Hypoglycemic Effect of Radix Et Rhizoma Salviae Miltiorrhizae, Guixi Green Tea and Their Compatibility on Mice

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Abstract [**Objectives**] To explore the hypoglycemic effect of Radix Et Rhizoma Salviae Miltiorrhizae, Guixi green tea and their compatibility on mice, so as to provide more options for the adjuvant treatment of diabetes and improve the comprehensive utilization value of Guixi green tea. [**Methods**] After being fed adaptively for 7 d, 60 SPF-grade male mice were intraperitoneally injected with alloxouracil (180 mg/kg) to induce hyperglycemia model, from which 35 mice with a successful model were selected. They were randomly divided into 5 groups (Danshen group, green tea group, compatible group, positive control group and blank control group), with 7 animals in each group. The mice in all groups were administered according to the dose of 200 mg/kg once a day for 15 d. After the last administration, their body weight was measured on the 15th day after fasting for 12 h, and their fasting blood glucose was measured by tail clipping. [**Results**] After modeling, the mice in each group showed typical hyperglycemia symptoms, namely obvious polydipsia, polyphagia, polyuria and weight loss. Compared with the blank control group, Danshen group and green tea group had obvious hypoglycemic effect ($P<0.05$), and compatible group had significant hypoglycemic effect ($P<0.01$). Compared with the blank control group, the weight loss of diabetic mice could also be inhibited in green tea group and compatible group ($P<0.05$). [**Conclusions**] Danshen, Guixi green tea and their compatibility had the effect of lowering blood sugar, among which the compatible group had the most obvious effect.

Key words *Salvia miltiorrhiza*, Guixi green tea, Compatibility, Blood sugar

1 Introduction

Salvia miltiorrhiza, which was first recorded in *Sheng Nong's Herbal Classic*, was listed as a top-grade medicinal material, and was recorded in previous herbal works[1]. As a traditional Chinese medicinal material in China, *S. miltiorrhiza* has been used as a medicine with its dried roots, and has a remarkable effect on cardiovascular diseases. Modern studies have found that *S. miltiorrhiza* has the functions of avoiding cardiac ischemia and hypoxia, improving microcirculation, inhibiting thrombosis, sedation, antibacterial and anti-inflammatory, *etc.*, and has been widely used in the clinical treatment of cardiovascular diseases at home and abroad[2]. At present, a large number of studies have shown that the preparation of *S. miltiorrhiza* or the combination of *S. miltiorrhiza* with other drugs has the effect of improving blood sugar and blood lipid, and it has been also widely used in clinical practice. On the basis of routine treatment of lowering blood sugar and blood pressure, Liu Bijian[3] treated 60 inpatients with diabetic nephropathy by combining Huangqi injection with Danshen injection. The results showed that after one cycle of treatment (30 d), fatigue, edema, back pain, loss of appetite and other symptoms were improved, or the adverse symptoms disappeared; the quantity of urine protein, blood urea nitrogen and serum creatinine decreased significantly within 24 h after the treatment. Yao Xinhui *et al.*[4] summarized the effects of *S. miltiorrhiza* extract and its components (tanshinone A, tanshinone B, tanshinone IIA, *etc.*) verified by experiments of various diabetes models in recent years, such as improving glucose and lipid metabolism, enhancing diabetic patients' heart function, alleviating motor and sensory nerve function defects caused by diabetes, preventing the occurrence of diabetic retinopathy, restoring the structural and functional damage of liver and kidney associated with diabetes, and helping the islets resist the atrophic vacuolation induced by high sugar.

Green tea, which is a traditional beverage in China, has become one of the three major non-alcoholic beverages in the world, and has various effects such as anti-oxidation, regulation of glucose and lipid metabolism disorders, and anti-atherosclerosis[5]. According to the difference of production process, tea can be divided into six categories such as green tea, yellow tea, and white tea, among which more of the natural ingredients in the fresh leaves of green tea are retained due to the different processing technology. In recent years, with the continuous development of social economy and continuous increase of people's health awareness, the health efficacy of green tea is increasingly concerned. For instance, Wang Changgang *et al.*[6] fed obese mice with green tea and white tea water extracts, and found that the blood lipid and blood sugar values of the experimental mice reduced to varying degrees. Tsuneki *et al.*[7] conducted a glucose tolerance test on the general population, and found that green tea could promote carbohydrate metabolism in the human body; animal experiments showed that green tea could reduce blood sugar level in db/db diabetic mice and streptomycin diabetic mice. The more famous varieties of green tea in western Guangxi are Baise green tea, Lingyun

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pekoe tea, Debao green tea and so on.

