A STUDY ON HYPOGLYCAEMIC HEALTH CARE FUNCTION OF *STIGMA MAYDIS* POLYSACCHARIDES

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Abstract

The objective of this paper was to study the therapeutic effect of *Stigma maydis* polysaccharides in diabetic mice. Mouse models of types 1 and 2 diabetes were established. The body weight, food intake, water intake as well as blood sugar level and glucose tolerance of mice were measured. *Stigma maydis* polysaccharides can improve the symptoms of weight loss and polydipsia in diabetic mice, and had an obvious antagonistic effect on alloxan-induced hyperglycaemia. The glucose tolerance test also showed that the *Stigma maydis* polysaccharides had very good effects on suppression and prevention of acute hyperglycaemia. *Stigma maydis* polysaccharides have some improvement effect on alloxan-induced types 1 and 2 diabetes.

Key words: *Stigma maydis* polysaccharides; ultrasonic extraction; hypoglycaemia

Introduction

Stigma maydis, also called Yu Mi Mai or Bang Zi Mao, originates from "South Yunnan Materia Medica". It is the stylus of Zea mays L. in the genus Zea of the family Poaceae, which is included in the "Standards for Medicinal Materials of the Ministry of Health of the People's Republic of China" (Standards for Medicinal Materials of the Ministry of Health of the People's Republic of China, 1986) 1985 Edition (Vol.1). As a commonly used medicinal species, it is mainly grown in the Northeast, Sichuan, Hebei and Shandong provinces of China. Over thousands of years, hundreds of dietary therapeutic methods have been formed with *Stigma maydis* as major ingredient. The US Food and Drug Administration (Fed Regist, 1991) has confirmed its safety and non-toxicity. Drugs made from its extract are non-prescription drugs. Scholars from various countries have carried out a large number of studies on its chemical constituents, contents and biological activities, and found out that the main constituents of *Stigma maydis* are crude fibre (Tsukunaga Kazue, 1923), polysaccharides (Li et al., 2008;), β-sitosterol (Zhao et al., 2010;) alkaloids, flavonoids, allantoin, organic acids, saponins, minerals, tannins that have convergence activity, zeaxanthin that have vitamin A activity and other nutrient substances and effective constituents, which can be used in the treatment of hypertension, nephritis, gallstone, diabetes, jaundice, measles, etc.

With the improvement of living standards, diabetes mellitus, which is closely related to people's living standards, has become a third common chronic disease causing serious harm to human health after tumours and cerebrovascular diseases in the world. The World Health Organisation (WHO) has listed diabetes mellitus as one of the world's three major difficult diseases, and designated November 14 of each year as "World Diabetes Day". Analysing from the pathological point of view, diabetes can be divided into type 1 and type 2. In the type 1 diabetes, islet function is completely lost, and this requires insulin treatment. In the type 2 diabetes, glucose tolerance is impaired mainly due to the relative lack of insulin secretion or

insulin resistance, and this requires treatment with insulin secretion promoting drugs (such as sulfonylurea hypoglycaemic agents) or insulin sensitizers. Diabetes need long-term or lifelong control by drugs, but some chemical drugs used at present for the treatment of diabetes have some serious adverse reactions if used long-term. Such serious adverse reactions include severe hypoglycaemia, anaphylactic shock, liver damage, cardiovascular toxicity, etc. Therefore, the development of drugs which can lower blood glucose while having little adverse reactions for diabetes treatment is of important clinical significance. *Stigma maydis* polysaccharides are high polymers composed of ten or more monosaccharides (Tian et al, 2005; Fang et al., 2007; Bioactivities, 2007) which are one of the main active constituents of *Stigma maydis*. Studies (Liu et al., 2006; Yu et al., 2009) have shown that most of the plant polysaccharides have a significant hypoglycaemic activity. In this paper, polysaccharides were extracted from *Stigma maydis* by ultrasonic extraction method, and then purified to investigate the effect of *Stigma maydis* polysaccharides on diabetes.

