Systemic metabolomics characterization of the ascending and descending property

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Research Article

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Abstract

Background According to the theory of TCM, ascending medicine has the effects of elevating yang, raising drooping, and inducing sweating. Descending medicine has the effects of relieving dyspnea and hiccup, stopping reflux, and calming nerves. In conclusion, the ascending/descending properties of TCM serve as a guide for the use of TCMs in clinical practice. However, at present, there are few studies on the ascending/descending properties of TCM. This study aimed to compare the effects of 6 ascending and 8 descending medicine on the main organs (heart, liver, lung, spleen, and kidney) of normal rats, characterizing the ascending and descending properties. Methods The organ samples of all experiment group were analyzed based on ultra performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-Q/TOFMS). Then, to character the ascending and descending properties, the effects of 6 ascending and 8 descending medicine on the main organs (heart, liver, lung, spleen, and kidney) of normal rats were compared. Results The systemic metabolomics results indicated that linoleic acid metabolism and arginine and proline metabolism were the major differential pathways affected by ascending/descending medicine in the heart; nicotinic acid and nicotinamide metabolism, glutamic acid and glutathione metabolism, and L-carnitine metabolism were the major differential pathways affected by ascending/descending medicine in the liver; tryptophan metabolism, phenylalanine metabolism, and pyroglutamic acid and glutamic acid metabolism were the major differential pathways affected by ascending/descending medicine in the lung; tryptophan metabolism, phenylalanine metabolism, and glutamic acid metabolism were the major differential pathways affected by ascending/descending medicine in the kidney; and glutamic acid and glutamine metabolism were the major differential pathway affected by ascending/descending medicine in the spleen. Conclusions Ascending medicine exhibited positive effects on nerve stimulation, immunity, and reproductive function by promoting energy metabolism in the heart and liver, whereas descending medicine had the opposite effect. Descending medicine exhibited a positive effect on diuresis by promoting blood circulation, whereas ascending medicine had the opposite effect. Both descending medicine and ascending medicine intervened with pulmonary inflammation, but the underlying mechanisms may be different. This study demonstrated that systemic metabolomics technology can be used for the characterization of traditional Chinese medicine properties.

Introduction

The properties of traditional Chinese medicine (TCM) include cold/hot [1–3], ascending/descending [4], and meridian tropism [5, 6]. In the previous study, we investigated the effects of typical hot and cold medicine on normal animals. We found that energy metabolism could be stimulated by typical hot TCM and inhibited by typical cold TCM. Moreover, a system to evaluate the typical hot and cold properties of Chinese medicine was established [7].

The characteristics of cold/hot and ascending/descending are properties of TCM. According to the theory of TCM, ascending medicine has the effects of elevating yang, raising drooping, and inducing sweating; therefore, they are often used to treat diseases with a downward tendency, such as diarrhea, uterine
prolapse, and a lack of sweat. Descending medicine has the effects of relieving dyspnea and hiccup, stopping reflux, and calming nerves; therefore, they are often used to treat diseases with an upward tendency, such as coughing, vomiting, irritability, edema, and defecation difficulty. In conclusion, the ascending/descending properties of TCM serve as a guide for the use of TCMs in clinical practice. However, at present, there are few studies on the ascending/descending properties of TCM. The ascending/descending properties are rarely taken advantage of in clinical practice, partly due to the lack of clear quantitative indicators to facilitate evaluation.

TCM generally has multiple components and multiple targets, and it is difficult to comprehensively evaluate the properties of TCM based on a single biochemical index [8]. Therefore, it is necessary to find an appropriate method to study the properties of TCM. Metabolomics analysis has a holistic nature and is suitable for comprehensive TCM research [9]. Liquid chromatography–mass spectrometry (LC-MS) includes separation through ultra-high-performance LC (UPLC) and analysis through MS [10]. Complex multi-component systems can be separated effectively, quickly, and accurately. The use of LC-MS technology accelerates the development of metabolomics [11].

