Rhubarb Extract's Inhibiting Effects on α-Glucosidase's Activities in the Small Intestines of Type 1 Diabetic Rats

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ABSTRACT

The objective of this study was to investigate rhubarb extract's inhibiting effects on α-glucosidase activities in the small intestines of rats with Type 1 diabetes mellitus. In group A, the rats were given normal salt. Group B received acarbose, while group C received rhubarb extract for seven days. Fasting blood-glucose was measured from the first to the sixth day. Four fixed rats were selected to measure the level of blood glucose two hours after the meal. On the seventh day, two rats from each group respectively, were selected, half an hour after a meal, an hour after the meal and two hours after a meal to measure their blood glucose, C-peptide and insulin as well as extracting and then determining the alpha glycosidase enzymes in their small intestines. The results from each of the three groups showed no significant differences in terms of insulin and C-peptide; and in the case of daily fasting blood-glucose and the fixed four rats' blood-glucose two hours after meal from the first day to the sixth day, and in the acarbose (group B) and the rhubarb extract (group C), the inhibiting effects on α-glucosidase's activities in the small intestines of Type 1 diabetic rats were superior to those with normal salt. Group A and the former two groups, showed no remarkable differences in these inhibiting effects. In conclusion, rhubarb extract, similar to acarbose, can inhibit α-glucosidase activities in the small intestines of Type 1 diabetic rats. There was little effect on the effect of insulin, C-peptide as well as blood glucose after a meal or in the case of rats with Type 1 diabetes that were hungry.

Keywords: Acarbose, α-glucosidase, diabetic rats, rhubarb extract

INTRODUCTION

In the treatment of diabetes, α-glucosidase within the intestinal epithelial cells can hydrolyze the glycosidic linkage of saccharides like sucroses and maltoses, and plays a significant role in the decomposition, digestion and absorption of saccharides. Its activities can be inhibited in the control of blood glucose level after meal. So, it has a positive effect on the prevention and treatment of diabetes. Existing studies have researched Tinospora crispa leaf, Stenochlaena palustris, hemiphyllum and the activities of α-glucosidase with inhibiting effects serving as a hint (1–3).

Meanwhile, existing experiments both at home and abroad involve the following aspects: (a) the study of Rhubarb extract's inhibiting effects on the activities of alpha glycosidase in a laboratory environment are close to the effects of those of the original alpha glycosidase inhibitors; (b) the National Natural Science Foundations of many countries have proved that Rhubarb, the traditional Chinese medicine, has no effect on the absorption of other nutrients except starch; and (c) the existing domestic research shows that Chinese rhubarb's components causing diarrhoea have nothing to do with its main biological activities (4–6). The above three aspects show that rhubarb, with the removal of the ingredient causing diarrhoea, can inhibit the activities of alpha glycosidase reagent in a laboratory and has no effects on the absorption of other nutrients with the exception of starch.

Active monomers like chrysophanols, separated from the Himalayan rhubarb, one of the rhubarb plants, have certain inhibitory effects on the activities of yeast glycosidase enzymes (7). However, there is no report that rhubarb extract causes the inhibiting effects on the activities of α-glucosidase. So, in this experiment, by studying rhubarb extract's inhibitory effects on the activities of α-glucosidase within the small intestines of Type 1 diabetic rats, we expect to provide new experimental and theoretical evidence on the hypoglycaemic effects and the mechanism of rhubarb extracts.

SUBJECTS AND METHOD

Medicinal materials

Rhubarb polygonaceae, a massive and herbaceous plant, was used in this study. It was the dried root and rhizome of Polygonum cuspidatum Sieb Et Bbatch Number: 110757200206, and was used as a yellow and brown fine powder. Specification: 100 g, period of validity 20170209, Source: Xi 'an Conway bio-engineering Co, Ltd. Its preservation condition was at normal temperature.

Reagent

The reagent for detecting α-glucosidase activities in small mice was produced by Sigma-Aldrich and bought from sigma-aldrich Shanghai trading Company Ltd. The article number was MAK123. Acarbose was obtained from Bayer healthcare company, Beijing; the drug approval word H19990205. Double evaporate water, sucrose, glucose, *etc*.