Materials and Methods

Drugs and reagents

Stigma maydis (collected from Wafangdian region) was identified by Professor Jinshan Liu (Zhengzhou University). Reagents include alloxan (Sigma), metformin (Aohua, Shenyang), glucose (Beijing Chemical Works), and blood glucose kit (Yilikang Biotechnology Co., Ltd., Wenzhou). Other reagents were domestic products of analytical grade.

Main instruments

The instruments used for the experiment are as follows: KQ-250DE medical CNC ultrasonic instrument (Kunshan Ultrasonic Instrument Co., Ltd.); low-temperature refrigerated centrifuge (Eppendorf, Germany); rotary evaporator (SENCO Technology Co. Ltd., Shanghai); 101A-1B thermostatic drying oven (Shanghai Instrument Co., Ltd.); electronic balance (Sartorius AG, Beijing); and blood glucometer (ACCU-CHEK-Active, Germany).

Experimental animals

SPF km male mice, weighing 20 ± 2g, were provided by the Laboratory Animal Center of China Medical University. All experimental procedures were approved by the Animal Research Ethics Committee.

Ultrasonic extraction and purification of *Stigma maydis* polysaccharides (Liang et al., 2011) 500 g of *Stigma maydis* were taken, added with an appropriate amount of distilled water and soaked for 24 h. Water was added to make the solid-liquid ratio 1:30, followed by ultrasonic extraction at 60 □ for 60 min four times. Then, the extracts were combined, cooled to room temperature, and filtered with four-layer gauze. The resulting filtrate was centrifuged at 3000 r/min for 20 min. The supernatant was taken, transferred to a rotary evaporator in batches and concentrated to 2000 mL. The concentrate was added with anhydrous ethanol to 80% and allowed to stand for 24 h at 4□. It was then centrifuged at 3000 r/min for 10 min. The precipitate was crude polysaccharides, which were then decolorized by D101 macroporous resin, deproteinized by SEVAGE method, washed several times with anhydrous ethanol, ethyl ether and acetone, and dried to powder in a 60 □ vacuum oven to obtain 25.132 g of grey-white *Stigma maydis* polysaccharides.

Establishment of diabetic mice model

In reference to the literature (Frode and Medeiros, 2008; Zhang et al., 2008; Kodama et al., 1993), some improvements were made. As for the establishment of type 1 diabetes model, male mice were adapted to the feeding for 3 d. Then, 6 mice were randomly picked as the blank control group, and the remaining mice were intraperitoneally injected with alloxan for

several times. The dose for the first injection was 150 mg/kg, and second injection 150 mg/kg. 2 d later, blood was sampled from caudal vein to determine the blood glucose level 8 h after fasting. Mice with blood glucose≥25 mmol/L were regarded as the successful experimental animal of type 1 diabetes model. For the establishment of type 2 diabetes model, male mice were adapted to the feeding for 3 d. Then, 6 mice were randomly picked as the blank control group, and the remaining mice were fed with a high fat diet for 1 month. After an overnight fast, the modelling was continued with low-dose alloxan (90 mg/kg, intraperitoneal injection). 2 d later, blood was sampled from caudal vein to determine the blood glucose level 8 h after fasting. Mice with blood glucose≥10 mmol/L were regarded as the successful experimental animal of type 2 diabetes model.

Grouping and administration

The successfully modelled mice were divided into six groups to give a total of seven groups together with the blank control group. They were administered for four weeks, and the arrangements are as follows:
(1) Blank control group, administered with equal volume of distilled water, denoted by B
(2) Type 1 diabetes model group, administered with equal volume of distilled water, denoted by M1
(3) Type 2 diabetes model group, administered with equal volume of distilled water, denoted by M2
(4) Type 1 diabetes positive control group, administered with 50 mg/kg metformin, denoted by C1
(5) Type 2 diabetes positive control group, administered with 50 mg/kg metformin, denoted by C2
(6) Type 1 diabetes drug group, administered with 20 g/kg *Stigma maydis* polysaccharides extract, denoted by D1
(7) Type 2 diabetes drug group, administered with 20 g/kg *Stigma maydis* polysaccharides extract, denoted by D2

Measurement of food intake, water intake and body weight change

Mice were intragastrically fed once a day for 28 consecutive days. The water intake, food intake and body weight of mice were recorded once every 4 d, and the nutritional status of the mice in each group was observed.