Different organs have different functions. TCM can affect multiple organs at the same time [12]. Therefore, in order to systematically elucidate the changes in the functions of organs upon treatment with ascending and descending TCM, we conducted a metabolomics analysis to study the effects of typical ascending TCM (Ephedrae Herba, Bupleuri Radix, Cimicifugae Rhizoma, Puerariae Thomsonii Radix, Menthae Haplocalycis Herba, and Platycodonis Radix), typical descending TCM (Magnetitum, Caryophylli Flos, Armeniacae Semen Amarum, Cyathulae Radix, Poria, Canavaliae Semen, Inulae Flos, and Lepidii semen), and Chuanxiong Rhizoma (with two-way regulation) on the heart, liver, spleen, lung, and kidney of normal rats, characterizing the ascending and descending properties.

Materials and Methods

Chemicals and reagents

LC-MS grade acetonitrile was purchased from Fisher Chemical (USA). Purified water was prepared with a water purification system. LC-MS grade formic acid (FA) was purchased from Anaqua Chemical Supply Inc. (USA).

All 15 TCM were purchased from Beijing Tongrentang Pharmacy. Professor Chen Suiqing of Henan University of Chinese Medicine authenticated them. The voucher specimens were deposited at our lab in Henan University of Chinese Medicine.

Extraction

Medicine was decocted two times with ten folds volume of water at 100°C (1 h each time). The extract was obtained by combining the decoctions and then concentrating the solution[4].

Animals
All animal experiments were approved by the Animal Care and Use Committee of Henan Medical University. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Male SD rats (weighing 190–240 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) (animal license number: SCXK (Jing) 2016-0006). Rats were housed in an environmentally controlled breeding room (temperature 22 ± 2°C, humidity 60 ± 5%, 12/12 h dark/light cycle). Water and food were available ad libitum. All rats were randomly divided into the control group, the typical ascending TCM group (Herba Ephedra Sinica, 4.2 g/kg; Radix Bupleuri Chinensis, 7.0 g/kg; Rhizoma Cimicifugae Foetidae, 7.0 g/kg; Radix Puerariae Lobatae, 10.5 g/kg; Herba Menthae Haplocalycis, 4.2 g/kg and Radix Platycodi, 7.0 g/kg), the typical descending TCM group (Magnetitum, 21.0 g/kg; Flos Syzygii Aromatici, 2.1 g/kg; Semen Armeniacae Amarum, 7.0 g/kg; Radix Cyathulae, 7.0 g/kg; Poria, Canavaliae Semen, 10.5 g/kg; Flos Inulae Japonicae, 6.3 g/kg and Semen Lepidii Apetalii, 6.3 g/kg), and the Ligusticum chuanxiong (with two-way regulation, 7.0 g/kg) group, with seven rats in each group. Rats in the typical ascending, typical descending, and two-way regulation groups were given corresponding extracts for 14 days. The dosage of TCM was determined according to the 2020 Pharmacopoeia.

**Metabolomics analysis**

**Sample preparation**

Blood sample was taken from the abdominal aorta and viscera (heart, liver, spleen, lung, and kidney) were collected and stored at −80°C.

Prior to Ultra performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q/TOF-MS) analysis, the samples were thawed at 0°C. Each organ sample was mixed with three folds volume of cold carbinol and homogenized. The homogenized samples were stored at −20°C overnight, thawed, and centrifuged at 12000 × g and 4°C for 15 min. The supernatant was stored at 4°C overnight and centrifuged at 12000 × g for 10 min, and finally, 2 µL of the supernatant was injected into the UPLC-Q/TOF-MS for analysis.

Aliquots (60 µL) of all samples were pooled to prepare quality control (QC) samples.

