Regulatory effects of *Poria* on substance and energy metabolism in cold-deficiency syndrome compared with heat-deficiency syndrome in rats

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**[ABSTRACT]** Recent studies have revealed that the property of drug is mainly associated with the body’s substance and energy metabolism. The present study aimed to evaluate the drug property of *Poria*, called *Fuling* (FL) in traditional Chinese medicine (TCM), in terms of its effects on the substance and energy metabolism in rat models of cold-deficiency and heat-deficiency syndromes, compared with *Aconiti Lateralis Radix Praeparaia*, called *Fuzi* (FZ) in TCM, with hot property, and *Anemarrhenae Rhizoma*, called *Zhimu* (ZM) in TCM, with cold property, as reference drugs, respectively. The appearance score, toe and rectal temperatures of the animals treated were assessed at different time points. Several indices in vivo correlated with substance and energy metabolism (glucokinase, phosphoglycerate kinase, cytochrome c reductase, cytochrome c oxidase, and Na⁺-K⁺-ATPase), endocrine system (triiodothyronine, thyroxine, and 17-hydroxycorticosteroid), nervous system (acetylcholinesterase), and cyclic nucleotide system were determined. The changes in appearance score and indices in vivo suggested the successful establishment of cold-deficiency and heat-deficiency syndrome models. FZ reversed the decreased levels of indices (substance and energy metabolism and endocrine system) and alleviated the syndrome of cold-deficiency model, and ZM showed obviously therapeutic effect on heat-deficiency syndrome (appearance score, substance and energy metabolism, and endocrine system). FL could alleviate cold-deficiency syndrome and raise the decreased levels of glucokinases, phosphoglycerate kinase, cytochrome c reductase and triiodothyronine in cold-deficiency model, but had no significant effect on heat-deficiency syndrome. Drug property of FL was inferred as trending to “flat and warm”, which still need further study. It was advisable to adopt both cold-deficiency and heat-deficiency models to study the drugs with “flat” property.

**[KEY WORDS]** *Poria*; Substance metabolism; Energy metabolism; Cold-deficiency syndrome; Heat-deficiency syndrome

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**Introduction**

In the theory system of traditional Chinese medicine (TCM), the condition of the body is a subtle harmony of *yin* and *yang*. An imbalance of *yin* and *yang* leads to abnormal conditions of the body. To be exact, it is diagnosed as heat-deficiency symptom, when the body is deficiency of *yin*, leading to an apparent excess of *yang*, and mild but very specific heat symptoms appear such as constipation and hectic fever; it is diagnosed as cold-deficiency symptom, when the body is deficiency of *yang*, resulting in various symptoms such as cold limbs and hypopoactivity [1].

Four properties of TCM referred to *cold, cool, hot and warm*, are often applied to describe the efficacy of TCM. Generally speaking, herbs with the *cold* or *cool* property are deemed to clear away heat, eliminate toxic substances, nourish *yin*, and remedy hot syndromes [2], including *Anemarrhenae Rhizoma* (Zhi-mu in TCM, cold property, ZM), the rhizome of *Anemarrhenae asphodeloides* Bge., which are generally used to cure heat-deficiency syndrome [3-4]. In contrast, herbs with the *hot* or *warm* property usually dispel cold, warm up the interior, support *yang*, and thus treat cold syndromes, such as *Aconiti Lateralis Radix Praeparaia* (Fu-zi in Chinese, hot property, FZ), the root of *Aconitum carmichaeli* Debx, which could be used in the treatment of cold-deficiency syndrome [5-6].

There also is a special kind of TCM property defined as “flat”, of which the cold and heat boundary is not obvious [7], but the understanding of “flat” property remains unclear. Since
the increasing use of drugs with “flat” property in the clinic, the study of “flat” property is recently taken more seriously. *Poria* (*Fuling* in Chinese, FL) is dry sclerotia of fungus *Poria cocos* (Schw.) Wolf, whose property is recorded as “flat” in Chinese Pharmacopoeia [8] and has been regarded as “flat and warm” based on textual research of FL [9]. The main efficacy of FL is clearing damp and promoting diuresis, which has been verified in experimental studies [10-11]. Clinically, FL is utilized extensively in prescriptions to cure syndrome of internal stagnation of fluid-dampness [12-13]. However, at present, there is a tendency to desalinate the drug property of FL in clinical practice. Therefore, the present study was carried out to explore the property of FL and provide an experimental basis for the clinical application of FL.

