The effect of different proportions of astragaloside and curcumin on DM model of mice

Mingsan Miao *, Jing Liu, Tan Wang, Xue Liang, Ming Bai

Departments of Pharmacology, Henan University of Chinese Medicine, Zhengzhou 450000, China

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A B S T R A C T

This paper aims to study the effects of different proportion of astragaloside and curcumin on STZ induced Diabetes Mellitus (DM) model of mice, and to select a better proportion of active components. Its ultimate purpose is to lay a basis for the follow-up research on astragaloside-curcumin capsule. Increase-decrease baseline geometric proportion design method and comprehensive performance evaluation utilised to study the effect of different proportion of astragaloside and curcumin on DM mice models, which have an intravenous tail injection of STZ. The proportions of the two components are 10:0, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10 respectively. And we will screen out the optimal composition. Blood glycated serum protein (GSP), hepatic glycogen and insulin tested to observe pathological changes in the pancreas. The mice DM model was copied successfully. Compared with the model group, groups treated with the metformin and with different proportions of astragaloside and curcumin help lower the blood glucose levels and GSP levels, increase glycogen stores of model mice by different degrees, and avoid pathological changes of pancreas in the model mice. The ratio of 3:7 was selected as the optimal one, based on the comprehensive performance evaluation method, followed by the ratio of 4:6. The optimal proportion of DM models is 3:7, followed by 4:6. The ratio of total astragaloside and curcumin can lower blood glucose levels, GSP levels, promote the formation of glycogen, and improve the pathological changes of pancreas in the model mice.

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1. Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders caused by absolute or relative insufficiency of insulin or an excess of glucagon, the counter-regulatory hormone of insulin. Symptom of this disease includes hyperglycemia, polyuria, polydipsia, polyphagia and weight loss. When it further develops, DM will also affect heart, brain, kidney and other organs. At present, most of clinical treatments for diabetes are western medicines (Ali et al., 2017; Shamsudin et al., 2017; Ghafar et al., 2017). Hypoglycemic agents mainly include insulin, sulfonylurea, biguanides, α-glucosidase inhibitors, and insulin sensitizers. Western medicine’s hypoglycemic effect is significant, but its effect on DM complications is minimal. Problems such as low glucose level, weight gaining, drug resistance, and high cost inhibit its further application. TCM doctors believe that diabetes which cause internal stagnation of the blood and deficiency in both yin and yang, is resulted from the deficiency of yin for kidney, the heat in lung and stomach, and blood astringent and stagnation. Therefore, the treatment should focus on supplementing qi, nourishing yin, tonifying spleen and absorbing clots (Pang et al., 2013). Chinese medicine originates from life practice, it is mild with minimal side effects and long efficacy. It has been successfully used to control diabetes, and has become a safe and effective source of developing new hypoglycemic drugs.

Astragaloside is a common drug for treating “the deficiency of qi, the depletion of fluid and diabetes” (Miao et al., 2011). Pharmacology experiments have verified that astragaloside can regulate glucose metabolism, insulin release, improve serum adiponectin levels, and inhibit apoptosis, thus having a synergistic effect on the treatment of diabetes (Li et al., 2012). Curcumin has antioxidant, anti-infective, anti-inflammatory, de-coagulant, lipid-lowering properties. It helps improve the disorders of glucose levels and fat metabolism of mice models, and relieve the
symptoms of diabetic peripheral neuropathy (Zhou et al., 2016). Curcuma and Astragalus have heat-clearing and detoxifying effects, they can upbear the clear and downbear the turbid, induce diuresis to alleviate edema, invigorate spleen and replenish qi. They are the most commonly used herbs for clinical practice, such as: “Modified Shengjiangsan” can treat diabetic nephropathy effectively and has a proven efficacy (Liu, 2012).

Chinese herbal compound of astragalosides and turmeric has a good therapeutic effect on diabetes. Previous studies also suggested that curcumin and astragalosides could improve the glucose and lipid metabolic disorders. It has antioxidantive constituent and helps improve pathological changes of pancreas on DM model mice. This study will focus on the hypoglycemic feature of different proportion of astragalosides and curcumin.