**UPLC-Q/TOF-MS analysis**

A Dionex Ulti-Mate 3000 UPLC system (Thermo Scientific, USA) was coupled with a maXis HD Q/TOF-MS platform (Bruker, Germany). Chromatographic separation of herbal extracts was performed on an Acclaim™ RSLC 120 C18 column (2.2 µm, 100 × 2.1 mm) at 40°C at a flow rate of 0.3 mL/min. The mobile phase consisted of water containing 0.1% FA (phase A) and acetonitrile (phase B). The gradient program was as follows: 0–3 min, 5–7% B; 3–5 min, 7–60% B; 5–6 min, 60–80% B; 6–10 min, 80–90% B; 10–24 min, 90–95% B. The MS conditions were as follows: the capillary voltage was 3.5 kV in ESI+ mode and...
3.2 kV in ESI\(^{-}\) mode, the dry gas temperature was 230°C, the nebulizer pressure was 2.0 bar, and the flow rate was 8 L/min.

**Data processing and statistical analysis**

Noise reduction, peak alignment, normalization, and baseline correction using Profile Analysis software (version 2.1, Bruker Germany) were performed on the raw UPLC-Q/TOF-MS data. PCA of the clean data was carried out using Simca-P 14.0 software (Umetrics, Sweden) to initially observe whether there were differences in metabolic profiles between different groups. To further explore the metabolic differences caused by typical ascending and descending TCM, orthogonal partial least squares discriminant analysis (OPLS-DA) was carried out. The variable importance in projection (VIP) value of each variable in the OPLS-DA model was calculated to determine its contribution to the classification. The molecules with VIP > 1 and \( P < 0.05 \) (t-test) were further analyzed in METLIN (http://metlin.scripps.edu/), HMDB (http://www.hmdb.ca/), and KEGG (http://www.genome.jp/kegg/) to identify potential biomarkers. According to the obtained potential biomarkers, a metabolic network was constructed to analyze the mechanisms underlying the effects of typical ascending and descending TCM. Finally, to visualize the variation trends of the metabolite levels among different groups, heatmaps of the shared metabolites among the ascending and descending TCM groups were produced according to the semi-quantitative biomarker results (Fig. 6B). The differential metabolic pathway network was established using the KEGG database to explain the effects of typical ascending and descending medicine.

**Results**

**Principal component analysis of organ samples**

**Principal component analysis of the control and ascending TCM groups**

In the PCA score plot, points at different shapes can represent different groups. The distance between points represents the difference in the metabolic characteristics of the two samples; the shorter the distance between two sample points, the higher the similarity of the metabolic characteristics of the two samples.

In the PCA score plots of the five organs (heart, liver, spleen, lung, and kidney), the sample points of the ascending TCM group were grouped into one category, indicating that the metabolic profile of each typical ascending TCM group is similar. There is a good separation between the sample points of the ascending TCM group and the control group (Fig. 1), indicating that the metabolic characteristics of normal rats have changed significantly after the administration of the typical ascending medicine.

**Principal component analysis of the control and descending TCM groups**
As shown in Fig. 2, the results of PCA of the five organs (heart, liver, spleen, lung, and kidney) showed that the sample points of the descending TCM group were grouped into one category, indicating that the metabolic profiles of the typical descending TCM groups are similar. There is a good separation trend between the sample points of the descending TCM group and the control group, indicating that the metabolic characteristics of normal rats have changed significantly after the administration of typical descending medicine.

**Principal component analysis of the ascending and descending TCM groups**

As shown in Fig. 3, in the PCA score plots, the sample points of the five organs (heart, liver, spleen, lung, and kidney) of the ascending and descending TCM groups were grouped separately, indicating that there is a difference in the metabolic profile between the ascending and descending TCM groups. Moreover, there is a certain overlap between the two groups, which needs further analysis in combination with different biomarkers.