Recent studies have revealed that the property of drug is mainly concerned with the body’s energy and substance metabolism by different pathways [14-15]. It has also been confirmed that some hot (or warm) drugs could improve the body’s basal metabolic rate or excite endocrine system and central nervous system [16-18], providing a new way to explore the efficacy of TCM. Our study was mainly focused on the relationships between drug property, drug efficacy, energy metabolism, and substance metabolism [19-20] and aimed to evaluate the drug property of FL. Both cold-deficiency model and heat-deficiency model built by classical modeling methods [21-22] were adopted using FZ (hot property) and ZM (cold property) as the reference drugs, respectively.

Several indices correlated with substance metabolism and energy metabolism, endocrine system, nervous system and cyclic nucleotide system, together with appearance score, toe and rectal temperatures, were determined in the rat models of cold-deficiency and heat-deficiency syndromes, respectively, and PCA method was employed to generate an overview for the tendency of effects. Then the specialty and tendency of the effects were analyzed to explore the drug property of FL. The present study provided a new way to adopt both cold-deficiency model and heat-deficiency model to explore the property of “flat” drugs, although a comprehensive system and effective ways remain to be established to interpret the scientific connotation of drug properties of TCM in the future.

**Materials and Methods**

**Instruments, chemicals, and reagents**

TDZ4-WS low speed centrifuge was purchased from Xiangyi Centrifuge instrument Co., Ltd. (Changsha, China). Heparin anticoagulative tube (Batch No. 20150116) was purchased from Kehua Inspection of Medical Products Co., Ltd. (Shanghai, China). 5-mL injectors were purchased from Kang Medical Devices Co., Ltd. (Zhenjiang, China). PCJ-10 Series ultrapure water machine was purchased from Pinchenge Science and Technology Ltd. (Chengdu, China). AccuLab analytical balance was purchased from Sartorius group (Goettingen, Germany). UV-2100 spectrophotometer was purchased from Unico Co., Ltd. (Shanghai, China). Normal saline was obtained from Kelun Pharmaceutical Ltd. (Heilongjiang, China). Microplate Reader was purchased from Kate Biomedical Medical Electronics Technology Co., Ltd. (Shenzhen, China). U570-86 Premium series of ultra-low temperature freezer (−80 °C) was purchased from NBS companies (Edison, New Jersey, USA).

The glucokinas (GCK) assay kits (Batch No. 20160621), phosphoglycerate kinase (PGK) assay kits (Batch No. 20160621), citrate synthase (CS) assay kits (Batch No. 20160621), cytochrome c reductase (CCR) assay kits (Batch No. 20160621), cytochrome c oxidase (COX) assay kits (Batch No. 20160621), triiodothyronine (T3) assay kits (Batch No. 20160621), thyroxine (T4) assay kits (Batch No. 20160621), 17-hydroxycorticosteroid (17-OHCS) assay kits (Batch No. 20160621), acetyl-coenzyme A (A-CoA) assay kits (Batch No. 20160621), adipose triglyceride lipase (ATGL) assay kits (Batch No. 20160621), cyclic adenosine monophosphate (cAMP) assay kits (Batch No. 20160621), and cyclic guanosine monophosphate (cGMP) assay kits (Batch No. 20160621) were purchased from Beijing Cheng Lin Institute (Beijing, China). The pyruvic acid (PA) assay kits (Batch No. 20160621), acetylcholin esterase (AchE) assay kits (Batch No. 20160505), and Na+−K+−ATPase assay kits (Batch No. 20160505) were purchased from Nanjing Jian Chen Bioengineering Institute (Nanjing, China).