Animal

Strain and level: Sprague-Dawley rat, SPF level, number 30 and gender - male. The weights of the animals were from 160g~180g. They were provided by the medical experimental animal centre of Guangdong province.

The method of identification: The coat staining method was adopted. The rats were numbered with saturated picrate, with painting spots on the coats on the different parts of the body surfaces of the rats with different numbers, and identifications according to the dyeing of the rats' skins and the dual serial number tags of the baskets.

Feeding and management: SD rats were raised in SPF animal room in the medical laboratory animal centre of Guangdong province. The licence of the experimental animals was B: SYXK (guangdong), 2013–0002.

The feeding conditions of the animals were as follows: single cage, the temperature and humidity of 20~26 ℃, continuous day and night lighting of 10 to 14 hours. The conditions of the feeding room remained stable to ensure the reliability of the experimental results. The animals were free to eat and drink. Feeding and drinking water were provided by the medical laboratory animal centre of Guangdong.

Quarantine: Quarantine inspections of the rats were done for four days, during which the animals were checked once every day. The unhealthy animals were immediately removed and replaced with other healthy rats.

Instruments

We used the following instruments: BBS3000A electronic balance, with precision 0.1 g from Shanghai Weighing Apparatus Co, Ltd Products, BS223S electronic balance with precision 0.001g from Sartorius company of Germany, RE-type 2000 rotary evaporator from Shanghai Rong Biochemical Instrument Plant, SI - IZ a Ⅲ circulating water vacuum pump from the Bright and Chromatography Equipment Factory in Shanghai, ZK - 82 a type of vacuum dryer from the experimental instrument factory of Shanghai, 722 spectrophotometer from the third analysis instrument factory of Shanghai city, TL-16R speed refrigerated centrifuge from the research institute of centrifuge machinery in Shanghai, RlGL-16G bench centrifuge from An Ting scientific instrument factory of Shanghai, and WI - I. 851 type vortex mixer from the world chemical factory of Shanghai.

Compounding methods of test sample and reference substance

For the preparation of citrate buffer solution with 0.1 mol/L, 2.1 g citric acid was accurately dissolved in 100 mL distilled water to make solution A; 2.94 g of sodium citrate was dissolved in 100 mL distilled water to make solution B. We mixed solutions A and B

according to certain proportions (1:1 ~ 1.2), and adjusting with pH test-paper to a pH of 4.2 ~ 4.5 and preserved the mixture in a refrigerator at 4 $^{\circ}$C with 1% STZ. Two hundred miligrams of STZ solution was put into two sterile centrifuge tubes respectively with the external wrapped in tinfoil. Precooled citric acid buffer was added and STZ bottles in an ice bath were taken to the animals' room. Streptozotocin has the expiration time of 30 minutes. So, STZ was dissolved in 20 mL of 0.1 mol/L citric acid buffer solution.

Building methods: The rats were fed overnight for 12 hours and an intraperitoneal injection of 1% STZ solution at the rate of 6 mL/kg was given to them. After the intraperitoneal injection of STZ, an abrosia of five hours was given to the injected animals; on the seventh day, their fasting glucose was measured with a blood glucose metre. Twelve animals with fasting plasma glucose of 11.0 mmol/L or more, were chosen to make 12 models and those with too much or too little blood sugar were eliminated. The selection was randomly divided into the control group (saline), and the acarbose and rhubarb extract groups with eight rats in each group. The rats in the acarbose and rhubarb groups were given gavage of acarbose 20 mg/kg and rhubard 0.1 g/kg, respectively, for eight consecutive days.

Lab-measuring indexes

The observation and recording of the general performance of the animals were made, and their weights were measured once every two days. The rats were given an ambrosia but not water at 10:00 am from Day 1 to Day 7 and four hours later. We gave them feed and gavage at the same time and correspondingly, their daily fasting blood glucose was measured. From Day 1 to Day 6, four out of the eight rats were selected to measure the levels of their blood glucose two hours after meal. On the seventh day, two rats were selected from each group, respectively. In the case of the overnight rats, half an hour after meal, an hour after meal and two hours after meal, we measured their blood glucose, C-peptide and insulin as well as

extracted and then determined the alpha glycosidase enzymes in their small intestines.

