The intervention effect of different distribution ratio of Astragalus total saponins and curcumin on the DM rats model

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Abstract

Through the study of the effect of different ratio of Astragalus Total Saponins (ATS) and curcumin on Diabetes Mellitus (DM) model rats, we want to improve the screening model of the distribution ratio in Chinese Medicine, screening out the optimal proportion of the prevention and control of DM. By injecting streptozotocin (STZ) in the tail vein of rats to induct the DM rats model, we measured the dynamic change, insulin and insulin antibody (IAA) levels, glycosylated serum protein (GSP) content, lipid metabolism and the changes of renal pathology in DM model rats. The different distribution ratio of ATS and Curcumin can significantly reduce GSP, total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL), significantly increased serum insulin levels and high density lipoprotein (HDL) content, significantly decreased IAA level on the DM rats model induced by STZ. Different proportions of ATS and Curcumin have a good therapeutic effect on DM model rats, and 6:4 is the optimal proportion for the prevention and treatment of DM.

1. Introduction

DM is a common metabolic disease of endocrine disorder disease, which is difficult to cure, with many complications, high mortality and high disability rate. It has become a serious threat to human health (Tang et al., 2010). At present, the prevention and treatment of DM has become an urgent task for the medical profession (Liu et al., 2016; Seshasai et al., 2011; Gohar et al., 2017). Chinese medicine is irreplaceable by western medicines for its prevention and treatment of DM, because is mild with long efficacy and minimal side effects. TCM physicians believe that the DM was caused by Yin depletion, and because the course is long, it often results in Yang deficiency and even in the deficiency of Qi and blood. So its therapeutic principle is to tonify Qi and nourish Yin, invigorate spleen and remove blood stasis (Sarfraz et al., 2016; Chen et al., 2016; Pang et al., 2013; Muhammad et al., 2017). Astragalus and curcumin are common clinical medicines. They have long been used as clinical prescription form to prevent and treat DM and early diabetic nephropath (Iftakhar et al., 2015; Nawaz et al., 2017). They have definite curative effect, which is beneficial to diuresis and etumescencend, strengthening the middle warmer and tonifying qi. In this paper, the optimal ratio of ATS and curcumin was studied. We selected the optimal ratio, in order to provide new ideas for further screening DM drugs and developing new drugs, and promote clinical research.

2. Experimental materials

2.1. Drug reagents

Curcumin was provided by Henan Guangye Natural Pigment Co. Ltd., and its percent purity was higher than 90%; ATS was provided by Nanjing Zelang Pharmaceutical Co. Ltd., and its purity was more than 50%; Metformin Hydrochloride Tablets was produced by Xinyi Pharmaceutical Factory of Shanghai Pharmaceutical Group Co., Ltd.; STZ is produced by Sigma company; the blood glucose testing kit was produced by j.i.c. Polytron Technologies Inc; the GSP kit is produced by Nanjing Institute of Biological Engineering; Insulin test kit of R&D company; IAA test kit of R&D company; TC Reagent box was produced by Beijing North Kang clinical Reagent Co. Ltd; TG reagent, Beijing North Kang clinical reagent co.; HDL reagent kit, Beijing North Chemical Reagent Co., Ltd, LDL kit, Beijing North Chemical Reagent Co., Ltd.

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2.2. Experimental apparatus

We used KDC-160HR high speed refrigerated centrifuge, which was produced by Zhongjia Company, the subsidiary of USTC Chuangxin Co., LTD. Zongkia Branch; we also used BIORAD-680 elisa, which is produced by American BIO-RAD Company; OLYMPUS BX61 electric microscope, produced by Japan OLYMPUS Company; and adjustable pipette analysis instrument produced by Shanghai Leibo Company.

2.3. Experimental animals

Wistar rats, male, weighting 150–200 g, provided by the Laboratory Animal Center of Hebei Province, qualified certificate number: 1208113, Certificate No. 2010-001. (Yu) SYXK.