2. Experimental material and methods

2.1. Druggery reagents and instruments

Both curcumin and total astragaloside were provided by the Chemistry Laboratory at Henan University of Chinese Medicine. The content of curcumin was greater than 90%, with the batch number of 110809 and the content of total astragaloside was greater than 50%, with the batch number of 111008. Metformin hydrochloride tablets were produced by the Xinyi Pharmaceutical Factory which was the subsidiary company of Shanghai Pharmaceutical Group Co., Ltd. Its batch number was 120523; STZ was produced by the Sigma Company with the lot number of Z120315; Citric acid LOT20081019 and sodium citrate LOT20100502 were both purchased from Shanghai Pudong Chemical Co., Ltd.; CMC, Tianjin Hengxing Chemical Reagent Manufacturing Co., Ltd. production, with the lot number of 20110728. The blood glucose kit is produced by the Baoding Great Wall Clinical Health Beijing Biotechnology Co., Ltd. with the batch number of 120421; glycosylated serum protein kit of LOT20120807 and hepatic glycogen kit of LOT20120728 were both purchased from Nanjing Jiancheng Bioengineering Institute; Insulin Kit, is produced by the US R&D Company, with the LOT number of 20120301A.

UV-2000 UV-visible spectrophotometer was from the Shanghai Tianmei Science Instrument Co., Ltd.; BIORAD-680 Microplate Reader was produced by the USA BIO-RAD Company.

2.2. KM mice

KM mice, male, weight 18–21 g, are provided by Henan Laboratory Animal Research Center, and with its certificate number of 0008350. Its laboratory certificate number is SYXK (Yu) 2010-001.

2.3. Experimental methods

We took male KM mice 200, with an average weight of 18–21 g. We fed them normally for 3 days. After fasting 12 h, these mice received intravenous tail injection of STZ (citric acid buffer solution; pH = 4.2) (Bu et al., 2012), lucifuge, 80 mg/kg, 0.02 ml/10 g). We also dissolved metformin hydrochloride tablets in the 0.5% CMC solution (500 mg/kg, 0.2 ml/10 g). We used another 12 mice as the blank group, and administer them with the same volume of 0.5% CMC suspension as the model group. They were given the drug once a day for 30 consecutive days. We collected blood from the tail veins at the 10th, 20th and 30th dosage day then we conducted centrifugation and test the blood sugar levels of the mice. After dosing 2 h on the 30th day, we collected the blood and centrifuged the blood samples to get supernatant for blood sugar and insulin testing. The fresh liver was weighed and the hepatic glycogen was measured. The pancreas was fixed with formalin, and the sections were taken for pathological examination.

The data were analyzed with SPSS 17.0 for windows, and the average standard ± deviation (x ± s) was used for the measurement. The variance was analyzed by one-way ANOVA among the groups. The homogeneity of variance was performed using least significant difference (LSD), heterogeneity of variance was examined by Games-Howell method, and the rank data was examined by the Ridit test.

3. Results

3.1. Effects of different proportions of total astragaloside and curcumin on blood glucose levels of DM model mice

The results are shown in Table 1:

Known from Table 1: there was no significant difference in the blood glucose before each group except the blank. Compared with the blank group, the blood glucose of model group increased significantly (P < 0.01) on the 10th, 20th and 30th day. Compared with the model group, the blood glucose level was significantly reduced by metformin, astragaloside and curcumin (P < 0.01) on the 10th, 20th and 30th day after administration, and the effect of 4:6 group was the best, followed by the group with a component ratio of 3:7.

3.2. Effects of all components of total astragaloside and curcumin on hepatic glycogen levels, GSP levels and insulin levels on DM mice

The results are shown in Table 2:

Known from Table 2: compared with blank group, the levels of hepatic glycogen and serum insulin in model group were significantly decreased (P < 0.01), GSP level was obviously increased (P < 0.01). Compared with model group, the content of hepatic glycogen and insulin (P < 0.01) were clearly increased and the level of GSP (P < 0.01) was markedly reduced by different components of total astragaloside and curcumin. The most optimal group is the one with the component ratio of 4:6, followed by the group with the ratio of 3:7.