**Plant materials**

*Poria* (Batch No. 201211) was purchased from Yunnan Xianghui Biotechnology Co., Ltd. (Yunnan, China), Aconiti Lateralis Radix Preparaaria (Batch No. 130101) was purchased from Henan Renhe herbal pieces Co., Ltd. (Henan, China), Anemarrhenae Rhizoma (Batch No. 130506), Zingiberis Rhizoma (Batch No. 130506), Cinnamomi Cortex (Batch No. 130506), Phellodendri Chinensis Cortex (Batch No. 130506), Gentianae Radix et Rhizoma (Batch No. 130506) and Glycyrrhizinum Fibrosum (Batch No. 130506) were purchased from Anhui Songshan Hall herbal pieces Co., Ltd. (Anhui, China). All the samples were identified and authenticated by Prof. WANG Bing (Department of Pharmaceutical Botany, College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China), demonstrating that the quality of all samples were in accordance with the requirements of Chinese Pharmacopoeia (The Pharmacopoeia Commission of PRC, 2015).

**Animals**

Male Sprague-Dawley (SD) rats (weighing 170−210 g) were purchased from the Laboratory Animal Center of Changsheng Bio-Technique Co., Ltd. (Benxi, Liaoning, China; qualification number SCXK 2015-0001). The animals were
kept in an air-conditioned room (temperature, 22 °C; relative humidity, 55%) and fed ad libitum with standard feed and water during the entire course of the present study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study protocol was reviewed and approved by the ethics regulations of the Animal Core & Welfare Committee in Liaoning University of TCM (131/2010; NO. 2100092016026, date: 20161010).

**Sample preparation**

The crude herbs of FZ and ZM were separately immersed with 8-fold distilled water for 1 h and then boiled for 1 h. After filtered, 6-fold distilled water was added and boiled again for 1 h. The filtrates were combined and concentrated to the volume at final concentrations of crude drugs (FZ, 0.828 g mL\(^{-1}\); ZM, 0.553 g mL\(^{-1}\)). The samples were kept at 4 °C before use.

The crude herb of FL was immersed with 10-fold distilled water for 1 h and then boiled for 1 h. After filtered, 10-fold distilled water was added and boiled again for 1 h. The filtrates were combined and concentrated at 65 °C to the volume at a final concentration of 1.34 g mL\(^{-1}\). The solution of 0.69 g mL\(^{-1}\) was obtained by appropriately diluting the solution of 1.38 g mL\(^{-1}\). The samples were kept at 4 °C before use.

In the preliminary work, the chemical constituents in the water decoction of FL were studied and a split-fractions method of the chemical constituents was established. All the chemical components were divided into fractions of polysaccharide (CPF), petroleum ether (PEF), ethyl acetate (EAF), alcohol eluate from macroporous resin (AEF), and water eluate from macroporous resin (WEF). The fingerprints for these constituents were established and each constituent was characterized. Briefly, CPF was mainly composed of polysaccharides, PEF was mainly composed of phthalic acid bis-(2-ethylhexyl) ester, dibutyl phthalate and other Liposoluble compounds; EAF was composed of pachymic acid, dehydrochymic acid and other tripterpenoids; AEF was mainly composed amino acid; and WEF was composed of monosaccharides and inorganic salt \(^{[21-25]}\).

**Decotion of cold-deficiency modeling drug:** This experiment adopted the classical modeling method of cold-deficiency syndrome rats \(^{[21]}\). In our study, *Gypsum Fibrosum, Gentianae Radix et Rhizoma, Phellodendri Chinensis Cortex* and ZM were weighed by 2 : 1.2 : 1 : 1.5 ratio, soaked in 8-times water for 1 h, quickly heated to boiling, and then kept the state of micro-boiling 20 min, hot filter; 6 times water was added to the remaining dregs, rapidly heated to boiling, and then kept micro-boiling for 15 min, hot filter. All the filtrates were combined and then concentrated at 65 °C to the volume at a final concentration of 4 g mL\(^{-1}\). The samples were kept at 4 °C before use.