**Identification of biomarkers**

To identify the differential biomarkers between groups in the five organs, PCA was conducted on different organ samples. As shown in Fig. S1–S5, there is a separation trend between the sample points of each administration group and the control group. Based on the PCA results, OPLS-DA and S-Polt analysis were conducted (Fig. S6–S10). In the S-Polt score plots, the points highlighted in red represent potential biomarkers. Furthermore, 200 permutations tests were conducted to ensure that there is no overfitting in each OPLS-DA (Fig. S11–S15). Finally, based on our OPLS-DA and S-Polt analysis results, an online database was used to identify the differential markers (Tables S1–S5).

**Quantitative and cluster analysis of biomarkers in multiple organs**

The quantitative results of biomarkers in the five organs of each ascending and descending TCM groups were imported into MeV 4.9.0. The heatmap cluster analysis results are shown in Fig. 4. In the heart, spleen, and kidney (Fig. 4A, D, E), the ascending and descending TCM groups are clustered on the left and right sides of the thermogram respectively. In the organs of heart, kidney and spleen, the regulation trend of two-way regulating medicine on markers is close to that of descending medicine, and in the organs of liver and lung, the regulation trend of two-way regulating medicine on markers is closer to that of ascending medicine. To sum up, the marker cluster level can be used to evaluate the effects of ascending and descending medicine.

**Metabolic pathway enrichment analysis and network construction**
The biomarkers in the heatmaps of the five organs were imported into the MetaboAnalyst 14.1 online database for pathway enrichment analysis, and the metabolic pathways in which these biomarkers in the five organs are involved were identified. Based on the biological functions of biomarkers, important metabolic pathways were screened, and metabolic network diagrams related to biomarkers in the five organs were constructed using the KEGG Mapping module (Fig. 5).

Discussion

Effects of ascending and descending medicine on heart

The metabolomics study indicated that linoleic acid metabolism and arginine and proline metabolism were the major differential pathways affected by ascending and descending medicine in the heart.

The heart consumes much energy. Therefore, glucose and fatty acids are continuously degraded [13]. When cardiac failure occurs, energy metabolism and ATP production are impaired [14]. Linolenic acid is a polyunsaturated fatty acid [15]. ATP can be produced through oxidative phosphorylation of linolenic acid in the mitochondria, and it can be degraded into unsaturated fatty acids. Unsaturated fatty acids can esterify cholesterol, reducing cholesterol and triglyceride levels in the blood at the same time, thus preventing atherosclerosis and other cardiovascular and cerebrovascular diseases [16]. The level of linolenic acid in normal rats was significantly increased after the administration of ascending medicine, indicating that ascending medicine may stimulate ATP production by promoting linoleic acid metabolism, enhance myocardial contractility, and prevent heart failure. The level of linolenic acid in the descending TCM group was decreased (Fig. 6).

Betaine is an important metabolite in arginine and proline metabolism. Betaine can protect the cardiovascular system by reducing the levels of lysosomal enzymes and lipid peroxidation and also protects against isoproterenol-induced myocardial infarction [17]. When myocardial infarction occurs, some myocardial cells become locally necrotic due to ischemia and the heart cannot pump blood normally. The betaine level in the heart of rats was significantly increased after administration of descending medicine, indicating that descending medicine can play a protective role in the cardiovascular system by increasing the level of betaine.

Effects of ascending and descending medicine on liver

The metabolomics study indicated that nicotinic acid and nicotinamide metabolism, glutamic acid and glutathione metabolism, and L-carnitine metabolism were the major differential pathways affected by ascending and descending medicine in the liver.

