Effects of Radix Puerariae, Radix Rehmanniae and Their Compatibility on Blood Glucose and Blood Lipids in Mice

Yumei ZHANG[1△], Yulin ZHAO[1△], Yanmei QIN[1], Jialing WEI[1], Xiang QIN[1], Suoyi HUANG[2,3*]

1. College of Clinical Medicine, Youjiang Medical University for Nationalities, Baise 533000, China; 2. Key Laboratory of Research on Characteristic Ethnic Medicine in the Youjiang River Basin of Guangxi Colleges and Universities, Baise 533000, China; 3. College of Pharmacy, Youjiang Medical University for Nationalities, Baise 533000, China

Abstract [**Objectives**] To explore the effects of the compatibility of Radix Puerariae and Radix Rehmanniae on blood glucose and blood lipids in diabetic mouses. [**Methods**] Diabetic mouse model was established. The body weight and fasting blood glucose of mice were measured after 7 and 14 d of administration, and the biochemical indicators of blood lipids (TC, HDL-C, and LDL-C) were detected after 14 d of administration. [**Results**] Compared with the Radix Puerariae group and Radix Rehmanniae group, the compatibility group (1 : 2) had the best hypoglycemic effect ($P < 0.05$), and TC and LDL-C in the compatibility group (2 : 1) significantly decreased ($P < 0.05$), while HDL-C in the compatibility group (1 : 1) significantly increased ($P < 0.05$). [**Conclusions**] Radix Puerariae, Radix Rehmanniae and their combination can reduce the blood glucose of diabetic mice. The compatibility group (1 : 2) had a significant hypoglycemic effect ($P < 0.05$), and LDL-C in the compatibility group (2 : 1) significantly declined, while HDL-C in the compatibility group (1 : 1) rose significantly.

Key words Radix Puerariae, Radix Rehmanniae, Compatibility, Hypoglycemic effect, Blood lipids

1 Introduction

Diabetes mellitus (DM) is a metabolic disorder, which is mainly characterized by chronic increase of glucose level due to insulin dysfunction and absolute or relative insufficiency of insulin secretion. The typical symptoms of DM are drinking, urinating and eating too much. The number of diabetic patients in China ranks first in the world[1], and the imbalance and disorder of human metabolism caused by DM can lead to myocardial damage, large blood vessel and microvascular lesions[2-3]. Due to long-term hyperglycemia, DM can result in an obstacle to wound repair[4], as well as various chronic complications. For example, hyperlipidemia not only has a high disability rate and fatality rate, but also requires long-term treatment, which aggravates the life and economic pressure of many patients[5]. There is no cure for DM, and early detection and diagnosis, intervention and treatment are the key to the prognosis. From pharmacological studies, it have been found that Radix Puerariae (the root of kudzu vine) has the functions of activating channels and collateral, relieving muscle and reducing fever, raising yang and preventing diarrhea, and helping produce saliva and slake thirst, among which puerarin plays a major role. Radix Puerariae has certain therapeutic effects on DM, atherosclerosis, cerebral ischemia and other diseases[6]. The pharmacological effects of Radix Puerariae also include anti-oxidation, immune regulation, and lowering blood sugar and blood lipids[7]. Other studies have shown that the combination of puerarin and berberine can improve glucose tolerance and reduce blood sugar and lipids in diabetic rats[8]. Originally published in the *Sheng Nong's Herbal Classic*, Radix Rehmanniae has the function of clearing heat and cooling blood, nourishing yin and promoting fluid[9]. With the in-depth study of pharmacology, active ingredients such as terpenoids, flavonoids and iridoid glycosides rich in it have been discovered. Radix Rehmanniae has significant effects on regulating blood sugar and lipids, improving the central and blood systems, anti-tumor, bacteriostat and ameliorating aging[10]. Radix Puerariae and Radix Rehmanniae have a wide range of pharmacological effects, but few scholars at home and abroad have studied their compatibility on the treatment of DM, which is helpful to further develop their medicinal value. In this paper, the effects of Radix Puerariae and Radix Rehmanniae extracts and their compatibility on the body weight, blood sugar and blood lipids of diabetic mice were studied.

