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# Metabolomics Study on the Mechanism of Junfang Tea in Regulating Glucose and Lipid Metabolism

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## ABSTRACT

Objective: This study aims to explore the regulatory effects and potential mechanisms of Junfang tea extract on metabolic disorders induced by a high-sugar, high-fat diet. Methods: A high-sugar, high-fat mouse model was established, and 220 healthy male mice were randomly divided into seven groups: normal control group (NC), model control group (MC), positive control group (PC), and four Junfang tea extract groups (DYA, DYB, DYC, DYD). The NC and PC groups consisted of 35 mice each, while the other groups had 30 mice each. The NC group was fed a standard diet, while the other groups were fed a high-sugar, high-fat diet for 8 weeks to induce the model. Starting from the 9th week, the observation groups were administered different doses of Junfang tea extract by gavage, while the PC group received metformin and lovastatin as positive controls. In the 15th week, blood samples were collected, and serum was separated for the measurement of glucose (Glu), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), urea, and creatinine levels. Results: Tea polyphenols and catechins are the main active components in Junfang tea, which regulate several metabolic pathways, including fatty acid oxidation, fat synthesis, and glucose metabolism, by modulating the AMPK, PPAR, and SREBP signaling pathways. Differential metabolite analysis showed that the abundance of Sinapoylhexoside and other metabolites was significantly higher in the NC group than in the MC group, suggesting its potential role in maintaining metabolic balance. Pathway Impact analysis revealed that Junfang tea significantly alleviated metabolic disorders induced by a high-sugar, high-fat diet through the regulation of key signaling pathways, such as AKT, mTOR, NF-κB, and JNK. Multi-group comparison analysis indicated that the metabolic state of group D showed noticeable improvement, approaching that of the normal control group (NC), although it did not fully return to normal levels. Conclusion: Junfang tea extract significantly reduced blood glucose and lipid levels in high-sugar, high-fat model mice and regulated glucose and lipid metabolism through multiple metabolic pathways, demonstrating its potential value in improving metabolic diseases.

## KEYWORDS

Junfang tea; Glucose metabolism; Lipid metabolism; Metabolomics

## 1. INTRODUCTION

Pu-erh tea (Camellia sinensis var. assamica) is primarily produced in Xishuangbanna, Yunnan Province, and is made from sun-dried large-leaf tea within the geographical indication protection area of Yunnan. It is processed using specific techniques within this designated geographical area, resulting in tea with unique quality characteristics. According to processing methods and quality traits, Pu-erh tea is divided into two types: raw tea and ripe tea. Raw tea is made from fresh tea leaves,

which undergo processes such as fixation, rolling, sun drying, steaming, and compression to form pressed tea. Ripe tea, on the other hand, is produced from sun-dried large-leaf tea using specific techniques and post-fermentation processes (either rapid or slow post-fermentation) to create loose or pressed tea [1]. Dayi Junfang tea belongs to the Pu-erh ripe tea category and is fermented using dominant microbial strains during the pile fermentation process.

It has been reported that a high-fat diet leads to obesity, and Pu-erh tea significantly upregulates P-AMPK and inhibits FAS, potentially playing a role in suppressing fatty acid synthesis [2]. Pu-erh tea extract can inhibit harmful ectopic lipogenesis in the liver of diet-induced obese mice, thereby protecting them from obesity. Furthermore, Pu-erh tea extract significantly reduces body weight and subcutaneous fat in high-fat diet-fed mice [3].

In this study, a high-sugar, high-fat mouse model was established to investigate the therapeutic effects of four types of Dayi Junfang tea on metabolic disorders induced by a high-sugar, high-fat diet. The findings aim to provide a theoretical basis for the medicinal value of Pu-erh tea.

# 2. MATERIALS AND METHODS

## 2.1. Experimental Animals

A total of 220 healthy male Kunming mice (SPF grade), weighing between 18-22 g, were used for the experiment. The certificate number for the animals is SCXK (E) 2010-0007. The mice were housed in standard cages under routine conditions.