Determination of glucose level

Blood was sampled from hyperglycaemic model mice on the 7th, 14th, 21st and 28th days after the administration, and blood glucose level was directly measured with blood glucometer.

Oral glucose tolerance test

After four weeks of administration, the mice were fasted for 6 h. Then, blood was collected from caudal vein. Serum was separated by centrifugation; 2.0 g/kg glucose was orally administered to determine the blood glucose levels before administration and 0.5, 1 and 2 h after administration. The changes in the glucose tolerance in each group were studied, and graphs were plotted by analysing the data.

Data analysis

Data were processed using SPSS 11.5 software, and were expressed as mean ± SD. Independent sample T-test was used for comparison of test groups and control groups, and significance levels of 0.01 and 0.05 were chosen. Hypoglycaemic data were analysed using one-way ANOVA.
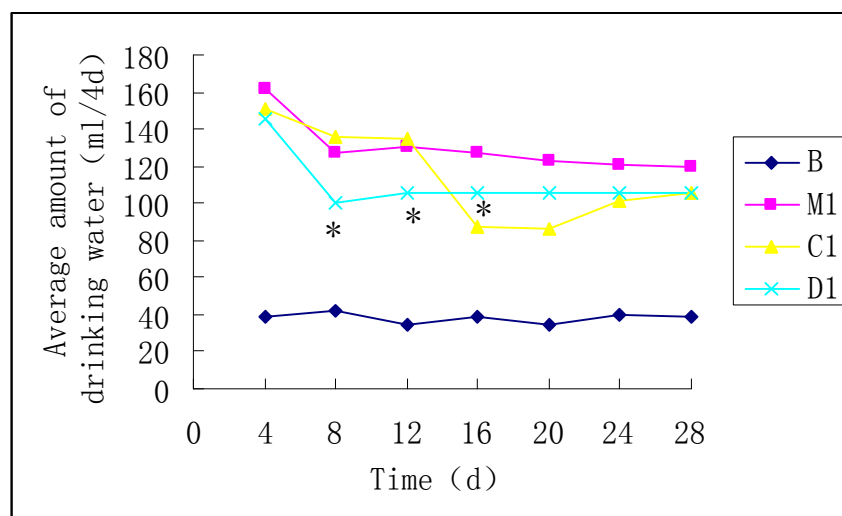
Results

Effects of *Stigma maydis* polysaccharides on appearance, body weight, food intake and water intake of diabetic mice