Based on previous studies, this paper aims to verify the control effect of *S. miltiorrhiza* and Guixi green tea on hyperglycemia when being used alone or in combination, and explore whether the compatibility of the two has a synergistic effect, so as to provide more options for the adjuvant treatment of diabetes and improve the comprehensive utilization value of Guixi green tea.

2 Materials and methods

2.1 Experimental animals 70 SPF-grade Kunming male mice were provided by Tongxiang Branch of Zhejiang Weitong Lihua Experimental Animal Technology Co., Ltd., with license No. of SCXK (Zhe) 2020-0002. Feed was provided by Guangdong Zhiyuan Biomedical Technology Co., Ltd. The mice were fed and tested under normal environment.

2.2 Drugs and reagents Radix Et Rhizoma Salviae Miltiorrhizae (hereinafter referred to as Danshen) was purchased from Zhengyang Pharmacy, Baise City, Guangxi, and green tea was purchased from Tianlin Badu Bamboo Shoot Wholesale, Baise City, Guangxi. Identified by Professor Huang Suoyi of Youjiang Medical College for Nationalities, Danshen was the dried rhizome of *S. miltiorrhiza*, a perennial upright herb of Salvia, Labiaceae, and the green tea was Lingyun pekoe tea (hereinafter referred to as green tea) produced locally in Baise. Metformin, alloxouracil (ALX): Beijing Solarbio Science & Technology Co., Ltd.; citric acid: Chengdu Jinshan Chemical Reagent Co., Ltd.; sodium citrate: Tianjin Aopusheng Chemical Co., Ltd.; blood glucose test paper: Sinocare Inc.; 75% ethanol solution: Chengdu Colon Chemical Co., Ltd.; 0.9% sodium chloride injection: Guangxi Yuyuan Pharmaceutical Co., Ltd.

2.3 Instruments DF-15 crusher: Jilin Dajixie Co., Ltd.; ZN-10002 electronic balance: Hangzhou Youheng Weighing Equipment Co., Ltd.; HH—S4 digital display constant temperature water bath: Shanghai Shangdeng Experimental Equipment Co., Ltd.; rotary evaporation instrument: Shanghai Yarong Instrument Factory; GA-6 blood glucose analyzer: Sinocare Inc.

2.4 Preparation of liquid medicine

2.4.1 Danshen liquid. Danshen was washed and left in a cool place for 4 – 8 h. After the surface moisture was dried, it was crushed into coarse powder with a grinder and decocted with water twice. 10 times of water was added to the powder for the first time, and then it was cooked for 1.5 h; 6 times of water was added to the powder for the second time, and it was cooked for 1 h. All decoction was mixed and filtrated, and the filtrate was concentrated. A certain amount of pure water was added to the concentrated liquid to prepare 200 mg/kg Chinese medicine Danshen. It was refrigerated for use.

2.4.2 Green tea extract. Green tea was boiled twice with water. For the first time, 10 times of water was added to the green tea and was boiled for 1.5 h; for the second time, 6 times of water was added to the green tea and was boiled for 1 h. All decoction was mixed and filtrated, and the filtrate was concentrated. A certain amount of pure water was added to the concentrated liquid to prepare 200 mg/kg green tea extract. It was refrigerated for use.

2.4.3 Compatible liquid medicine. Danshen and green tea were mixed in a ratio of 1∶1, and decocted in water twice. For the first time, 10 times of water was added to the mixture and was decocted for 1.5 h; for the second time, 6 times of water was added to the mixture and was decocted for 1 h. All decoction was mixed and filtrated, and the filtrate was concentrated. A certain amount of pure water was added to the concentrated liquid to prepare 200 mg/kg compatible liquid. It was refrigerated for use.

