitive Hypertension Rat Model. Nutrients. 2018
490   Aug 23;10(9):1154. doi: 10.3390/nu10091154. PMID: 30142973; PMCID: PMC6164908.

491   [26]   Ke C, Zhu X, Zhang Y et al., "Metabolomic characterization of hypertension and
492   dyslipidemia," *Metabolomics,* vol. 14(9), no. 117, PMID: 30830367, 2018.

493   [27]   Durante W. The Emerging Role of l-Glutamine in Cardiovascular Health and Disease.
494   Nutrients. 2019 Sep 4;11(9):2092. doi: 10.3390/nu11092092. PMID: 31487814; PMCID:
495   PMC6769761.

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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.