## 2.2. Main Reagents and Instruments

Dayi Junfang tea concentrated extracts A, B, C, D (provided by Yunnan Dayi Microbial Technology Co., Ltd.); M-007 Automatic Biochemistry Analyzer (Shenzhen Technology Co., Ltd.); Ultra-pure Water System (Chengdu Youpu Technology Co., Ltd.); Mass Spectrometer (Q Exactive Plus); Ultra-High Pressure Liquid Chromatography (Nexera X2 LC-30AD).

## 2.3. Experimental Methods

### 2.3.1. Preparation of Dayi Junfang Tea Concentrates

Each of the four Dayi Junfang tea samples (A, B, C, D) weighing 1.0 kg was mixed with 20,000 mL of purified water and heated to a boil for 1 hour. After cooling, the solution was filtered, and the filtrate was freeze-dried to obtain the concentrated extract of Junfang tea. The extraction yields for the A, B, C, and D Junfang tea samples were 191, 180, 133, and 179 $g \cdot kg^{-1}$, respectively.

### 2.3.2. Animal Grouping

To evaluate the hypoglycemic and hypolipidemic effects of Dayi Junfang tea concentrates, 220 healthy male mice were randomly divided into seven groups: normal control group (NC), model control group (MC), positive control group (PC), and four observation groups (DYA, DYB, DYC, DYD). The NC group was fed a standard diet, while the MC, PC, and observation groups were fed a high-sugar, high-fat diet[4]. After 8 weeks of model establishment, from the 9th week onwards, the observation groups were administered Dayi Junfang tea concentrates by gavage: DYA group received 0.318 $g \cdot kg^{-1}$, DYB group received 0.300 $g \cdot kg^{-1}$, DYC group received 0.222 $g \cdot kg^{-1}$, and DYD group received 0.298 $g \cdot kg^{-1}$. The PC group was administered 83.33 $mg \cdot kg^{-1}$ metformin and 3.33 $mg \cdot kg^{-1}$ lovastatin by gavage.

### 2.3.3. Animal Treatment and Sample Collection

At the end of the 8th week, five mice were randomly selected from both the NC and MC groups. Subsequently, ten mice were randomly selected from each group during the 11th, 13th, and 15th

weeks. The mice were fasted overnight before sample collection, and their body weights were recorded the following morning. The mice were then anesthetized via intraperitoneal injection of 10% chloral hydrate. Blood was collected by cardiac puncture from the inferior vena cava after opening the abdominal cavity. The collected blood samples were centrifuged at 3,000 r/min for 10 minutes to separate serum. Biochemical indicators in the serum were analyzed using an automated biochemical analyzer, and the remaining serum samples were stored at -80°C for further analysis.

To analyze metabolites in the serum, the blood samples were first preprocessed. For each sample, 100 μL of serum was mixed with 400 μL of pure methanol, followed by thorough vortexing. The mixture was sonicated in an ice bath for 20 minutes to ensure complete extraction of metabolites. The samples were then left at -20°C for 2 hours to precipitate proteins. Afterward, the samples were centrifuged at 16,000g for 20 minutes at 4°C, and the supernatant was collected for further analysis. The supernatant was then dried using a high-speed vacuum concentrator. Before mass spectrometry analysis, 100 μL of 50% methanol solution was added to reconstitute the samples, which were centrifuged again at 20,000g for 15 minutes at 4°C. The supernatant was subsequently subjected to UPLC-MS/MS for metabolite analysis.

#### 2.3.4. Statistical Analysis

Data were analyzed using SPSS 12.0 software. Measurement data were expressed as the mean ± standard deviation (x±s). Independent t-tests were used for comparisons between two groups, while analysis of variance (ANOVA) was employed for comparisons among multiple groups. A P-value of less than 0.05 was considered statistically significant.