2.4.4 Metformin solution. Firstly, 4 g of metformin hydrochloride was placed in a 200 mL beaker, to which distilled water was added and stirred thoroughly. Afterwards, a certain amount of distilled water was added to the beaker until the volume was up to 200 mL to obtain 0.02 g/mL metformin solution. Finally, it was refrigerated for use.

2.4.5 ALX solution. At first, 2.10 g of citric acid (FW: 210.14) was placed in a 100 mL beaker, and a certain amount of distilled water was added to the beaker until the volume was up to 100 mL to obtain 0.1 mol/L limonic acid (liquid A). Secondly, a certain amount of distilled water was added to 2.94 g of sodium citrate (FW: 294.1) until the volume was up to 100 mL to obtain 0.1 mol/L sodium citrate solution (B solution). After the mixture of liquids A and B were mixed in the ratio of 1∶1.32, pH was determined with a pH test paper, and then was adjusted to 4.3 to obtain the citric acid-sodium citrate buffer. The demand for modeling was calculated according to the dose of 180 mg/kg. Before modeling, the citric acid-sodium citrate buffer was used to prepare 1% ALX solution.

2.5 Establishment of hyperglycemia mouse model A total of 70 SPF-grade Kunming male mice were selected. After 7 d of adaptive feeding and 12 h of fasting, 60 mice were randomly selected and weighed for model induction experiment. 1% ALX solution was intraperitoneally injected at 180 mol/L according to mouse body weight for 2 consecutive days, while another 10 mice were intraperitoneally injected with equal volume of normal saline as control. After 72 h, the mice were fasted for 12 h, and the tail tip was clipped to collect blood. The fasting blood glucose (FBG) value of each mouse was measured by a glucose meter. FBG≥11.1 mmol/L meant it was a successful model, and 35 mice were selected and included in the experiment.

2.6 Animal grouping and administration method The selected 35 mice were randomly divided into 5 groups, with 7 mice in each group, including Danshen group, green tea group, compatible group, positive control group and blank control group. Continuous intragastric administration was conducted once a day for 15 days. Danshen group: Danshen Chinese medicine liquid (200 mg/kg) was given. Green tea group: green tea extract (200 mg/kg) was given. Compatible group: compatible liquid (200 mg/kg) was given. Positive control group: metformin liquid (200 mg/kg) was given. Blank control group: 0.9% sodium chloride solution was given. All groups were administrated by intragastric

administration of 0.1 mL/10 g.

2.7 FBG determination After the mice were fasted for 12 h on the 1st and 15th day of intragastric administration, the blood was collected by aseptic surgical clipping, and FBG was measured by blood transfusion with aseptic surgical clipping.

2.8 Statistical methods The experimental data were represented by average ± standard deviation, and were processed by SPSS 24.0 software. The comparison between two groups was conducted by paired t test. $P<0.05$ means statistical difference, and $P<0.01$ indicates significant difference.

3 Results and analysis

3.1 Effects on the body weight of mice The experimental results show that compared with the blank control group, the green tea group and the compatible group could inhibit the weight loss of diabetic mice induced by intraperitoneal injection of ALX, and the difference had statistical significance ($P<0.05$) (Table 1).

Table 1 Effects on the body weight and FBG of mice in each group ($\bar{x}\pm s$, $n=7$)

Group	Body weight//g		FBG//mmol/L	
	Before treatment	After treatment	Before treatment	After treatment
Blank control	31.23 ± 3.30	23.91 ± 3.44	20.56 ± 2.68	22.13 ± 4.60
Positive control	32.24 ± 3.83	26.33 ± 3.81	20.39 ± 3.95	16.74 ± 5.60
Danshen	32.00 ± 2.54	27.31 ± 2.20*	17.96 ± 3.95	12.63 ± 3.00*
Green tea	31.13 ± 2.94	29.11 ± 2.70*	16.41 ± 3.77	13.00 ± 5.13*
Compatibility	30.69 ± 2.60	29.37 ± 3.66*	19.20 ± 3.39	11.51 ± 3.77**

NOTE Compared with the blank control group, *$P<0.05$, **$P<0.01$.