Regarding the preparation of liquid alpha glycosidase enzyme, the rats' small intestines after anaesthesizing and scarifying's them, were removed and the mucous membrane were put on an ice plate and a homogenate of phosphate buffer added to five times the amount at 4 ^{o}C at pH value at 6.8 at 5000 rpm/minute. Twenty centrifugalizations at 4 ^{o}C was carried out and the supernatant stored at - 20 ^{o}C.

Determination of alpha glycosidase enzyme activity

The reagent of detecting α-glucosidase's activities in the big rats, was produced by Sigma-Aldrich and bought from sigma-aldrich (Shanghai) Trading Co Ltd. Their article number was MAK123. The value of absorbance A was measured and the activities of the alpha glycosidase enzymes determined.

Processing of data

The experimental data were analysed with the single factor of variance using SPSS 21.0 statistical software, making a multiple comparison of LSD (L) testing with assumed homogeneity of variance and making a multiple comparison of Tamhane's T2 testing without assumed homogeneity of the variance. The level of significant difference remains $p < 0.05$.

RESULTS

Feeding did not have a big influence on the insulin levels in the three groups and there was also no obvious secretion peaks. So, there were no significant differences when we compared the pairs among the three groups (Fig. 1).
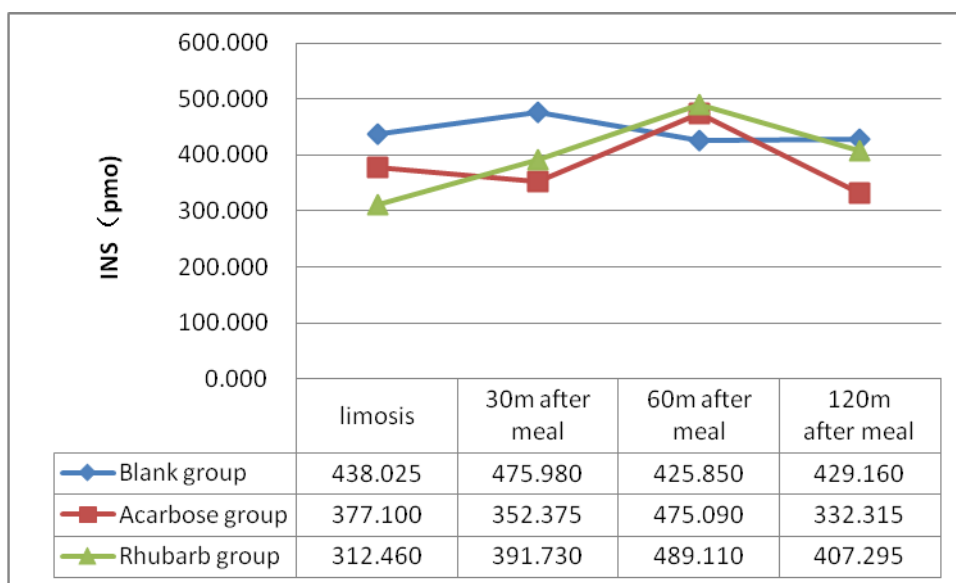
Fig.1:   The comparison of the levels of insulin among the three groups.