3. Experimental methods

3.1. Modeling and administration

We took 150 Wistar rats that weigh 150–200 g, and fed them normally for 3 days. The rats were anesthetized after fasting for 12 h, under the condition of STZ. We put them under dark rooms, and injected STZ (with the dosage of 40 mg/kg, 0.2 ml/100 g) dissolved by citric acid (pH is 4.2) in the rat venae sublingualis. Seven days after the injection, we let those mice fast for 12 h, and then took the blood from their tail end. Blood glucose was measured when the stomach of those mice were empty. We selected 132 mice whose blood glucose value ≥16.7 mmol/L and ≤21 mmol/L, because these mice drink more water, eat more food, and urinate more than usual. The selected 132 rats were randomly divided into 11 groups based on their blood sugar value. The group name were based on different component ratio of the medicine: metformin group and model group. The mice were administered respectively with different ratio of AST and curcumin suspension, metformin suspension and 0.5% CMC suspension.

Preparation method of different component ratio of AST and curcumin: with a constant total amount of 0.27 g/kg, we took AST of 0.27 g, 0.216 g, 0.189 g, 0.162 g, 0.135 g, 0.108 g, 0.081 g, 0.054 g, 0 g and curcumin of 0 g, 0.054 g, 0.081 g, 0.108 g, 0.135 g, 0.162 g, 0.189 g, 0.216 g, 0.27 g. Based on the baseline geometric changes, we mixed AST and curcumin at the ratio of 10:0, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10. Before use, we added 0.5% CMC solution and made the medicine of same concentration (13.5 mg/ml) but different proportion of components.

Positive control group: melbin (DMBG) (330 mg/kg, 2 ml/100 g, equivalent to 10 times of the clinical dosage). The blank control group and model group were administered with the same volume of 0.5% CMC suspension, given the medicine 1 time per day, with a continuous administration of 30 d. When administered 10 d, 20 d, 30 d, we took the blood from the rat’s tail. Then we took the serum and measured their blood glucose. After 2 h at 30 d, we took the serum, and quickly took the kidney. We rinsed the kidney with saline, and fixed it with 10% formalin, then we performed HE staining and pathological histology observation on the kidney.

4. Experimental results

As can be seen from the above table: except for the blank control group, the blood sugar levels were almost the same for other groups, so the group is evenly divided. Compared with the control group, the blood glucose level of model group was significantly higher at 10, 20, and 30 d (P < 0.01). This result indicates that the model is successful (see Table 1).

![Picture from above shows: the blank group, the islet cell nucleus of 8:2,5:4:6:3:7' groups are more loose, islet cell cytoplasm is more abundant, as can be seen in photo 1, 5, 8, 9 and 10; islet cell nucleus of model group animals is more densely located, islet cell cytoplasm is more atrophy, as can be seen in photo 2; The islet cell nucleus in the metformin group is more densely spread, and the cytoplasm of islet cells is atrophy, as can be seen in photo 3; Islet cell nucleus in 10:0 group is relatively concentrated, and islet cell cytoplasm completely shrinks, as can be seen in photo 4; Islet cell nucleus in 7:3, 0:10 group is relatively dense, part of islet cell cytoplasm shrinks, as can be seen in photo 6,12; In the group of 6:4, the islet cell nucleus is more intensive, and the cytoplasm of islet cells is shrinking, as can be seen in photo 7. In the 2:8 group, the islet cell nucleus is more densely distributed, and the cytoplasm of islet cells is shrinking, as can be seen in photo 11.](Picture from above shows: the blank group, the islet cell nucleus of 8:2,5:4:6:3:7' groups are more loose, islet cell cytoplasm is more abundant, as can be seen in photo 1, 5, 8, 9 and 10; islet cell nucleus of model group animals is more densely located, islet cell cytoplasm is more atrophy, as can be seen in photo 2; The islet cell nucleus in the metformin group is more densely spread, and the cytoplasm of islet cells is atrophy, as can be seen in photo 3; Islet cell nucleus in 10:0 group is relatively concentrated, and islet cell cytoplasm completely shrinks, as can be seen in photo 4; Islet cell nucleus in 7:3, 0:10 group is relatively dense, part of islet cell cytoplasm shrinks, as can be seen in photo 6,12; In the group of 6:4, the islet cell nucleus is more intensive, and the cytoplasm of islet cells is shrinking, as can be seen in photo 7. In the 2:8 group, the islet cell nucleus is more densely distributed, and the cytoplasm of islet cells is shrinking, as can be seen in photo 11.)
The effect of different ratio of ATS and curcumin on the blood sugar level of the DM rats model.