## 3. RESULTS

### 3.1. Comparison Between Two Groups

#### 3.1.1. Principal Component Analysis (PCA)

Based on both two-dimensional and three-dimensional PCA analyses, there were significant differences in metabolite levels between the Junfang tea-treated group (Group B) and the normal control group (Group A). The model's R2X and Q2 values indicated that PCA could explain a substantial proportion of the variance in metabolite levels and exhibited a certain level of predictive capability. These findings suggest that the regulatory effect of Junfang tea on metabolites in mice may be related to the modulation of lipid metabolism, glucose metabolism, and other metabolic pathways.

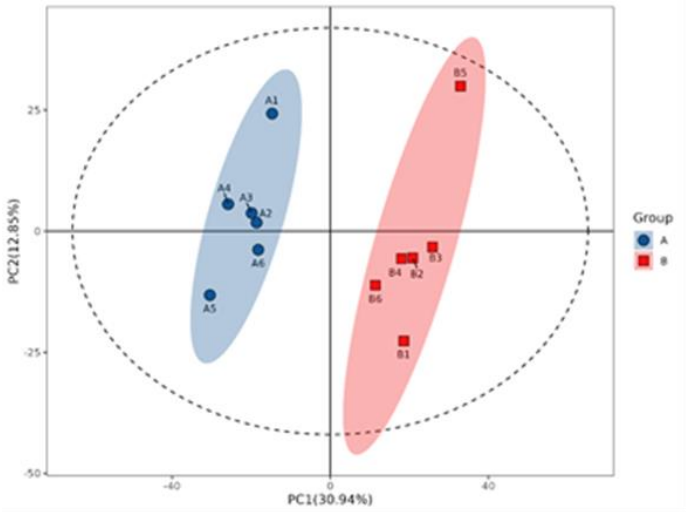


**Figure 1.** PCA Score Plot for Comparison Between Group A and Group B

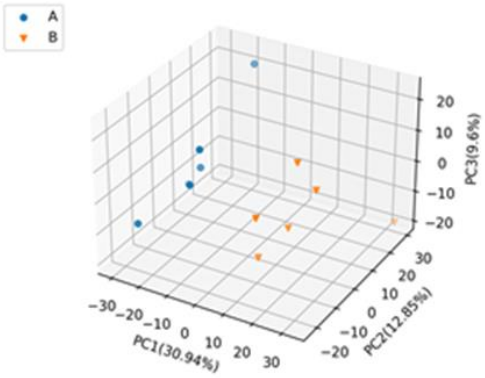


**Figure 2.** 3D PCA Score Plot for Comparison Between Group A and Group B

### 3.1.2. Partial Least Squares Discriminant Analysis (PLS-DA)

As observed in the results, both R2X and R2Y values are close to 1, indicating that the PLS-DA model has a high explanatory power for the data and can effectively reveal the differences in metabolites between the two groups. The Q2 value is also within a reasonable range, demonstrating that the model has good predictive capability and can effectively differentiate the metabolite profiles of the normal control group and the model group. Additionally, the estimated Root Mean Square Error of Estimation (RMSEE) shows low error, further supporting the robustness of the model.

Through PLS-DA analysis, significant differences in metabolite levels between Group A (NC group) and Group B (MC group) were clearly observed. The model's R2X, R2Y, and Q2 values indicate strong explanatory and predictive abilities, enabling effective differentiation of the metabolic characteristics of the two groups. These findings suggest that Junfang tea may play a significant role in regulating glucose and lipid metabolism, as well as related metabolic pathways, highlighting its potential application in improving metabolic diseases.