After successful modelling, types 1 and 2 diabetic mice both showed varying degrees of mental depression, lustreless body complexion, slow response and other symptoms, and complicated with typical symptoms of "polyuria, polydipsia, polyphagia, weight loss". In comparison, mice in the normal control group had bright fur and hair, good spirit, normal reaction, and did not show the typical symptoms of "polyuria, polydipsia, polyphagia, weight loss". Body weight of type 1 fodiabetic mice in each group is shown in Figure 1. The body weight of mice in M1 group was significantly lower than that in group B, which was in a gradual downward trend, and the trend of weight loss of mice in D1 group was significantly inhibited by *Stigma maydis* polysaccharides. Water intake and food intake status of type 1 diabetic mice are as shown in Figure 2 and Figure 3. At the beginning, the water intake in D1 group was lower than that in M1 group, but there was no significant change. After 8-16 d of administration, the water intake in D1 group was significantly lower than the M1 group ($P<0.05$). Meanwhile, starting from the 12th day, the food intake in Dl group was also significantly lower than M1 group. The experimental results indicated that the *Stigma maydis* polysaccharides can improve the weight loss, polydipsia and polyphagia symptoms of type 1 diabetic mice to a significant extent.
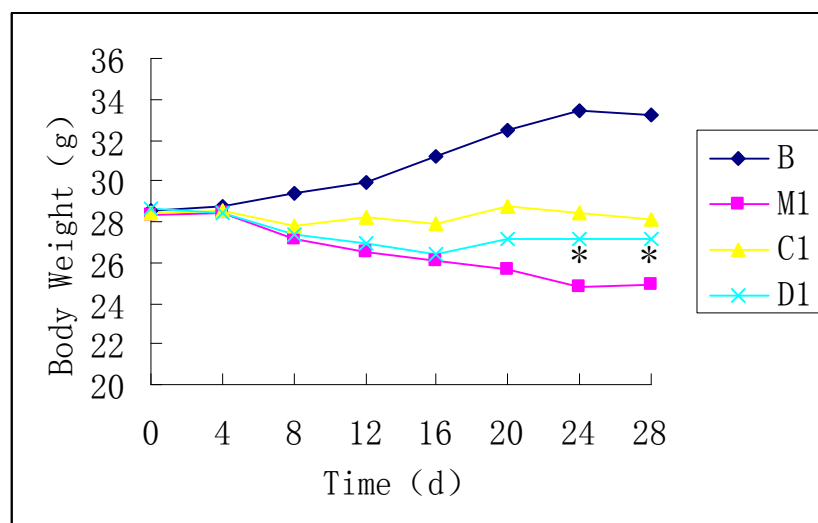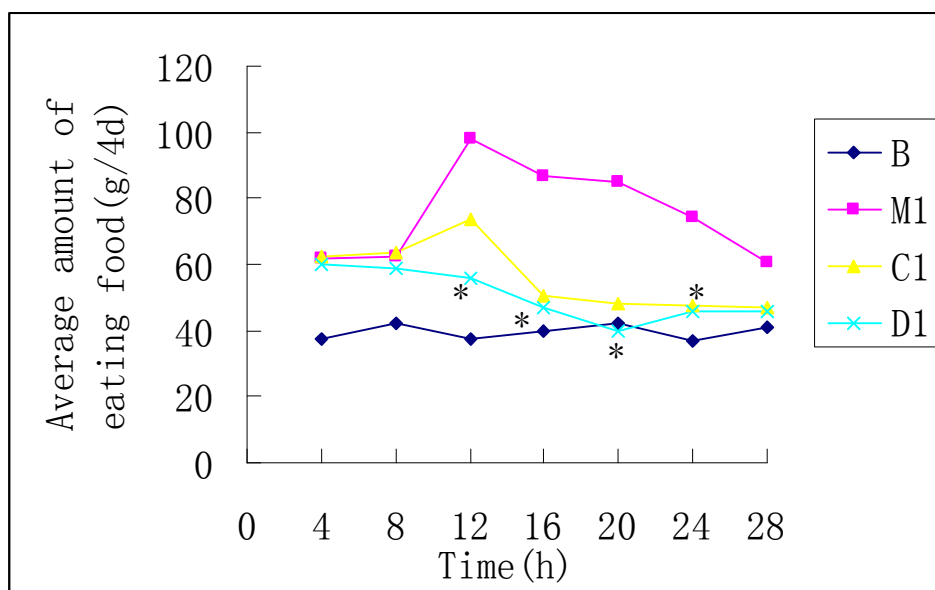

Comparison with M1, * $P<0.05$

Figure 1: Change in the body weight of type 1 diabetic mice during the administration period