| Group          | n   | Dose (mg/kg) | Blood sugar (mmol/L) | Initial blood glucose | Tenth days | Twentieth days | Thirtieth days |
|----------------|-----|--------------|----------------------|----------------------|------------|----------------|----------------|
| Blank group    | 12  | –            | 22.93 ± 2.464        | 16.795 ± 3.402       | 1.564 ± 0.059 |                |                |
| Model group    | 12  | –            | 9.798 ± 2.248        | 20.000 ± 2.189       | 3.362 ± 0.151 |                |                |
| Metformin group| 12  | 330          | 16.024 ± 0.740       | 15.599 ± 2.094       | 3.085 ± 0.087 |                |                |
| 10:0 group     | 12  | ATS: curcumin = 270: 0 | 13.411 ± 1.106 | 17.196 ± 3.292 | 2.617 ± 0.233 |                |                |
| 8:2 group      | 12  | ATS: curcumin = 216:54 | 11.551 ± 2.779 | 17.115 ± 3.369 | 2.465 ± 0.089 |                |                |
| 7:3 group      | 12  | ATS: curcumin = 189:81 | 12.175 ± 1.452 | 17.035 ± 2.998 | 2.698 ± 0.047 |                |                |
| 6:4 group      | 12  | ATS: curcumin = 162:108 | 13.293 ± 1.710 | 17.115 ± 2.971 | 2.667 ± 0.044 |                |                |
| 5:5 group      | 12  | ATS: curcumin = 135:135 | 13.952 ± 1.165 | 17.756 ± 3.118 | 2.543 ± 0.034 |                |                |
| 4:6 group      | 12  | ATS: curcumin = 108:162 | 14.612 ± 1.013 | 16.474 ± 2.555 | 2.286 ± 0.108 |                |                |
| 3:7 group      | 12  | ATS: curcumin = 81:189 | 14.447 ± 1.433 | 16.875 ± 2.563 | 2.139 ± 0.109 |                |                |
| 2:8 group      | 12  | ATS: curcumin = 54:216 | 13.811 ± 1.172 | 17.676 ± 2.854 | 2.058 ± 0.068 |                |                |
| 1:10 group     | 12  | ATS: curcumin = 0:270 | 13.811 ± 1.555 | 17.276 ± 2.994 | 2.425 ± 0.060 |                |                |

* Indicates that compared with model group P < 0.05.
** Indicates that compared with model group P < 0.01.

5. Discussion

DM is a chronic metabolic disease with many causes. According to the different pathogenesis can be divided into insulin dependent (type1) DM, non-insulin-dependent (type2) DM, pregnancy DM and special type DM, type II DM is the most common one. Chinese physician believe that DM is due to yin deficiency and it is closely related the deficiency of Yin and Yang, Qi and blood, which also involves the spleen and kidney. They believe that to treating DM, we need to supplement Qi, nourish Yin, invigorate spleen and remove blood stasis. Curcumin can inhibit free radical mediated lipid peroxidation, protect the role of biofilm, which can significantly improve the sugar and fat metabolism in the model mice. So curcumin has a good control effect on DM and DM-related chronic complications (Liu et al., 2014; Samad et al., 2017). In modern pharmacological research, STA has the function of reducing blood sugar and increase the level of serum insulin, which has a protective effect on renal oxidative damage (Liu et al., 2014, Li et al., 2006). They are commonly used in clinical medicine to cure DM, and become part of the formula of DM medicines. Astragalus membranaceus has curative effect in clinical application, in the prevention and treatment of DM and early diabetic nephropathy for years.