Figure 2 indicated that feeding did not have a big influence on the C-peptide levels of the three groups and there was also no obvious secretion peaks. So, there were no significant differences when we compared the pairs among the three groups (Fig. 2).
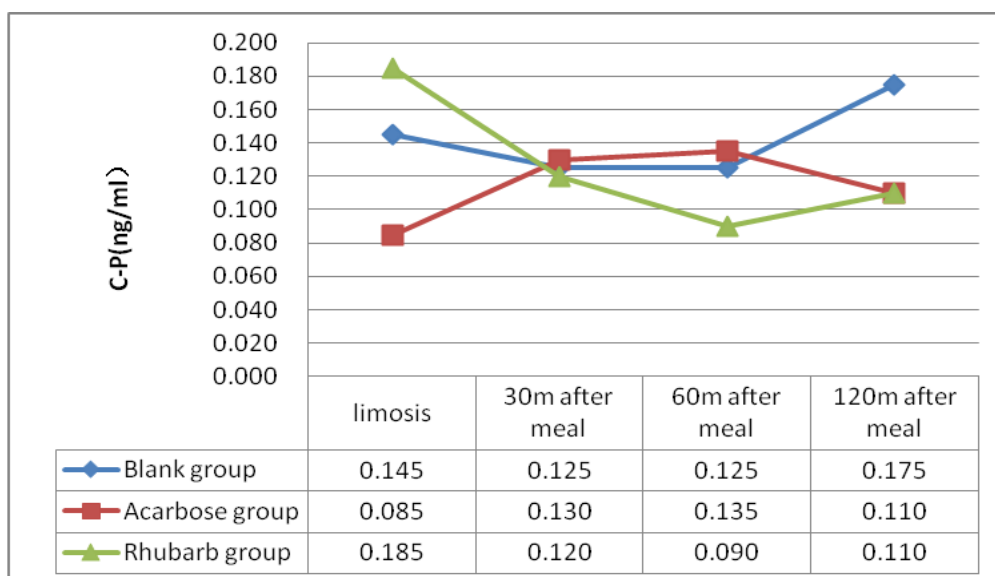

Fig. 2: Comparing C-peptide levels among the three groups.

Tamhane's T2 was used for the multiple comparisons of the three groups. Comparing groups A and B, Band C, and A and C on the fasting blood glucose levels of the same four rats for 120 minutes after meals from Day 1 to Day 6, the results were not statistically significant ($p < 0.05$). So, there were no significant differences in the rats' fasting blood glucose level (Table 1).

Table 1: Comparing fasting blood glucose levels among the three groups every day(x ± s, mmol/L)

Groups	D1	D2	D3	D4	D5	D6	D7
Blank Group（A）	20.90±1.894	20.16±2.672	22.25±2.178	24.01±1.756	21.89±1.067	23.31±2.515	22.46±2.743
Acarbose group（B）	22.36±4.043	21.53±1.291	22.71±2.745	21.98±3.972	21.66±1.909	24.49±5.338	24.13±3.244
Rhubarb extraction（C）	22.68±3.975	23.74±3.740	22.93±3.217	23.26±2.684	21.08±2.823	19.58±4.463	22.71±3.944

Tamhane's T2 was used to put up multiple comparisons. Comparing groups A and B, B and C, and A and C on the blood glucose levels of the same four rats at 120 minutes after meals from Day 1 to Day 6, the result showed that their mean scores are very close. So, there were only minor differences in their blood glucose levels.

Table 2: Comparing the blood glucose levels of the same four rats in the 120 minutes after meals from Day 1 to Day 6 among the three groups(x ± s，mmol/L)

Groups	D1	D2	D3	D4	D5	D6
Blank Group（A）	26.48±2.998	26.95±3.133	28.03±1.580	25.93±2.084	25.73±1.389	26.67±3.077
Acarbose group（B）	24.78±1.987	26.28±4.924	26.13±3.313	26.80±3.795	27.05±4.015	24.98±1.528
Rhubarb extraction（3）	27.78±2.142	22.45±1.323	24.03±5.044	29.93±1.135	28.68±3.785	26.60±4.280

Through the vertical comparison, the enzyme activities on the empty stomach of group A was lower than the enzyme activities on the empty stomach of groups B and group C, because we thought that when enzymatic activities were inhibited in the process of gavage during the seven days, they would produce negative feedback effects. Through the lateral comparisons, the enzyme activities of group A tended to be higher than the enzyme activities of groups B and C. The enzyme activities of groups B and C tended to be obviously lower. Comparing the three groups, in the 120 minutes after the meal, the enzyme activities of groups B and C tended to be clearly lower than the enzyme activities of group A. So, there was no significant difference