**Decotion of heat-deficiency modeling drug:** This experiment adopted the classical modeling method of heat-deficiency syndrome rats \(^{[22]}\). In our study, FZ, *Zingiberis Rhizoma* and *Cinnamomi Cortex* were weighed by 1 : 1 : 1 ratio. First, FZ was soaked in 8-times water for 1 h, quickly heated to boiling, and then kept the state of micro-boiling to taste no hemp, hot filter; 6 times water was added to the remaining dregs, rapidly heated to boiling, and then kept micro-boiling for 20 min, hot filter; the filtrates were combined; 8-times water was added to *Zingiberis Rhizoma* and *Cinnamomi Cortex* for 1 h, rapidly heated to boiling, and then kept micro-boiling for 30 min, hot filter; 6-times water was added to remaining dregs, rapidly heated to boiling, and kept micro-boiling for 20 min, hot filter. All the filtrates were combined and then was concentrated at 65 °C to the volume at a final concentration of 2 g mL\(^{-1}\). The samples were kept at 4 °C before use.

**Dose calculation for rats**

The doses of different samples were calculated through equivalent dose ratio of human to rat according to body surface area. The one-day maximum dose of FZ for humans is 15 g in accordance with the dose in 2015 Edition of Chinese Pharmacopoeia. Therefore, the equivalent six-fold dose of FZ for rats was calculated as 8.28 g kg\(^{-1}\). The one-day dose of ZM for humans is 12 g in accordance with the dose in 2015 Edition of Chinese Pharmacopoeia, while the equivalent eight-fold dose of FZ for rats was 8.88 g kg\(^{-1}\). The one-day dose of FL for human is 15 g in accordance with the dose in 2015 Edition of Chinese Pharmacopoeia, while the equivalent ten-fold dose of FL for rat was 13.8 g kg\(^{-1}\) and equivalent five-fold dose of FL was 6.9 g kg\(^{-1}\).

**Drug administration**

Based on cold-deficiency model, 50 healthy male SD rats, SPF grade, were housed for a week in an air-conditioned room (temperature, 22 °C; relative humidity, 55%) and were randomly divided into 5 groups (n = 10 per group): blank, control, FZ, HWDF (10-fold dose for rat of *Poria* water decoction), and LWDF (5-fold dose for rat of *Poria* water decoction) groups.

Based on heat-deficiency model, 40 healthy male SD rats, SPF grade, were housed for a week in an air-conditioned room (temperature, 22 °C; relative humidity, 55%) and were randomly divided into 4 groups (n = 10 per group): blank, model, ZM, and WDF (water decoction of *Poria*) groups.

The model drugs (13.8 g kg\(^{-1}\)) were given by oral gavage (i.g.) every morning for 14 days, respectively, while blank group was administered i.g. with the equivalent volume of distilled water. The administration volume was 16 mL·kg\(^{-1}\) of body weight. Since the 15th day, each administration group was administered i.g. with the dose as above every afternoon for 7 consecutive days, while the blank and control groups were given the equivalent volume of distilled water, the administration volume was 10 mL·kg\(^{-1}\) of body weight.

**Determination of temperature and appearance score**

The toe temperature and rectal temperature of rats in cold-deficiency and heat-deficiency groups were measured on Days 0, 14, and 21 (7th day of treatment). Rectal temperature...
was measured with an electronic rectal thermometer (Acorn Trading Co., Ltd., Shanghai, China). Simultaneously, toe temperature was measured with infrared thermometer (CEM Co., Matthews, North Carolina, USA).

The appearance score of each rat was measured on the Days 7, 14, and 21 (7th day of treatment), according to the Standard of TCM syndrome [26] and National standard for clinical diagnosis and treatment of TCM (State Bureau of Technical Supervision, GB/T 16751.2-1997).

**Blood and tissue sampling**

At 1h after the last drug administration, the rats were intraperitoneally injected with 1% pentobarbital sodium (4 mL·kg\(^{-1}\) body weight). Under anesthesia, the abdominal cavity was exposed, and then blood was collected from the abdominal aortic and then cryogenically centrifuged at 2500 r·min\(^{-1}\) at 4 ºC for 20 min to prepare the plasma. The liver was removed and approximately 0.1 g of the liver was homogenized in 9 times of saline and then centrifuged at 3,500 r·min\(^{-1}\) for 15 min. All the dissection parts of the liver were taken from the same spot of the liver of the rats and kept at –80 °C before use.