Comparison with M1, * $P<0.05$

Figure 2: Change in the water intake of type 1 diabetic mice during the administration period.
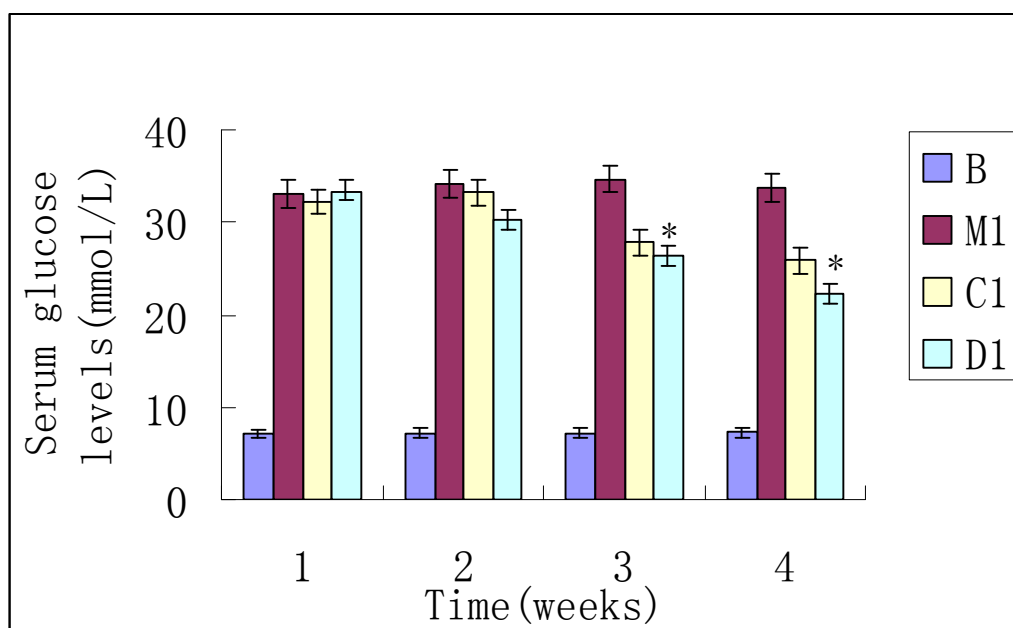

Comparison with M1, * P<0.05

Figure 3: Change in the food intake of type 1 diabetic mice during the administration period

Results for measurement of blood glucose level

Figures 4 and 5 show the measurement results of the blood sugar levels. Compared with the B group, blood glucose concentrations in model groups M1 and M2 were significantly elevated. 3-4 weeks after the administration, the blood glucose levels in drug administration groups D1 and D2 were significantly lower than the model groups, indicating that *Stigma maydis* polysaccharides have different degrees of inhibitory effects on blood glucose rise in diabetic mice.

Comparison with M1, * P<0.05

Figure 4: Effect of *Stigma maydis* polysaccharides on blood glucose of type 1 diabetic
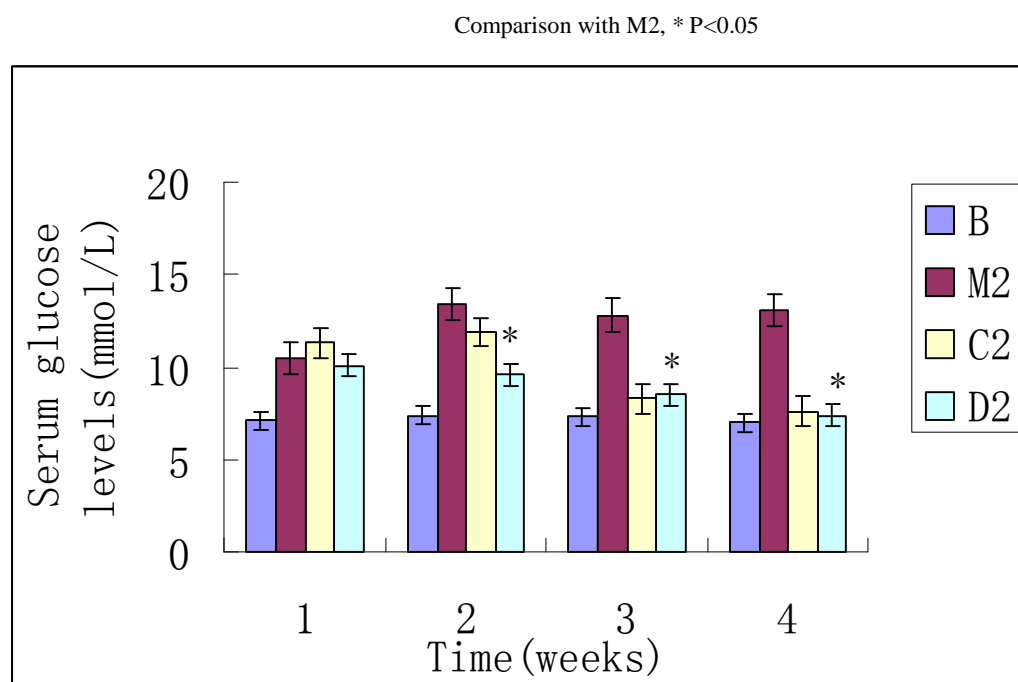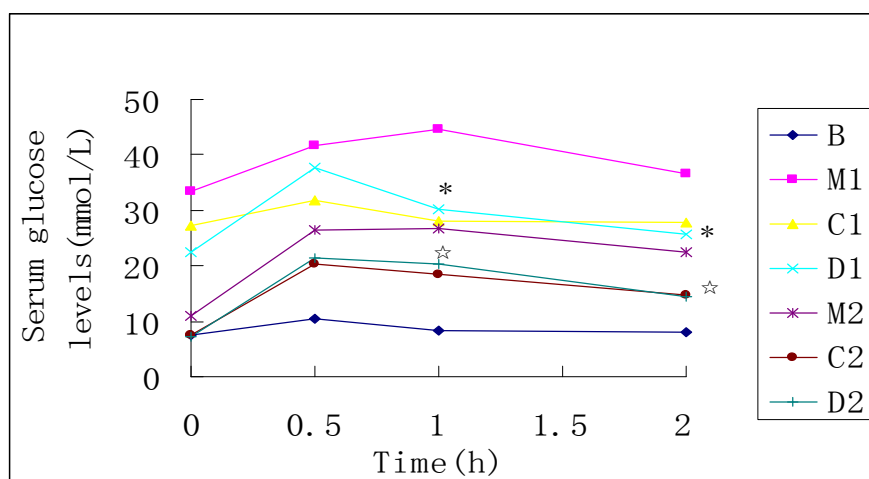
Comparison with M2, * P<0.05