3.3. Effects of components of total saponins and curcumin on the pancreas of DM model mice

The pancreatic tissues of mice were as follows (the pictures are shown in Appendix A): The distribution of islet cells in the blank group was loose and the cytoplasm was mostly full, as can be seen in picture 1; The distribution of islet cells in the model group was extremely dense, and most cytoplasm were atrophy, as can be seen in picture 2; In metformin group, most of the islet cell nucleus was more densely spread, the cell cytoplasm was most atrophy, see picture 3: 10: 0 group, islet cell nucleus was more concentrated, the cytoplasm was all atrophy, as can be seen in picture 4; 8: 2 group, islet nucleus was more concentrated, the cytoplasm completely shrinks, as can be seen in picture 5; 7: 3 groups of total islets of the nucleus was more concentrated, with all the cytoplasm weakened, as can be seen in the picture 6; 6: 4 group, islet cell was...
Effects of all components of total astragaloside and curcumin on pancreatic tissue in DM mice.

Hepatic glycogen levels, GSP levels and insulin levels in DM mice.

Pathological changes of the pancreas in the experimental group measured by the semi-quantitative standard, as can be seen in Table 3.

As can be seen from Table 3: The Ridit test showed that model group had significant pathological changes (P < 0.01) compared with the blank group. Compared with the model group, the pathological changes of the pancreas were considerably improved by groups of 10:0, 8:2, 7:3, 5:5, 4:6, 3:7 and 2:8 (P < 0.01). The pathological changes of the pancreas were significantly improved in groups of metformin, total astragaloside and curcumin (6:4, 0:10 group). The effect on group 3: 7 was better than that of group 4:6.
The total score of astragalus saponins and curcumin was evaluated by the comprehensive weight method. Improvement rate of DM model in mice. Results of DM model in mice. 4. Discussion

3.3.3. According to the formula of total evaluation score \( P \) value, the evaluation score of comprehensive weight of different matching group is calculated, the results are shown in Table 5

The total score of 3:7 was the highest, followed by the group of 4:6, which indicated that the improvement effect of total astragalosides and curcumin 3:7 group on DM model mice was better than that of astragalus saponins and curcumin was excellent, followed by the group of 4:6.

4. Discussion

STZ has a high selectivity to islet \( b \) cells; the dose and frequency of injection can damage the \( b \) cells in varying degrees, and will also affect the secretion of insulin. A small amount of subcutaneous administration of STZ by several times can establish animal models of type 1 DM; large amount of administration for once can establish a \( II \) model. Intravenous injection with less dosage of drugs is for quick absorption and the risk of infection is relatively small, but we should pay attention to the speed of administration. The tail vein injection of large amount of STZ has an advantage of less time consuming, simple operation, good repeatability, etc. Most people are suffering from type \( II \) DM. Therefore, we used a single large number of STZ to cause type \( II \) DM on the animal model. The blood glucose level is a commonly used as an indicator of DM diagnosis and its hypoglycemic effect, but it is fluctuated due to various factors (Abbas et al., 2017; Jamal et al., 2017). GSP reflects the level of glycemic control during the past 2–3 weeks, and this measurement is not affected by short-term blood glucose fluctuation. These two indicators combined overcome the limitations of one single index, so this method has high clinical value. Liver glycogen metabolism affects the body's homeostasis of glucose metabolism. Insulin is the only antidiabetic substance that is secreted by pancreatic \( b \) cell. The excessive intake of sugar will stimulate insulin secretion and peripheral glucose uptake, which will be part of the synthesis, and insulin will also inhibit glycogen decomposition. So that the high blood sugar is prevented. Pathological sections of pancreas can directly reflect the success of the model, the pathological degree of the disease and the hypoglycemic effect of such kind of medicine.

According to the multi-index evaluation method established by the research group, the selected index was divided into the core, the important and the related indexes. We use the evaluation of strong, moderate and weak effect of the drug to determine the weight coefficient of each index (Ma et al., 2011). According to the mathematical formula calculated at different ratio of total astragalosides and curcumin, the 3:7 group had highest evaluation score \( P \), which indicated that group with the ratio of 3:7 had the best effect on improving the DM model. The efficacy of drug substance, mechanism of action, the clinical indications relatively clear, non-pharmacological effects of less interference, a variety of feedback mechanisms, targeted, efficacy is better than Pieces compatibility, quality control, suitable for industry promotion (Sindhu et al., 2017; Miao and Miao, 2012; Deng and Huang, 2016). Astragalus for tonifying Qi and lifting yangqi to drug, turmeric is qi stagnation and blood stasis in a variety of pain syndrome. The early study of various components shows that curcumin and astragalus saponins could repair microvessels, relieve \( b \) cell damage, and stimulate insulin secretion. Other effects on the DM multi-link reflects their multi-target, multi-channel characteristics. But the two component ratio of each specific index in the corresponding effect in different weight, if the deviation is large, it may affect the final results of the judgment. So we should consider the weight of each index, and different factors; as for the properties of the component ratio and odor, we have no idea. The