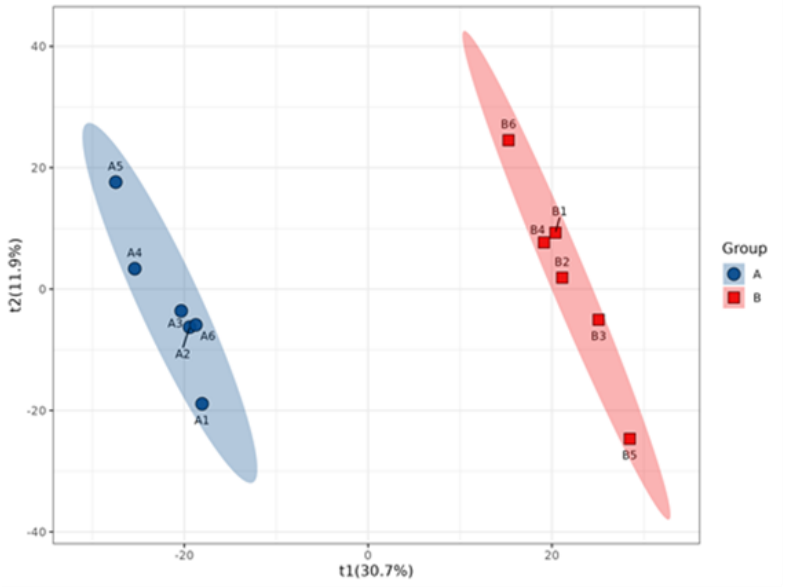


**Figure 3.** PLS-DA Score Plot for Comparison Between Group A and Group B

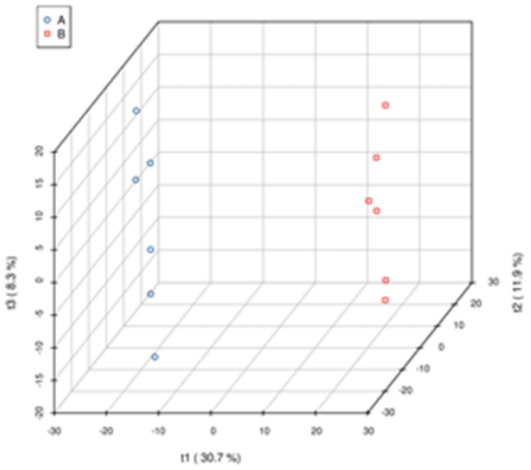


**Figure 4.** 3D PLS-DA Score Plot for Comparison Between Group A and Group B

### 3.1.3. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)

Using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), we observed significant differences in metabolite levels between the Junfang tea-treated group and the normal control group. The R2X, R2Y, and Q2 values of the OPLS-DA model indicate that the model has strong explanatory power as well as high predictive ability, allowing for accurate differentiation between the control and treatment groups. Further S-plot analysis helped identify the metabolites that contributed most to the observed differences between the groups, providing important clues for further exploration of the metabolic regulatory mechanisms of Junfang tea.

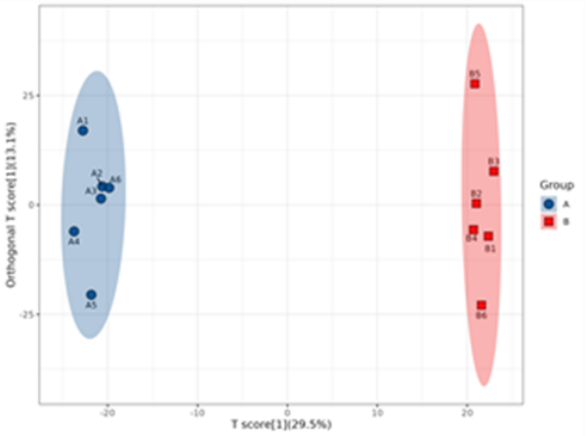


**Figure 5.** OPLS-DA Score Plot for Comparison Between Group A and Group B

## 3.2. Comparison Among Multiple Groups

### 3.2.1. Principal Component Analysis (PCA)

Principal component analysis (PCA) of metabolites from the three groups of mice (A, B, and D) revealed significant differences in metabolic profiles. The clear separation between Group A and Group B indicates a marked difference in metabolic status between the model group and the normal control group, reflecting the impact of the metabolic disorder. After treatment with Junfang tea, the metabolic characteristics of Group D showed improvement, with its distribution falling between Groups A and B. This suggests that Junfang tea exerts a regulatory effect on metabolism, although it does not fully restore normal metabolic function. The PCA results provide critical data supporting the role of Junfang tea in the regulation of metabolic disorders.