**Determination of indicators in vivo**

All the indices were measured strictly according to manufacturers’ instructions.

The contents of T3, T4, cAMP, cGMP, pyruvic acid, and AchE in plasma were evaluated by enzyme linked immunosorbent assay (ELISA). The contents of CCR, COX and Na\(^{+}\)-K\(^{-}\)-ATPase in liver were assayed to evaluate the effects on energy system. The contents of CS, A-CoA, PGK, GCK, 17-OHCS and ATGL in liver were assayed to evaluate the effects on substance system.

**Statistical analysis**

Measurement data were expressed as means ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) with the Least Significant Difference (LSD) test using SPSS version 20.0. The values of \(P < 0.05\) were considered statistically significant.

**Analysis of drug property attribution by PCA method**

The data in vivo were analyzed by principal component analysis (PCA) method to attribute the drug property of FL. Multivariate analysis was performed with EZInfo Software of Masslynx V4.1 version (Waters Corp., Milford, USA). PCA was performed to generate an overview for group clustering of all the index data. Results were visualized in the form of the score plots, where each point represented an individual sample.

**Results**

**Effects on endocrine system**

The main function of thyroid is to synthesize thyroid hormone and regulate the body metabolism. Thyroid secretes biologically active hormones such as T4 and T3, which are the main factors affecting the body's energy metabolism, regulating substance metabolism increasing rate of intracellular oxidation and improving oxygen consumption [27]. 17-OHCS is a metabolite of adrenal glucocorticoids and mineralocorticoids, and the content of 17-OHCS reflects the function of adrenal cortical. As shown in Fig 1 (A, B, and C), the T3, T4, and 17-OHCS levels of the model group were significantly decreased compared with that of the blank control group (\(P < 0.05\) or \(P < 0.01\)). The levels of T3, T4 and 17-OHCS in the FZ group were increased compared with that of model group (\(P < 0.05\), and the level of T3 in HWDF group was increased obviously, compared with that of the model group (\(P < 0.05\)).

**Fig. 1  Effects of blank, control, FZ, HWDF and LWDF treatments on the levels of T3, T4, and 17-OHCS in cold-deficiency syndrome on the 21st day (7th day of treatment).**

**Effects on substance metabolism**

Glycolysis and aerobic oxidation are the major pathways of glucose metabolism. Glycolysis is an effective way to replenish energy in anaerobic condition, while aerobic oxidation is mostly associated with the tricarboxylic acid cycle (TCA). Pyruvic acid (PA) plays an important role for the three major nutrients (sugar, fat, and amino acids) metabolism.
GCK, PGK, and CS are key enzymes of glucose metabolism. As shown in Fig. 2 (A, B, C, D), the levels of GCK, PGK, CS, and PA of the model group were significantly decreased compared with that of the blank group \( P < 0.01 \) or \( P < 0.05 \). The levels of PGK, CS, and PA of the FZ group were increased compared with that of the model group \( P < 0.05 \) or \( P < 0.01 \), and the level of PGK in HWDF group was increased, compared with that of the model group \( P < 0.05 \).

The level of A-CoA of the model group was increased obviously compared with that of the blank group \( P < 0.05 \), and the level of A-CoA of the FZ and LWDF groups were decreased compared with that of the model group \( P < 0.01 \; \text{Fig. 3} \).

Effects on energy metabolism

CCR and COX are present in the respiratory chain complex III and complex IV, respectively. They participate in the respiratory chain of electron transport, whose content or activity can reflect the mitochondrial respiratory status and energy release level. CCR catalyzes electron passing from coenzyme Q to cytochrome C. COX is a mitochondrial marker enzyme, whose enzymatic activity plays an important role in maintaining mitochondrial structure and function as well as the production of cellular energy \[28\]. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is a kind of transmembrane protein extensively existing at the cell membranes of all most epithelial cells. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase actively extrudes three Na\textsuperscript{+} out and imports two K\textsuperscript{+} into the cell for the hydrolysis of every ATP. The results from the present study showed that the levels of COX, CCR, and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in the model group were significantly decreased, compared with that of the blank group \( P < 0.05 \), the levels of CCR, COX and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in the FZ group were increased compared with that of the model group \( P < 0.05 \), and the level of CCR in FL group was increased compared with that of the model group \( P < 0.05 \; \text{Figs. 4A, 4B, and 4C} \).