In this study, the principle of STZ modeling is that the STZ model was used to study the toxicity of STZ on the highly selective islet beta cells. STZ has toxic effects on the highly selective

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**Table 1**
The effect of different ratio of ATS and curcumin on the blood sugar level of the DM rats model.

**Table 2**
The effect of different ratio of ATS and curcumin on insulin level, IAA and GSP of the DM mice model.

**Table 3**
The effect of different ratio of ATS and curcumin on TG, TC, HDL and LDL of the DM rats model.
Pancreatic Islet \( \beta \) Cells of mice (Tao et al., 2016; Xie et al., 2016; Mirandola et al., 2006). Once the islet cells have been destroyed, a serious shortage of insulin secretion will make the model animals develop diabetes. By adjusting the size of the injection dose and injection times, Pancreatic Islet \( \beta \) Cells can be destroyed in varying degrees, so that the secretion function of insulin can be badly affected. A small dose of STZ can establish animal model of human type II diabetes (Feng et al., 2016; Wang et al., 2012; Zaheer et al., 2017). Large doses of STZ, once given to the experimental subject, will establish an animal model similar to human type I diabetes.

The level of Fasting blood glucose (FBG) and glycosylated serum protein (GSP) reflects blood glucose levels of diabetic patients at different time points. It is a common indicator of blood glucose control and monitoring. GSP is produced by non-enzymatic glycation reaction between blood glucose and albumin. Its content is proportional to the concentration of blood glucose. So the level of serum GSP can reflect the latest blood glucose level in patients (Safti et al., 2015; Li et al., 2012).

Insulin is the only hypoglycemic hormone secreted by pancreatic \( \beta \) cells. IAA is a kind of hormone secreted by pancreatic islet cells. The two hormones play an important role in maintaining the balance of blood glucose. At the onset of diabetes, the insulin biological activities were affected and became absolutely or relatively insufficient, which results in the reduction of the fatty acid transportation. The plasma removed less TG, so the fat synthesis is inhibited. The lack of insulin causes the decomposition of a large number of fat tissues, resulting in fat metabolism disorders. Type II diabetes mellitus is often accompanied by abnormal metabolism of serum lipids, with increased TG, TC and LDL levels (Zhu et al., 2010). Meanwhile, the use of glucose in the liver, muscle and adipose tissue decreases, while the liver glycogen output increases, thus the formation of high blood sugar (Yine et al., 2015; Zhang et al., 2012; Zaidi et al., 2017). If we observe the microstructure of islet tissue under light microscope, we will have a more intuitive understanding about whether the islet tissue is damaged or not. This is a measurement of a successful DM model. Therefore, this study regards a number of hormone levels in the treatment of diabetes as drug efficacy indicators.

Medicine with different ratio of ATS and curcumin can significantly reduce the abnormal increase of blood glucose, GSP, TC, TG, and LDL; It significantly increases serum insulin levels and HDL content, significantly reduces IAA on the DM mice model induced by STZ. It suggests that different ratio of ATS and Curcumin on DM model rats can maintain blood glucose balance, correct the disorder of lipid metabolism, and have a certain therapeutic effect for DM. What's more, the 4:6 group and the 3:7 group is the optimal ratio of the components. This study shows that a combined use of drugs in promoting blood circulation will
remove blood stasis and replenish qi. In the prevention and treatment of DM animal model it has important application value. This paper will provide an important basis for the research and development of Jiang Qi capsule, providing a new idea for the development of new DM drugs.

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