The liver is the main metabolic organ of the body. It has important functions in a variety of physiological processes, such as digestion, metabolism, detoxification, secretion, and immunity. The water, salt, sugar, and amino acids absorbed by the digestive tract are transported to the liver through the portal vein. After
undergoing liver metabolism, they pass through the inferior vena cava to reach the heart to enter the systemic circulation. Non-nutrient substances can be transformed and detoxified in the liver and then eliminated from the body [18, 19]. Nicotinamide participates in energy metabolism. Nicotinamide is the precursor of coenzyme-I and coenzyme-II. Coenzyme-I and coenzyme-II are coenzymes of dehydrogenase and transfer material in the body for lipid metabolism and glycolysis [20, 21]. In the present study, the level of nicotinamide in the liver samples of normal rats was increased after the administration of ascending medicine, indicating that ascending medicine can promote energy metabolism in the liver by enhancing the production of nicotinamide, while the nicotinamide level was decreased in the descending TCM group. During the oxidation of fatty acids, acyl coenzyme A is first generated. Long-chain acyl coenzyme A requires the transport of L-carnitine into mitochondria for further oxidation to produce ATP [22]. The liver is the most important organ for energy production and the main site for carnitine synthesis. When L-carnitine is deficient, this will lead to energy supply disorder and accumulation of various intermediate acid metabolites of fatty acid metabolism. In the present study, the level of L-carnitine was increased after administration of ascending medicine (Fig. 6), indicating that ascending medicine can promote energy metabolism in the liver, reflecting its ascending property. The intervention trend of descending medicine to L-carnitine was opposite to that of ascending medicine.

Depressive-like behavior is a typical symptom of liver qi stagnation [23]. Ascending medicine has an effect on liver qi stagnation. Glutamic acid plays an important role as a major excitatory neurotransmitter in the central nervous system [24]. The level of glutamic acid in the liver was significantly increased after administration of ascending medicine, indicating that ascending medicine may promote neural excitation and improve depression-like behavior caused by liver qi stagnation through regulating glutamic acid and glutathione metabolism, thus reflecting its ascending property. The glutamic acid level was decreased in the descending TCM group.

**Effects of ascending and descending medicine on lung**

The metabolomics study indicated that tryptophan metabolism, phenylalanine metabolism, and pyroglutamic acid and glutamic acid metabolism were the major differential pathways affected by ascending and descending medicine in the lung.

Tryptophan and its metabolites regulate immunity [25]. Tryptophan can inhibit the production of the pro-inflammatory cytokine IL-17 and the maturation and differentiation of T helper 17 (Th17) cells, thereby alleviating the inflammatory response of the lung [26, 27]. In the present study, the level of tryptophan in normal rats was significantly increased after administration of ascending medicine, indicating that ascending medicine may promote tryptophan metabolism, thereby inhibiting the inflammatory reaction of the lung. The level of tryptophan in normal rats was significantly increased after administration of descending medicine. Other studies have shown that the level of phenylalanine in patients with acute inflammatory disease is higher than that in normal controls [28]. Severe pulmonary inflammation leads to decreased phenylalanine-4-hydroxylase and 5,6,7,8-tetrahydrobiopterin activity, causing the accumulation of phenylalanine, thus promoting inflammation [29]. In the present study, the level of phenylalanine in normal rats was significantly decreased after administration of descending medicine, indicating that
descending medicine may promote phenylalanine metabolism, thereby inhibiting pulmonary inflammation. The level of phenylalanine in normal rats was significantly increased in ascending medicine group. Thereby both descending and ascending TCM influence pulmonary inflammation, but the underlying mechanisms may be different.

Gamma-aminobutyric acid (GABA), a glutamate metabolite, can activate the GABA$_A$ receptor in airway smooth muscle tissues, relaxing the trachea and improving the symptoms of dyspnea in patients with lung diseases [30]. Compared with normal rats, the levels of pyroglutamic acid and glutamic acid in the lung samples were significantly increased after administration of ascending and descending medicine (Fig. 6), indicating that ascending and descending medicine could relax the trachea and improve the symptoms of dyspnea by enhancing glutamic acid metabolism.

**Effects of ascending and descending medicine on kidney**

The metabolomics study indicated that tryptophan metabolism, phenylalanine metabolism, and glutamic acid metabolism were the major differential pathways affected by ascending and descending medicine in the kidney.