2 Materials and methods

2.1 Experimental subjects 80 male SPF-grade ICR mice aged 6 – 7 weeks, with the body weight of 28 – 34 g, were purchased from Guangdong Weitonglihua Laboratory Animal Technology Co., Ltd., with the production license number of SCXK (Yue) 2022-0063. The animals were kept in an SPF-grade environment, with ambient temperature of 20 – 24 ℃) and humidity of 50%. They ate food and drank water freely. Animal experiments were conducted in accordance with the regulations of the Ethics Committee of Youjiang Medical University For Nationalities.

2.2 Drugs Radix Puerariae and Radix Rehmanniae were purchased from Zhengyang Pharmacy, Baise City; alloxouracil (5 g/bottle, batch number: 227A021) was purchased from Beijing Solarbio Experimental Technology Co., Ltd.; metformin hydrochloride enteric-coated tablets (0.25 g each, batch number: 20220401) were purchased from Guizhou Tian'an Pharmaceutical Co., Ltd.

2.3 Main equipment and consumables Sinocare blood glucose meter and test paper, TWCL magnetic stirrer (Shanghai Biaohe Instrument Co., Ltd.); Rotary evaporation instrument

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△These authors contributed equally to this work.
* Corresponding author. E-mail: huangsuoyi@163.com

RE-3000A (Shanghai Yarong Biochemical Instrument Factory); ZN-10002 electronic balance (Hangzhou Youheng Weighing Equipment Co., Ltd.); TH-UP-10 laboratory special ultrapure water machine (Shanghai Hetai Instrument Co., Ltd.); TG16-WS centrifuge (Hunan Xiangyi Instrument Experimental Development Co., Ltd.); crusher (Yongkang Jiupin Industry and Trade Co., Ltd.), and clean grade laboratory rat maintenance feed (Beijing Ke'aoli Feed Co., Ltd.). T-CHO, HDL-C, and LDL-C kit, kit batch number: 20221122 (Nanjing Jiancheng Bioengineering Institute).

2.4 Establishment of diabetic mouse model According to the method of reference[11], the mice were deprived of food and water for 16 h, and then given intraperitoneal injection of alloxouracil (180 mg/kg). After eating food and drinking water freely for 72 h, the mice were deprived of food for 8 h. After tail clipping, venous blood was taken to measure the fasting blood glucose of the mice, and the fasting blood glucose not less than 11.1 mmol/L was as the success criterion for modeling.

2.5 Grouping and administration of experimental animals A total of 56 mice successfully modeled were randomly divided into 7 groups, namely positive control group, blank control group, Radix Puerariae group, and Radix Rehmanniae group, their compatibility groups (the ratio of Radix Puerariae to Radix Rehmanniae was 1 : 1, 1 : 2 and 2 : 1), and there were 8 mice in each group. The weight of the mice was measured after one week of adaptive feeding. The mice in the positive control group were given metformin hydrochloride solution by gavage (200 mg/kg), while the mice in the blank control group was given 0.9% normal saline of the same volume as the positive control group by gavage, and the rest of the experimental groups was given the drug solution by gavage according to body weight (0.1 mL/10 g). The gavage was conducted once a day and lasted for 14 d.

2.6 Drug extraction Radix Puerariae and Radix Rehmanniae were dried and crushed into powder with a grinder. 75% anhydrous ethanol with 10 times of the volume was poured into the Radix Puerariae powder, heated in a water bath at 65 ℃ and congealed for 1.5 h. The filtered filter residue was added with 75% anhydrous ethanol with 5 times of the volume. The above steps were repeated three times, and the filtrate obtained was mixed. The extract was obtained by evaporation and concentration, prepared into 1 g/mL liquid drug, and stored in a refrigerator at 4 ℃ for use. The preparation method and concentration of Radix Rehmanniae were the same as Radix Puerariae.

2.7 Measurement of experimental indicators One day before administration, 7 and 14 d after administration, the mice were deprived of food for 8 h, and the fasting blood glucose of the mice in each group was measured by tail clipping and blood collection. 14 days after administration, referring to reference[12], the eyeballs of the mice were taken for blood collection, and the whole blood was placed in a centrifuge tube (1.5 mL), cultured in an incubator at 37 ℃ for 30 min, and centrifuged at a speed of 3 000 r/min for 15 min, and the serum obtained was stored in a refrigerator at −80 ℃ for use. The contents of TC, HDL-C and LDL-C in the serum were measured by kit method.