### Table 4
Results of DM model in mice.

| Grade | I level indicators | II level indicators | III level indicators |
|-------|--------------------|--------------------|---------------------|
| Pathological results of the pancreas | Blood sugar, GSP | Hepatic glycogen, insulin |
| Weight factor | 0.5 | 0.3 | 0.2 |

### Table 5
Improvement rate of DM model in mice.

| Improvement rate | <20% | 20–50% | 50–80% | >80% |
|------------------|------|--------|--------|------|
| Degree           | No improvement | More improvement | Improvement | Significantly improved |
| Weight factor    | 0.1 | 0.2 | 0.3 | 0.4 |

### Table 6
The total score of astragalus saponins and curcumin was evaluated by the comprehensive weight method.

| Group | Core indicators | Important indicators | Related indicators | \( P \) values |
|-------|-----------------|----------------------|--------------------|---------------|
| Pathological section(pancreas) | Blood sugar level | GSP | Hepatic glycogen | Insulin |
| 10:0 | More improvement | More improvement | No improvement | Improvement | Improvement | 0.31 |
| 8:2 | Improvement | More improvement | No improvement | Improvement | More improvement | 0.34 |
| 7:3 | More improvement | More improvement | No improvement | Improvement | More improvement | 0.29 |
| 6:4 | More improvement | More improvement | More improvement | More improvement | More improvement | 0.3 |
| 5:5 | Improvement | More improvement | More improvement | More improvement | More improvement | 0.37 |
| 4:6 | Improvement | More improvement | More improvement | More improvement | Significantly improved | 0.41 |
| 3:7 | Significantly improved | More improvement | More improvement | Significantly improved | Improvement | 0.46 |
| 2:8 | Improvement | More improvement | More improvement | Improvement | More improvement | 0.37 |
| 1:3 | Improvement | More improvement | More improvement | Improvement | More improvement | 0.24 |

\( P \) = I level indicators score * I level indicator weight factor + II level indicators score * II level indicator weight factor * III level indicators score * III level indicator weight factor = \( (m \cdot 0.4 + n \cdot 0.3 + p \cdot 0.2 + q \cdot 0.1)^{0.5} + (m \cdot 0.4 + n \cdot 0.3 + p \cdot 0.2 + q \cdot 0.1)^{0.5} \cdot 0.3 + (m \cdot 0.4 + n \cdot 0.3 + p \cdot 0.2 + q \cdot 0.1)^{0.2} \) 

(m: the number of significantly improved; n: the number of improvements; p: the number of higher improvement; q: the number of no improvement).
study of the components of traditional Chinese medicine is only from the mechanism of animal experiments and efficacy evaluation. As for its in-depth deficiencies at the cellular and molecular levels, further researches are needed.

5. Conclusions

This paper studies the ratio of total astragaloside and curcumin by using the comprehensive weighing method. The results shows that astragalosides and curcumin could lower the abnormal blood glucose levels and GSP levels at the ratio of 3:7. This is proven on DM mice model induced by streptozotocin; this proportion of the two herbs significantly promotes glycogen synthesis and insulin secretion, and relieves pancreatic β-cell pathological damage. This experiment combines Chinese medicine’s effects of blood activating and stasis-eliminating, tonifying qi and supplementing blood. From the prespective of modern cell medicine, it provides an experimental support for selecting better hypoglycemic drugs and preventing DM. It not only provides the basis for the research and development of Jiangqi capsules, but also promotes the development of new DM drugs.