The kidney is the main organ that produces urine. Taurine can exert diuretic effects by inhibiting the renin–angiotensin–aldosterone system (RAAS) [31–33]. In the present study, the level of taurine was significantly decreased in the ascending TCM group and increased in the descending TCM group, indicating that descending TCM may promote taurine metabolism, inhibit the RAAS, and exert diuretic effects, reflecting their descending nature. Previously, our study found that ascending medicine can promote the secretion of gonadotropin-releasing hormone (GnRH), which is synthesized and secreted by the hypothalamus. GnRH can regulate the animal’s estrus and reproductive activities. Relevant studies have found that excitatory amino acids can enhance the discharge of GnRH neurons and promote the secretion of GnRH [34, 35]. Glutamic acid and aspartic acid are the main excitatory amino acids [36]. Compared with the control group, the levels of glutamic acid and aspartic acid in most ascending TCM groups were significantly increased, indicating that ascending medicine increased the levels of GnRH by promoting alanine, glutamic acid, and aspartic acid metabolism. The levels of glutamic acid and aspartic acid in most descending TCM groups were significantly decreased (Fig. 6).

A related study showed that arginine can treat endocrine disorders, delayed sexual maturity, and sexual dysfunction induced by codeine [37], and it can significantly increase the sperm density, total sperm count, and effective sperm count of the body [38, 39]. Arginine is a precursor of nitric oxide (NO), which is an important signal and effector molecule in the body, plays an important role in the male reproductive system, and can promote vasodilation and penile smooth muscle relaxation. In addition, NO promotes blood flow in testis and synthesis and secretion of testosterone, upregulates the level of dopamine, and enhances sexual activity and the genital reflex [40–42]. Related studies have shown that NO can promote the secretion of GnRH to mediate the body’s estrus process, and estradiol stimulates the secretion of GnRH by increasing the level of NO [43, 44]. Compared with the control group, the arginine level in the ascending TCM group was significantly increased, indicating that the ascending medicine may improve
the body’s sexual function and sperm quality by regulating arginine and proline metabolism. Carnitine also has a significant regulatory effect on the body’s reproductive system. As discussed in Section 4.2, the liver is the main site for carnitine synthesis. However, humans cannot degrade carnitine. It is discharged through urine in free form, but most carnitine is reabsorbed in the kidneys, which maintain the carnitine balance in the body. Studies have shown that L-carnitine is the active form of L-acetyl-carnitine, which is an important antioxidant. L-carnitine has a protective effect on sperm, possibly by protecting sperm mitochondria from oxidative stress by removing toxic acetyl coenzyme A from the cell membrane, thereby improving sperm vitality and the probability of pregnancy [45–47]. In the present study, the levels of L-carnitine and arginine in the kidneys of normal rats were significantly increased after the administration of ascending medicine (Fig. 6) and decreased after the administration of descending medicine, indicating that ascending TCM could promote arginine and proline metabolism and L-carnitine metabolism, enhance the reproductive function of the body, improve sperm quality, and increase the pregnancy rate, reflecting its ascending property.

**Effects of ascending and descending medicine on spleen**

The spleen is the largest lymphoid organ in the body and participates in the immune response [48]. Glutamic acid can be converted into glutamine and vice versa. Under the action of glutamine synthetase, glutamic acid and ammonia can be converted into glutamine, while glutamine can be hydrolyzed into glutamic acid and ammonia under the action of glutaminase. Studies have shown that lymphocytes, neutrophils, and macrophages can also obtain energy through partial oxidation of glutamine, and an increase in glutamine levels can indirectly increase the proliferation rate of these immune cells. Moreover, glutamine can also promote phagocytosis, cytokine synthesis and secretion, and antigen presentation in macrophages and enhance the immune function of the body [49, 50]. Compared with normal rats, the levels of glutamic acid and glutamine in the spleen were significantly increased after the administration of typical ascending medicine (Fig. 6), while the glutamic acid and glutamine levels were decreased in the descending TCM group. Some typical ascending medicine may improve energy supply for the immune system, promote the proliferation of immune cells, and improve the immunity of the body by interfering with glutamic acid and glutamine metabolism.