2.8 Statistical methods The obtained data were represented by ($\bar{x} \pm s$), and statistical analysis was performed using SPSS 27.0 software. Data of various groups were compared by one-way analysis of variance, multiple comparison and paired sample t test.

3 Results and analysis

3.1 Comparison of weight between various groups As can be seen from Table 1, the body weight of mice in the experimental groups was significantly lower than that of the blank control group after 7 and 14 d of administration ($P < 0.05$), and the body weight of mice in the Radix Puerariae group and compatibility group (1 : 1) was significantly lower than that of the positive control group after 7 d of administration ($P < 0.05$).

3.2 Comparison of blood glucose between various groups Seen from Table 1, after 7 d of administration, the blood glucose of mice in the positive control group, Radix Puerariae group and compatibility group (1 : 2) was significantly lower than that of the blank control group ($P < 0.05$), and the blood glucose of mice in the Radix Puerariae group and compatibility group (1 : 1) was significantly lower than that of the positive control group ($P < 0.05$). After 14 d of administration, the blood glucose of mice in all experimental groups was significantly lower than that of the blank control group ($P < 0.05$). The blood glucose of mice in the compatibility group (1 : 2) was significantly lower than that of the positive control group ($P < 0.05$), and the blood glucose of mice in the compatibility group (1 : 1) was significantly higher than that of Radix Puerariae and Radix Rehmanniae groups ($P < 0.05$). Except for the Radix Rehmanniae group after 7 d of administration, the blood glucose of mice in the other experimental groups was significantly lower than that one day before administration ($P < 0.05$). It can be seen from the table that the hypoglycemic effect was better in the compatibility group (1 : 2).

3.3 Comparison of biochemical indexes of blood lipids between different groups As shown in Table 1, the TC value of serum in all experimental groups was significantly lower than that of the blank control group ($P < 0.05$), and the LDL-C value of serum in the compatibility groups (1 : 1 and 2 : 1) was significantly lower than that of the blank control group ($P < 0.05$). Compared with the Radix Puerariae group, the LDL-C value of serum in all experimental groups was significantly lower ($P < 0.05$). The LDL-C value of serum in the compatibility group (2 : 1) was significantly lower than that of Radix Rehmanniae group ($P < 0.05$). The HDL-C value of serum in the compatibility group (1 : 1) was significantly higher than that of the blank control group, positive control group, Radix Puerariae group, and Radix Rehmanniae group ($P < 0.05$), and the HDL-C value of serum in the compatibility group (2 : 1) was significantly higher than that of the Radix Puerariae group and Radix Rehmanniae group ($P < 0.05$). The TC and LDL-C values of serum in the compatibility group (2 : 1) was the lowest in the compatibility group (2 : 1), while the HDL-C value of serum in the compatibility group (1 : 1) was the highest.

Table 1 Comparison of body weight, blood sugar and biochemical indicators of blood lipids between different groups ($\bar{x} \pm s$, $n = 8$)