DISCUSSION

Normal saline (group A), acarbose group (group B), rhubarb extraction group (group C) were used to continuously treat the Type 1 diabetic rats with gavage after seven days. Measuring the insulin and C-peptide on the seventh day and daily fasting blood-glucose (FBG), there was no significant difference in the blood glucose levels of the same four rats 120 minutes after meals from day one to day six among the three groups. The FBG of the three groups of rats were all ≥ 11.0 mmol/L. Insulin and C-peptide did not have secretion peaks because of the feeding. The Type 1 diabetic rats were induced successfully. Impaired insulin function and insufficient insulin secretion were the main symptoms of the Type 1 diabetic rats. α-glucosidase activities inhibitor did not have a significant effectiveness in the treatment of the fasting and postprandial blood glucose.

The inhibitory effects of acarbose group (group B) and rhubarb extract group (group C) in the treatment of α-glucosidase activities of the Type 1 diabetic rats were better than in the normal saline control, group A. There was no significant difference between the inhibitory effects on the acarbose group B and rhubarb extract group C in the treatment of α-glucosidase activities in the Type 1 diabetic rats.

CONCLUSIONS

Diabetes mellitus is a disease characterized by polydipsia, polyphagia, polyuria and weight loss. The main tests involve measuring the blood glucose level and, examining the glycosylated haemoglobin. But the complications of diabetes, such as peripheral neuropathy, diabetic foot, diabetic nephropathy (DN) and *etc*, all happened on the basis of poor glucose

control which would seriously affect the psychology and physiology of the patients (8, 9). So, it is very important to seek effective and safe solutions and medicine to reduce blood glucose.

Research shows that inhibitors' inhibitory effects on related diseases were effectively controlling the doses of the inhibitors to improve the selectivity and reversibly decrease enzyme activities (10, 11), and at the same time not thoroughly inactivating the enzymes and decrease the other adverse reactions which can affect treatment. To find an effective α-glucosidase inhibitor from Chinese traditional medicine has great realistic and theoretical significance in the treatment of diabetes and in elucidating the hypoglycaemic mechanism.

Rhubarb is a kind of traditional Chinese medicine. In the prescription drugs, rhubarb can be used as the medicine which could remove stagnancy, eliminate dampness and heat, discharge fire, cool blood, remove stasis and detoxify. Based on making the full use and development of Chinese medicine resources, our experiment used rhubarb extracts as the raw

material to study α-glucosidase activities' inhibitory effects *in vivo* in Type 1 diabetic rats' small intestines. The results were as follows. (a) Rhubarb extract and acarbose were not effective in the improvement of Type 1 diabetes insulin, C-peptide, fasting and postprandial blood glucose in the two hours after meals. We thought the reason was related to the absolute deficit of Type 1 diabetes' insulin. From the aspect of treatment, we gave priority to insulin therapy. Rhubarb extract group and acarbose group can obviously inhibit α-glucosidase activities and there was no difference between the two groups. The function and mechanism of rhubarb extract in the treatment of diabetes are just like those of α-glucosidase inhibitor.

α-glucosidase inhibitor is a kind of relatively mature medicine which could be used to treat diabetes by deferring carbohydrate's absorption in the intestine and has been used wildly in clinics. The mode of action of α-glucosidase is in the endothelial brush border of the small

intestine (12) and its functions are to protect the intestinal tract, break down and absorb amylodextrin, polysaccharide, sucrose and maltose, and decompose other oligosaccharides into dextroglucose, galactose and D-fructose. α-glucosidase inhibitor can delay the changes of polysaccharide and disaccharides into simple sugars and relive postprandial blood sugar's rising by passing reversible inhibitor brush border α-glucosidase which includes: amylase, maltase, sucrose, and isomaltase.

Acarbose is directly extracted and separated from the secondary metabolite of actinoplanes. Voglibose and miglitol are produced, respectively, from the structural modification of actinomycete and bacillus's secondary metabolite (13). Research shows (14) that the root cause of α-glucosidase inhibitors in the human intestine is that α-glucosidase activities probably change the gut bacteria communities, though it has not been fully elaborated. We will study and elaborate on rhubarb extract's inhibitory effects and mechanisms about α-glucosidase in our next experiment.

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