To sum up, ascending medicine can promote energy metabolism in the heart and liver, which may underlie their stimulatory effects on the immune system, nervous system, and reproductive system. The generation of urine in the kidneys is closely related to blood circulation; therefore, the diuretic effects of descending medicine may be related to their protective effects on the cardiovascular system and their enhancing effects on blood circulation. Finally, both ascending medicine and descending medicine have anti-pneumonia effects, but the underlying mechanisms are different. The specific differences need further experimental exploration.

**Conclusions**
This is the first study to research the ascending/descending properties of TCM in a large cohort by using metabolomics technology. The effects of 15 TCM on five major organs in normal rats were comprehensively studied. The results indicate that ascending TCM exhibit positive effects on nerve stimulation, immunity, and reproductive function by promoting energy metabolism in the heart and liver, while descending TCM has the opposite effect; descending TCM exerts a positive effect on diuresis by promoting blood circulation, while ascending TCM has the opposite effect. Both descending and ascending TCM influence pulmonary inflammation, but the underlying mechanisms may be different. In summary, the properties of TCM, which often have multiple components and multiple targets, can be evaluated through systemic metabolomics.

**Abbreviations**

ASA
Armeniacae Semen Amarum
BR
Bupleuri Radix
CF
Caryophylli Flos
CRa
Cyathulae Radix
CRh
Cimicifugae Rhizoma
CR
Chuanxiong Rhizoma
CS
Canavaliae Semen
EH
Ephedrae Herba
GABA
Gamma-aminobutyric acid
GnRH
gonadotropin-releasing hormone
IF
Inulae Flos
LC-MS
Liquid chromatography–mass spectrometry
LS
Lepidii Semen
Mag
Magnetitum
MHH
*Menthae Haplocalycis Herba*
NO
Nitric oxide
OPLS-DA
Orthogonal partial least squares discriminant analysis
Por
*Poria*
PCA
Principal component analysis
PR
*Platycodonis Radix*
PTR
*Puerariae Thomonii Radix*
QC
Quality control
TCM
Traditional Chinese medicine
RAAS
The renin–angiotensin–aldosterone system
UPLC-Q/TOF-MS
Ultra performance liquid chromatography-quadrupole time of flight mass spectrometry.

**Declarations**

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Not applicable.

**Authors’ contributions**

Tong Liu: Carried out the experiments, Data curation, Writing. Xiaoke Zheng and Weisheng Feng: Supervision. Ning Zhou and Yang Fu: Revision, suggestion.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experiments were approved by the Animal Ethics Committee of Henan University of Chinese Medicine (DWLL2018080003).

Consent for publication

All authors agree to publish this paper.

Competing interests

All authors declared no competing interests.

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References


Figures

Fig. 1 PCA score plots of the main organs in the ascending TCM group and the control group. (A, B) Heart. (C, D) Liver. (E, F) Lung. (G, H) Kidney. (I, J) Spleen. Ascending TCM group ▲; control group ●.

Figure 1

See image above for figure legend
**Fig. 2** PCA score plots of the main organs in the descending TCM group and the control group. (A, B) Heart. (C, D) Liver. (E, F) Lung. (G, H) Kidney. (I, J) Spleen. Descending TCM group ▼; control group ●.

**Figure 2**

See image above for figure legend
Fig. 3 PCA score plots of the main organs in the ascending TCM group and the descending TCM group. (A, B) Heart. (C, D) Liver. (E, F) Lung. (G, H) Kidney. (I, J) Spleen. Ascending TCM group ▲; descending TCM group ▼.

Figure 3

See image above for figure legend
Figure 4

Figure 5

Metabolic networks indicating the effects of the ascending and descending medicine on main organs. Red indicates the screened common markers, and black indicates the undetected markers that play a role in connection. (A) Heart. (B) Liver. (C) Lung. (D) Spleen. (E) Kidney.
Figure 6

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