Effects on cyclic nucleotide system

The intracellular second messengers, cAMP and cGMP, play a critical role in cellular metabolism and physiological effect. The metabolisms of cAMP and cGMP present dynamic equilibrium under normal circumstances and help regulate hormone synthesis and secretion. After long-term research, it has won a widely recognition that cAMP belongs to yang and cGMP belongs to yin \[29\]. The data from the present study disclosed that cAMP and the cAMP/cGMP ratio of model group were significantly
decreased compared with that of the blank group \((P < 0.01)\), and the levels of cAMP and cAMP/cGMP in the FZ group were increased, compared with that of the model group \((P < 0.01)\), while other groups showed no significant difference from the model group (Figs. 5A, 5B, 5C, and 5D).

**Fig. 4** Effects of blank, control, FZ, HWDF, and LWDF treatments on levels of COX, CCR, and \(\text{Na}^{+}-\text{K}^{+}\)-ATPase in cold-deficiency syndrome on the 21st day (7th day of treatment). Cumulative values are reported as means ± S.E. for 10 rats in each group. *\(P < 0.05\) and **\(P < 0.01\) vs control group, #\(P < 0.05\) and ##\(P < 0.01\) vs blank group, using ANOVA with the LSD analysis. FZ, Aconiti Lateralis Radix Praepararia; HWDF, 10-fold dose for rat of Poria water decoction; LWDF, 5-fold dose for rat of Poria water decoction

**Drug properties as analyzed by PCA**

The results showed that model and blank groups were well separated, and, after treatment, the FZ and model groups were well separated (Figs. 6A and 6B). In addition, both HWDF and LWDF groups could be separated from the blank group, while HWDF and LWDF groups showed obvious overlap with cold-deficiency model group and obvious tendency to FZ group (Figs. 6A and 6B).

**Effects on heat-deficiency syndromes**

The changes in appearance score and indices *in vivo* suggested the successful establishment of heat-deficiency syndrome model. ZM showed obviously therapeutic effect on heat-deficiency syndrome (appearance score, substance and energy metabolism, and endocrine system), while FL had no significant effect on heat-deficiency syndrome.

**Fig. 5** Effects of blank, control, FZ, HWDF, and LWDF treatments on cAMP, cGMP, and cAMP/cGMP in cold-deficiency syndrome on the 21st day (7th day of treatment). Cumulative values are reported as means ± SE for 10 rats in each group. *\(P < 0.05\) and **\(P < 0.01\) vs control group, #\(P < 0.05\) and ##\(P < 0.01\) vs blank group, using ANOVA with the LSD analysis. FZ, Aconiti Lateralis Radix Praepararia; HWDF, 10-fold dose for rat of Poria water decoction; LWDF, 5-fold dose for rat of Poria water decoction

**Discussion**

In TCM, drug property is a unique standard to indicate drug’s efficacy. The exemplary way to explore the property theory of TCM is to study the characteristics and regularity of drug property based on the pharmacological effects. Recent researches have indicated that the drug properties are primarily associated with the metabolism of substance and energy of organism [14-15]. The present study was mainly focused on the effects of FL on substance and energy metabolism based on the models of cold-deficiency and heat-deficiency syndromes, compared with FZ (hot property) and ZM (cold property), respectively. The index data *in vivo* were analyzed by PCA to generate an overview for group clustering to attribute the drug property of FL. Drug property was considered as “hot or warm” when the drug reversed index changes in cold-deficiency model and had the same trend of effects as FZ; it was regarded as “cold or cool” property if the drug reversed index
changes in heat-deficiency model and showed the same trend of effects as ZM.