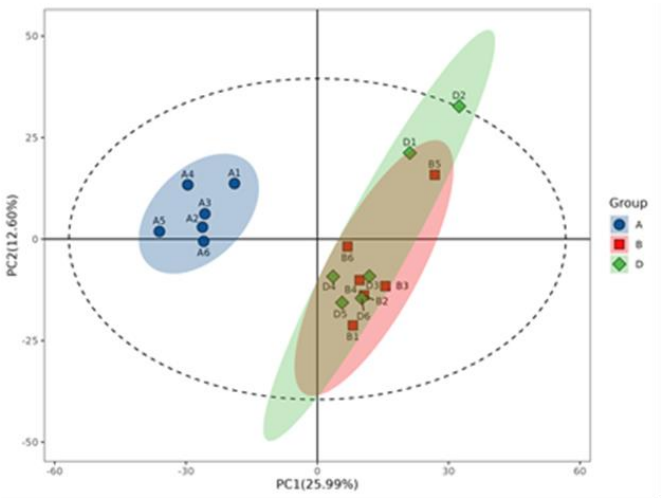


**Figure 6.** PCA Scatter Plot of the First and Second Principal Components for Comparison Among Groups A, B, and D

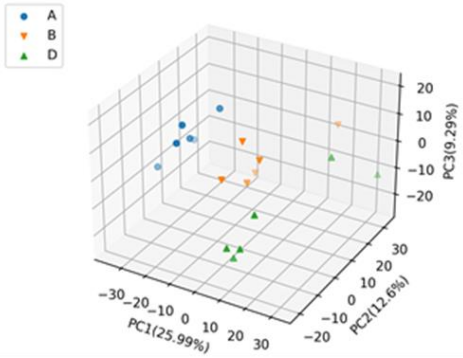


**Figure 7.** 3D PCA Scatter Plot for Comparison Among Groups A, B, and D

### 3.2.2. Partial Least Squares Discriminant Analysis (PLS-DA)

PLS-DA analysis revealed significant differences in metabolic profiles among Group A, Group B, and Group D. The metabolites in Group A were stably distributed, indicating a normal metabolic state. In contrast, Group B showed a marked deviation from Group A, suggesting significant metabolic changes in the model group, likely associated with the disease. Group D's metabolic profile was positioned between Group A and Group B, indicating the potential role of Junfang tea in regulating metabolic disorders. Further metabolite analysis will provide a deeper understanding of the specific mechanisms by which Junfang tea modulates metabolism and intervenes in disease processes.