Figure 5:   Effect of *Stigma maydis* polysaccharides on blood glucose of type 2 diabetic mice

Results for oral glucose tolerance test

The test results of the oral glucose tolerance are as shown in Figure 6. After administration of glucose, glucose levels of mice in each group were all significantly elevated. Compared separately with M1 and M2 groups, the glucose concentration values of mice in D1 and D2 groups were significantly lower than each model control group, and higher than B group. The trends of blood glucose level rise of mice in D1 and D2 groups after 0.5, 1 and 2 h were significantly inhibited.

Comparison with M1, * P<0.05; comparison with M2, ☆ P<0.05
Figure 6:   Effect of *Stigma maydis* polysaccharides on glucose tolerance of diabetic mice

Discussion

Diabetes is a common endocrine metabolic disease whose characteristic manifestations are high blood sugar and glycosuria, as well as disorders of fat, protein and water-electrolyte, which often lead to serious complications. In recent

years, its incidence has been in a gradually increasing trend. At present, chemosynthetic hypoglycaemic drugs are generally used in the treatment, and these drugs are not conducive to long-term medication of diabetes patients (Zhang, 2009). Alloxan is a kind of β-cytotoxic agent, which has a selective damaging effect on pancreatic β cells, thereby enabling the incidence of diabetes in mice. Because of its small tissue toxicity and high animal survival rate, it has become one of the commonly used methods for preparation of diabetic animal model currently at home and abroad. This animal model can well reflect the actions and effects of various hypoglycaemic drugs.

In this experiment, *Stigma maydis* polysaccharides were extracted using ultrasonic method to avoid breakdown of the polysaccharides by high temperature and to observe its effect on blood glucose of diabetic mice. It can be seen from the experiment results that the *Stigma maydis* polysaccharides have certain hypoglycaemic effect on diabetic mice and can improve weight loss and polydipsia symptoms of diabetic mice. But its hypoglycaemic effect was less significant than that of the positive control drugs. *Stigma maydis* polysaccharides have an obvious antagonistic effect on alloxan-induced elevated blood glucose, and a significant hypoglycaemic effect on diabetic mice. The glucose tolerance test also showed very good effects of *Stigma maydis* polysaccharides in suppression and prevention of acute hyperglycaemia, which may be as a result of the combined biological effect of multiple pathways such as glucose metabolism, lipid metabolism and insulin. *Stigma maydis* is extremely rich in resources. If fully utilised, it will be turning waste into treasure and can well serve the health of majority of the people.

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