The animal models of cold-deficiency syndrome and heat-deficiency syndrome are the basic models of common syndromes in clinical practices of TCM. This experiment adopted the modeling method of cold-deficiency syndrome rats [21], giving continuous lavage with water decoction (Gypsum Fibrosum, Gentianae Radix et Rhizoma, Phellodendri Chinensis Cortex, ZM by 2 : 1.2 : 1 : 1.5 ratio) for 14 days to rats. It was found that the model rats showed the characteristics of cold syndrome, the toe and rectal temperatures were significantly decreased, and the situation of diarrhea and loose stools, chilly curled up, fur filthy, dull, white fur, and so on. After treatment with FZ as a positive drug, the diarrhea state of rats in FZ group was reduced or even disappeared, and the body weight increased, and the toe and rectal temperatures were increased. In FL group, the syndrome of diarrhea was alleviated, body weight, rectal and toe temperatures had been rebounded, suggesting that FL had a certain extent to alleviate the state of cold-deficiency in rats.

This experiment adopted the modeling method of heat-deficiency syndrome rats [22], giving continuous lavage with water decoction (FZ, Zingiberis Rhizoma, Cinnamomi Cortex by 1 : 1 : 1 ratio) for 14 days to rats. It was found that model rats began to show the characteristics of heat-deficiency syndrome, which were slightly manic, irritable, with dry feces, and the increasing secretion around eyes, suggesting that the heat-deficiency model was built successfully. After treatment
with ZM for 7 days, the score of sign index was rebounded, suggesting that ZM could alleviate the state of heat-deficiency syndrome. FL did not show obviously effect on heat-deficiency syndrome rats.

Endocrine system plays an important role in regulating physiological function and homeostasis of body. Among them, T3 and T4 can increase the oxygen consumption and heat production of tissues, whose decrease may cause the reduction of heat production, leading to the appearance of "cold" syndrome. It was found that contents of T4 and T3 in cold-deficiency syndrome rats were reduced, indicating the cold-deficiency molding drug inhibited the function of thyroid hormone, thus affecting the substance and energy metabolism of body. After treatment, FZ could increase T3 and T4 significantly in cold-deficiency rats and HWDF could significantly increase the content of T3, indicating that FL could reversed the reduction of T3, thus affecting the endocrine system. In heat-deficiency model, the content of T3 in model rats was increased, showing that heat-deficiency molding drug could promote the secretion of thyroid hormone. After treatment, ZM could alleviate the state of the heat-deficiency syndrome. FL showed no obviously effects on endocrine system of heat-deficiency syndrome model.

Studies have shown that heat drugs can excite the sympathetic adrenal system and promote the production of 17-OHCS, while cold drugs can inhibit the sympathetic adrenal system and decrease the content of 17-OHCS. The level of 17-OHCS in cold-deficiency syndrome group was decreased, showing that the molding drug could inhibit the function of adrenocortical, while FZ showed the opposite action. FL showed no obviously effects on both cold-deficiency and heat-deficiency syndrome models.

Substance metabolism is the basic characteristic of life phenomenon. Through the process of substance metabolism, energy is provided for life activities to maintain organism metabolism. The increase of PA indicated the accelerating of glucose metabolism and the enhancement of energy metabolism of body. The PA level of the cold-deficiency model group was significantly decreased, which was in accordance with the literature reported, while the level of PA in FZ group was increased after treatment. The transformation of sugars, fats, and amino acids can be achieved with PA through A-CoA and TCA. A-CoA for citrate synthesis is generated by oxidation of pyruvate, and the generated A-CoA can activate pyruvate dehydrogenase. The present study found that content of A-CoA in cold-deficiency model group was increased, but A-CoA of heat-deficiency group was significantly decreased, and ZM could reverse the decreasing tendency of A-CoA activity.

The activity of GCK, PGK, and CS in the aerobic oxidation process of the cold-deficiency was decreased, which suggested that the cold-deficiency modeling medicine slowed the process of aerobic oxidation. The results indicated that heat-deficiency modeling drug improved the substance metabolism, and ZM could reverse the increasing tendency of GCK activity caused by heat-deficiency model drug.