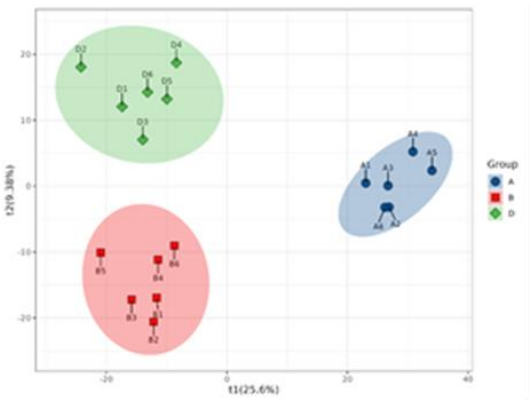


**Figure 8.** PLS-DA Score Plot for Comparison Among Groups A, B, and D

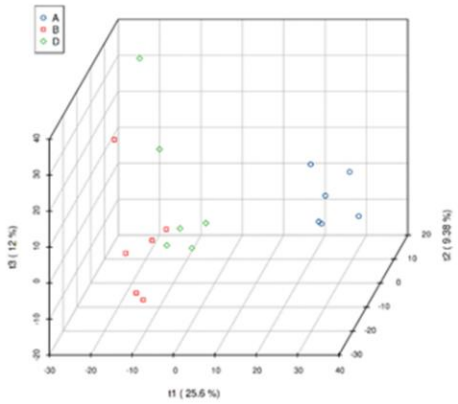


**Figure 9.** 3D PLS-DA Score Plot for Comparison Among Groups A, B, and D

### 3.2.3. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)

The results of Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) showed significant differences in metabolite profiles between Group A (normal group), Group B (model group), and Group D (treatment group). The metabolic features of Group A and Group B were clearly separated, indicating substantial changes in the metabolic state of the model group mice. After treatment with Junfang tea, the metabolic profile of Group D showed partial recovery, though it still differed from that of the normal group. This suggests that Junfang tea may have a restorative effect on metabolism, though it does not fully return the metabolic state to normal.

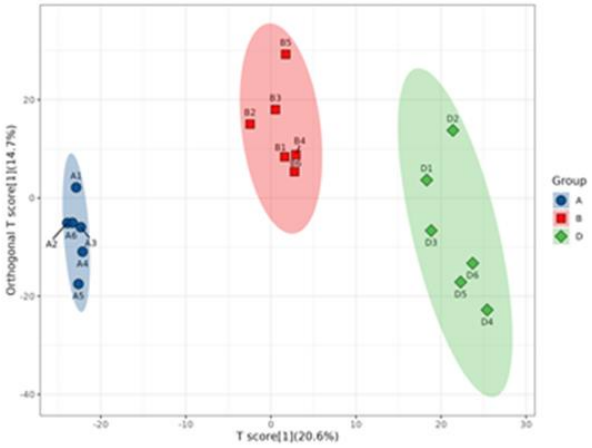


**Figure 10.** OPLS-DA Score Plot for Comparison Among Groups A, B, and D

## 4. DISCUSSION

Hyperglycemia is a common chronic disease, and its prevalence has been steadily increasing in recent years. Currently, China has the highest number of diabetes patients globally, and this trend is expected to continue for a considerable period of time [5]. Dyslipidemia is associated with various diseases, including cardiovascular diseases, hyperlipidemia, obesity, hypocholesterolemia, and coronary heart disease, most of which pose significant threats to human health. Hyperlipidemia, a disorder of lipid metabolism, is one of the primary causes of cardiovascular and cerebrovascular diseases [6]. Diabetes and hyperlipidemia often coexist, which can further lead to complications such as cardiovascular diseases and cancer. The complex pathophysiological mechanisms involved in these conditions have garnered significant attention across multiple scientific disciplines today.

Pu-erh tea is a type of tea that undergoes microbial fermentation and has long been consumed for its purported health benefits [7]. During its post-harvest fermentation stage, which occurs prior to drying and steaming, microorganisms play a critical role in the development of flavor, color, and functional

components. In recent years, Pu-erh tea and its constituents have attracted widespread attention due to their potential health benefits, such as mediating lipid metabolism, reducing the risk of cardiovascular diseases and cancer, lowering serum lipoprotein, total cholesterol (TC), and triglyceride (TG) levels, while increasing high-density lipoprotein cholesterol (HDL-C) levels and reducing low-density lipoprotein cholesterol (LDL-C) levels [8]. The health-promoting effects of Pu-erh tea are attributed to its bioactive polyphenols. Adequate intake of Pu-erh tea may prevent or reduce body fat by regulating fatty acid metabolism, thereby potentially lowering the risk of obesity-related diseases. Long-term consumption of Pu-erh tea has been shown to be beneficial in alleviating obesity and metabolic syndrome, and daily intake of Pu-erh tea may offer a safer and more effective means of countering diet-induced obesity and metabolic syndrome compared to other similar tea beverages [9]. Additionally, Pu-erh tea is considered effective in regulating blood lipids and preventing atherosclerosis. In apolipoprotein-deficient mice, Pu-erh tea was found to inhibit the progression of atherosclerosis by promoting apoptosis of macrophages within atherosclerotic plaques [10].