Group	Body weight//g			Blood sugar//mmol/L			Biochemical indicators of blood lipids//mmol/L		
	One day before administration	7 d after administration	14 d after administration	One day before administration	7 d after administration	14 d after administration	TC	LDL-C	HDL-C
Blank control	36.01 ± 1.14	39.69 ± 1.40	40.24 ± 1.76	19.36 ± 2.42	19.55 ± 3.38	19.30 ± 2.71	4.52 ± 0.87	$5.38 \pm 1.77^{3)}$	3.16 ± 1.80
Positive control	34.75 ± 1.21	$36.44 \pm 1.52^{1)}$	$36.45 \pm 1.15^{1)}$	19.03 ± 2.55	$15.14 \pm 2.68^{1)\#}$	$12.44 \pm 2.67^{1)\#}$	$3.37 \pm 1.57^{1)}$	$3.83 \pm 1.45^{3)}$	3.29 ± 1.05
Radix Puerariae	34.46 ± 3.22	$33.24 \pm 3.09^{1)2)}$	$37.71 \pm 2.53^{1)}$	21.18 ± 4.99	$15.04 \pm 2.51^{1)\#}$	$12.06 \pm 2.00^{1)\#}$	$3.16 \pm 1.03^{1)}$	7.31 ± 1.55	2.35 ± 0.92
Radix Rehmanniae	34.93 ± 2.13	$36.16 \pm 2.70^{1)}$	$37.63 \pm 2.49^{1)}$	19.09 ± 4.73	16.39 ± 3.25	$10.50 \pm 3.29^{1)\#}$	$3.15 \pm 0.98^{1)}$	$4.23 \pm 2.53^{3)}$	2.12 ± 0.73
Compatibility 1 : 1	35.41 ± 0.88	$34.03 \pm 1.15^{1)2)}$	$35.84 \pm 1.54^{1)}$	20.51 ± 2.83	$18.99 \pm 2.99^{2)3)\#}$	$15.33 \pm 2.51^{1)3)4)\#}$	$3.13 \pm 1.08^{1)}$	$3.44 \pm 2.92^{1)3)}$	$5.16 \pm 1.68^{1)2)3)4)}$
Compatibility 1 : 2	35.78 ± 2.90	$35.14 \pm 2.50^{1)}$	$37.23 \pm 2.71^{1)}$	18.66 ± 3.91	$14.13 \pm 4.64^{1)\#}$	$9.38 \pm 3.06^{1)2)\#}$	$3.40 \pm 0.74^{1)}$	$3.65 \pm 1.15^{3)}$	2.96 ± 1.23
Compatibility 2 : 1	35.59 ± 1.12	$37.26 \pm 1.77^{1)}$	$26.38 \pm 2.79^{1)}$	21.93 ± 5.99	$17.06 \pm 2.97^{\#}$	$11.86 \pm 3.97^{1)\#}$	$2.56 \pm 1.10^{1)}$	$2.06 \pm 1.31^{1)3)4)}$	$3.26 \pm 1.62^{3)4)}$

Note: Compared with the blank control group: $^{1)}P < 0.05$; compared with the positive control group: $^{2)}P < 0.05$; Compared with the Radix Puerariae group: $^{3)}P < 0.05$; compared with the Radix Rehmanniae group: $^{4)}P < 0.05$; compared with before administration: $^{\#}P < 0.05$.

4 Discussion

Blood sugar is an important indicator to evaluate the progression and treatment effect of diabetes patients. Long-term higher than normal blood sugar will cause lesions in various tissues and organs of the body, lead to multiple complications such as hyperlipidemia, and directly or indirectly result in a number of chronic diseases, such as atherosclerosis, cardiovascular and cerebrovascular diseases[13]. At present, the main method for controlling blood sugar and blood lipids in clinical practice is diet control supplemented by drug therapy, and this treatment has a good effect, but it requires patients' subjective cooperation and long-term persistence, which is a great test for patients' self-control and perseverance[14]. In order to seek a more effective treatment for hyperglycemia and hyperlipidemia and make diabetic patients eat and live freely, the compatibility of Radix Puerariae and Radix Rehmanniae was carried out based on previous studies, and whether their compatibility can produce synergistic effects was explored to provide a reliable basis for clinical drug use.

The results of this experiment suggest that the two herbs had a synergistic effect on reducing serum TC and blood glucose. The test results of blood glucose show that the compatibility group (1 : 2) had the best hypoglycemic effect, and the hypoglycemic effect was better than that of the group containing only Radix Puerariae or Radix Rehmanniae. According to the results of blood biochemistry, the serum LDL content in the compatibility group (2 : 1) was the lowest, so the group had a better lipid-lowering effect.

In conclusion, Radix Puerariae and Radix Rehmanniae as well as their compatibility had a good regulation effect on the metabolism of blood lipids and blood sugar in nutritional diabetic mice, and their compatibility can play a good regulation and improvement effect on the prevention and treatment of dyslipidemia caused by hyperglycemia. In this experiment, the effects of the compatibility of Radix Puerariae and Radix Rehmanniae on the body weight, blood glucose and blood biochemical indicators in mice were preliminarily studied, and the detailed mechanism needs to be studied further.

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