Mitochondria is the center of energy synthesis and supply, and the respiratory chain is the important structure for generating energy in mitochondria. Studies had found that activity of respiratory chain complex in cold-deficiency syndrome people is restrained. It was found that CCR and COX of cold-deficiency syndrome rats were reduced, suggesting that the oxidative respiratory chain complex III and IV were inhibited and the main way to synthesis of ATP was blocked. After treatment, FZ could reverse the decrease of the COX and CCR activity, so that the synthesis of ATP was restored. Meanwhile, FL could regulate energy metabolism of cold-deficiency syndrome though reversing the decrease of CCR activity.

Na⁺-K⁺-ATPase can maintain the different gradient concentrations of ions and adjust the transportation of amino acid and glucose. Since Na⁺-K⁺-ATPase activity was increased, the energy consumption and the heat production would be upwards. Our results manifested that the Na⁺-K⁺-ATPase activity of cold-deficiency syndrome was decreased, thus affecting the use and synthesis of ATP, which prompted that CCR, COX and Na⁺-K⁺-ATPase were the factors of energy metabolism in rats. FZ could reverse the decrease of Na⁺-K⁺-ATPase activity, which was in accordance with the idea that FZ could improve the energy metabolism in rats, by influencing the metabolic process of sugar, lipid and amino acid.

In the heat-deficiency model, the heat-deficiency modeling drug promoted energy metabolism and respiration metabolism. After treatment, ZM decreased the contents of CCX, COX, and Na⁺-K⁺-ATPase, thus decreasing the level of energy metabolism in heat-deficiency syndrome rats. FL showed no effect on energy metabolism of heat-deficiency syndrome rats.

The theory of TCM considers that the properties of hot and warm belong to yang, while the properties of cold and cool belong to yin. The present study found that the cAMP content of the cold-deficiency rats was decreased, while the cGMP content did not change significantly, so the cAMP/cGMP ratio was also decreased. This corresponded to the results of Zhang et al., suggesting that the yang deficiency caused by cold-deficiency was achieved and the model was built successfully. After treatment, cAMP and cAMP/cGMP of FZ group were increased, suggesting that FZ could correct the state of yang deficiency in rats and restore the normal physiological effects of cold-deficiency syndrome. Other groups showed no obvious difference.

In the present study, our results showed that there was no obvious relationship between nervous system with either cold-deficiency or heat-deficiency syndrome.

PCA was performed to generate an overview for group clustering of all the index data to attribute the drug property. In the cold-deficiency model, there were significant differences in index data of substance metabolism, energy metabolism, nervous system, and endocrine system between the model group and the normal group. After treatment, FZ group could reverse the tendency of index data in cold-deficiency model to
the normal group, which was in accordance with its hot property. The results of PCA showed that FL had a tendency to alleviate the state of the cold-deficiency of rats. In heat-deficiency model, ZM group could reverse the tendency of index data in heat-deficiency model to that of the normal group, which was in accordance with its cold property. There was no obvious tendency of effects seen in FL group.

To sum up, the property of FL was mainly dominated by cold-deficiency model, since the effect of FL on heat-deficiency rats was even less. Comprehensive analysis of the results of appearance and indices in vivo, FL could alleviate the cold-deficiency syndrome, reverse the appearance score of cold-deficiency and exert certain effects on substance metabolism, energy metabolism, and endocrine system of cold-deficiency rats. FL could not reverse the heat-deficiency syndrome and had no significant effect on indexes of heat-deficiency model. Finally, the property of FL was inferred as "flat and warm", but since the pharmacodynamic material basis and pharmacological action mechanism were very complex, further study should be undertaken to confirm it. The chemical constituents in the water decoction of FL were studied in the preliminary work; considering that the interactions between the components of TCM were complicated, further study should be undertaken to identify the certain components responsible for the effects. The present study provided a new way to explore the efficacy of TCM, but a comprehensive system and effective ways have not been established yet, in order to interpret the scientific connotation of drug property of TCM.

Conclusion

Drug property of FL was inferred as trending to “flat and warm” which still need further study. It was advisable to adopt both cold-deficiency model and heat-deficiency model to study the drugs with “flat” property. The present study provided an experimental basis and complement for the guidance to clinical application of FL in the future.

References

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