3.2 Effects on the FBG of mice The experimental results reveal that compared with the blank control group, the FBG of diabetic mice induced by intraperitoneal injection of ALX could be reduced in Danshen group, green tea group and compatible group, among which the difference of hypoglycemic effect in Danshen group and green tea group had statistical significance ($P<0.05$), and that of compatible group was significant ($P<0.01$) (Table 1).

4 Discussion

According to the International Diabetes Federation (IDF), the total number of people with diabetes worldwide is expected to reach 643 million by 2030 and 783 million by 2045[8]. In China, the number of diabetes patients is large, and the prevalence rate and incidence rate are also increasing year by year. Moreover, the two indicator is higher in rural areas compared with urban areas. Hypertension and diabetes have become the most important chronic diseases of Chinese residents[9-12].

Up to now, the world still lacks the means to completely cure diabetes, and in clinical practice, Western medicine combined with diet control is more used to stabilize blood sugar, which has many side effects and unsatisfactory effects[13-15]. In contrast, traditional Chinese medicine has the advantages of less side effects and adverse reactions. Based on previous studies, this study aims to verify the effect of Danshen and Guixi green tea on the control of hyperglycemia when they are used alone or in combination, and explore whether the compatibility of the two have a synergistic effect, so as to provide more options for the adjuvant treatment of diabetes and improve the comprehensive utilization value of Guixi green tea.

Before modeling, the mental state of the mice in each group was good, and they had smooth and glossy hair. Their activity was normal, and the body weight was physiologically increased, while their FBG was normal. During the modeling process, ALX could selectively destroy islet β cells, with strong and rapid effect, and also easily caused toxic damage to liver and kidney tissues, resulting in a high mortality of the modeling animals[16]. In this experiment, 4 mice died 3 days after two rounds of ALX injection. The model making success rate was close to that of Su Linjie *et al.*[17]. The rest of the mice were slightly sluggish, and had coarse hair. After modeling, they also showed typical hyperglycemia symptoms, namely obvious polydipsia, polyphagia, polyuria and weight loss.

The results of this hypoglycemic experiment indicated that compared with the blank control group, the FBG of diabetic mice could be reduced in Danshen group, green tea group and compatible group, among which Danshen group and green tea group had obvious hypoglycemic effect ($P<0.05$), and compatible group had significant hypoglycemic effect ($P<0.01$). Compared with the blank control group, the weight loss of diabetic mice could also inhibited in green tea group and compatible group ($P<0.05$).

In summary, the combination group had the most significant effect on lowering blood sugar, indicating that the combination of Danshen and Guixi green tea had a synergistic effect on lowering blood sugar. In this experiment, the effects of single use and combination of Danshen and Guixi green tea on the body weight and blood sugar of mice were preliminarily explored at the overall level, and the relevant mechanism of action needs to be studied further.

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improve the emotions of patients and help patients achieve the resonance of music and emotions. Five-element music therapy is of great significance for improving the lung function of COPD patients. From the point of view of modern clinical medicine, music therapy can help patients release dopamine in the body, thus helping patients to alleviate pain to the greatest extent.

5 Conclusions

In conclusion, the TCM treatment of COPD can mainly play a role in treating both symptoms and root causes. For the acute stage of COPD, we can choose to treat the symptoms urgently, that is, cough, expectoration, dyspnea, and choose some drugs for resolving phlegm, relieving cough and asthma. For example, Armeniacae Semen Amarum, Platycodonis Radix, Stemonae Radix and other related expectorant and antitussive drugs are often used in clinic. Besides, for the remission period, that is, the chronic period, we can choose some drugs to strengthen the treatment. This is also an advantage of traditional Chinese medicine in the treatment of COPD, mainly through the long-term application of traditional Chinese medicine, play a better prevention and treatment effect. Relatively speaking, Chinese medicine is safer and does not have as many side effects as Western medicine, but also can restrain some of the toxic side effects of Western medicine.

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