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Appendix A. Effects of different proportion of total saponins and curcumin on the pancreas of DM model mice

| Pic 1 | blank group pancreas HE×400 |
| Pic 2 | model group pancreas HE×400 |
| Pic 3 | metformin group pancreas HE×400 |
| Pic 4 | 10:0 group pancreas HE×400 |
| Pic 5 | 8:2 group pancreas HE×400 |
| Pic 6 | 7:3 group pancreas HE×400 |
| Pic 7 | 6:4 group pancreas HE×400 |
| Pic 8 | 5:5 group pancreas HE×400 |
| Pic 9 | 4:6 group pancreas HE×400 |
| Pic 10 | 3:7 group pancreas HE×400 |
| Pic 11 | 2:8 group pancreas HE×400 |
| Pic 12 | 0:10 group pancreas HE×400 |

References

Abbas, G., Salman, A., Rahman, S.U., Ateeq, M.K., Usman, M., Sajid, S., Zaheer, Z., Younas, T., 2017. Aging mechanisms: linking oxidative stress, obesity and inflammation. Matrix Sci. Med. 1 (1), 30–33.
Ali, W., Halib, M., Sajid, S., Khan, A.R.S., Mazhar, M.I., Khan, I.U., Saliba, U., Farooq, M., Shah, M.S.U.D., Muzzammil, H.M., 2017. A Reverse transcription-polymerase chain reaction (RT-PCR) based detection of foot-and-mouth disease in District Faisalabad, Pakistan during the Year 2016. Matrix Sci. Med. 1 (1), 27–29.
Bu, Y.J., Yang, J.J., Liu, S.F., et al., 2012. Effects of soybean isoflavones on renal ischemia reperfusion injury in diabetic mice. Chin. J. Gerontol. 32, 309–310. Deng, C.Q., Huang, X.P., 2016. Research progress and comment on the compatibility of effective Chinese herbs. W. Chin. Med. 11, 565–569.
Ghafar, F., Nazrin, T.T.N.M., Salieh, M.M.R., Hadi, N.N., Ahmad, N., Hamzah, A.A., Yusof, M.Z.A., Azman, I.N., 2017. Total phenolic content and total flavonoid content in moringa oleifera seed. Galeri Warisan Sains 1 (1), 20–22.
Jamal, M., Shareef, M., Sajid, S., 2017. Lincomycin and tetracycline resistance in poultry. Review. Matrix Sci. Pharma 1 (1), 33–38.
Li, X.T., Zhang, Y.K., Kuang, H.X., et al., 2012. Mitochondrial protection and anti-aging activity of astragalus polysaccharides and their potential mechanism. Int. J. Mol. Sci. 13, 1747–1761.
Liu, C.B., 2012. A comparative study on the treatment of diabetic nephropathy with modified Shengjiangsan combined with western medicine. J. Practical Tradit. Chin. Int. Med. 26, 46–47.
Ma, R.J., Miao, M.S., Wei, R.R., et al., 2011. Research methods and considerations of components of Chinese medicine. J. Henan Univ. (Med. Sci.) 30, 1–5.
Miao, M.S., Miao, J.X., 2012. Present situation, problems and thinking of component TCM research. J. Tradit. Chin. Drug. Res. Pharmacol. 9, 1116–1119.
Miao, M.S., Zhang, X.X., Cao, S., 2011. Application of astragalus membraneaceaeus in diabetes and its complications. J. Tradit. Chin. Med. 11, 1323–1325.
Pang, B., Wang, S.D., Zhao, J.X., et al., 2013. Re-discussion on the thought and method of Lu Renhe’s “Six Treatments on Treatment” of diabetes. W. Chin. Med. 8, 274–278.
Shamsudin, N.H., Wong, C.F., Rahman, R.A.R.N.Z., Ali, M.M.S., 2017. Tight repression of elastase strain K overexpression by Pir7 (A1/D04/D3) shuttle expression system. Galeri Warisan Sains 1 (1), 20–22.
Sindhu, Z.U.D., Shafiq, Z., Naseer, M.U., Khan, M.N., Aslam, M.S.K.B., Abbas, R.Z., Khan, M.K., 2017. Prevalence of ectoparasitic fauna and efficacy of two commercial acaricides against argus persicus in layer poultry. Matrix Sci. Pharma 1 (1), 39–40.
Zhou, Y.K., Zhang, Y.M., Han, Y., et al., 2016. Progress in clinical research of curcumin. J. Bingtuan. Med. 1, 63–66.