Dayi Junfang Tea, developed by the Dayi Company, is a type of Pu-erh tea fermented using dominant microbial strains during the "wo dui" fermentation process. Based on the different strains and quantities used in the fermentation process, four varieties—A, B, C, and D—were developed. After microbial fermentation, the content of tea polyphenols, beneficial elements, and other compounds in the tea changes, resulting in distinct effects. The results of this study show that all four varieties of Junfang tea can significantly reduce blood glucose levels in a high-fat, high-sugar diet-induced hyperglycemic and hyperlipidemic mouse model. Additionally, they increase insulin levels in the mice, improving the hyperglycemic condition. The teas also have a regulatory effect on blood lipids, lowering total cholesterol (TC) and triglyceride (TG) levels, while raising high-density lipoprotein cholesterol (HDL-C) levels, thus improving lipid metabolism.

## REFERENCES


[1] Sun Shili, Liu Yaqiong, Liu Jin, et al. In Vitro Hypoglycemic and Hypolipidemic Effects of Pu-erh Tea from Different Years and Its Material Basis [J]. Food Research and Development, 2018, 39(9): 152-158.

[2] Huang HC, Lin JK. Pu-erh tea, green tea, and black tea suppresses hyperlipidemia, hyperleptinemia and fatty acid synthase through activating AMPK in rats fed a high-fructose diet [J]. Food Funct, 2012, 3(2): 170-177.

[3] Liu C, Guo Y, Sun L, et al. Six types of tea reduce high-fat-dietinduced fat accumulation in mice by increasing lipid metabolism and suppressing inflammation [J]. Food Funct, 2019, 10(4): 2061-2074.

[4] Lu SY, Qi SD, Zhao Y, et al. Type 2 diabetes mellitus non-genetic rhesus monkey model induced by high fat and high sucrose diet [J]. Exp Clin Endocrinol Diabetes, 2015, 123(1): 19-26.

[5] Chen Zhongyun, Yang Shuyu. Progress in Research on the Pathophysiological Mechanisms of Traditional Chinese Medicine in Improving Insulin Resistance in Type 2 Diabetes [J]. Fujian Medical Journal, 2017, 39(3): 140-142.

[6] Liu Xueming, Huang Xiaoxia, Liao Sentai, et al. Comparative Study on the Antioxidant and Lipid-Lowering Activities of Three Anthocyanin-Rich Plant Extracts [J]. Journal of Chinese Institute of Food Science and Technology, 2014, 14(1): 20-27.

[7] Ku KM, Kim J, Park HJ, et al. Application of metabolomics in the analysis of manufacturing type of Pu-erh tea and composition changes with different postfermentation year [J]. JAgric Food Chem, 2010, 58(1): 345-352.

[8] Zeng L, Yan JN, Luo LY, et al. Effects of Pu-erh tea aqueous extract (PTAE) on blood lipid metabolism enzymes [J]. Food Funct, 2015, 15(45): 2.

[9] Shimamura Y, Yoda M, Sakakibara H, et al. Pu-erh tea suppresses diet-induced body fat accumulation in C57BL/6J mice by down-regulating SREBP-1c and related molecules [J]. Biosci Biotechnol Biochem, 2013, 77(7): 1455-1460.

[10] Xiao YH, He M, Liang X, et al. Pu-erh tea ameliorates atherosclerosis associated with promoting macrophage apoptosis by reducing NF-κB activation in apoE knockout mice [J]. Oxid Med Cell Longev, 2018, 23: 3197829.