Effects of pine bark procyanidins extract on blood glucose, blood lipid and antioxidation in diabetic mice

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Abstract. Objective: To study the effects of pine bark procyanidins extract on blood glucose, blood lipid and antioxidation in diabetic mice. Methods: Diabetic mice model was made by intraperitoneal injection of Streptozotocin (STZ). Diabetic mouse were randomly divided into model group, Metformin group (250mg/kg), pine bark procyanidins extract high-dose group (400mg/kg), middle-dose group (200mg/kg) and low-dose group (100mg/kg). Fasting blood glucose was measured after continuous intragastric administration 8 weeks. Blood samples were taken out from the tail of diabetic mice to measure fasting blood glucose;Then blood samples were taken out from the eyeball to measure the serum activity of superoxide dismutase (SOD), INS content, MDA content and blood lipid level. Results: Pine bark procyanidins extract high and middle-dose group can significantly lower the blood glucose level in STZ induced diabetic mice; Serum INS content in pine bark procyanidins extract high-dose group was higher than the model group significantly ($p<0.05$); Serum SOD content in pine bark procyanidins extract high and middle-dose group was higher than the model group significantly ($p<0.05$); Serum MDA content in pine bark procyanidins extract high-dose group was lower than the model group significantly ($p<0.05$); TG、TC in pine bark procyanidins extract middle-dose group and LDL-C content in pine bark procyanidins extract high-dose group were lower than the model group significantly ($p<0.05$); HDL-C content in pine bark procyanidins extract high-dose group was higher than the model group significantly ($p<0.05$). Conclusion: The pine bark procyanidins extract had a significant effects on reducing blood glucose, antioxidation and improving blood lipid level in STZ induced diabetic mice.

Introduction

Pine bark procyanidins extract is a large class of polyphenol compounds extracted from pine bark. Research shows that it has wide biological activity, including oxidation resistance, antineoplastic effect, antisepsis and blood lipid reduction etc. [1,2] However, its function for reducing blood glucose is not reported. To further develop and utilize the pharmaceutical value of pine bark extract fully, this paper primarily explores the function of pine bark procyanidins extract for reducing blood glucose.

Oligomeric procyanidins (OPC) is a powerful antioxidant with multiple pharmacological effects such as removal of free radical and immunoregulation [3,4].Diabetic model induced with STZ intraperitoneal injection method can simulate the pathogenetic process of diabetes effectively and is applicable to study on pathogenesis of organ-specific autoimmune disease with progressive injury of islet β cell and inadequate insulin secretion [6,7].

Material and method

Material

Experimental animal. Kunming mice: male, weight (25 ± 2)g, clean grade, provided by Experimental Animal Center of Southwest Medical University, production license number SCXK (Chuan) 2013-17.

Main reagents and drugs. Pine bark procyanidins extract, Shaanxi Huike Botanical Development Co., Ltd. (batch number: 20150915); melbine (batch number: AAG2883), Sino-US Shanghai Bristol-Myers Squibb Pharmaceuticals Co., Ltd.; streptozotocin (STZ) (batch number: 617D031), sigma company; sodium citrate; mice insulin (INS) kit, mice malondialdehyde (MAD) kit, mice superoxide dismutase (SOD) kit, Shanghai Lanpai Science and Technology Co., Ltd.

Key instrument. Glucometer, with blood glucose test strips, Acon Biotechnology Co., Ltd.; electronic scales; fully automatic biochemical analyzer.

Method

1. Preparation of diabetic mice model and grouping: 100 healthy mouse were fed with adaptive normal diet for 1 week. 10 mouse were randomly selected as animals in normal control group and were fed with normal diet again. The other 90 mouse were fed with high-fat diet for 4 weeks and subject to intraperitoneal injection with STZ solution of a concentration of 12mg/ml prepared with 0.1mol citric acid – sodium citrate buffered solution as per 90mg/kg after fasting without water deprivation for 12h.(Preparation of 0.1mol citric acid – sodium citrate buffered solution: prepare solution A by weighing 2.1g citric acid precisely and adding double distilled water 100mL, prepare solution B by weighing 2.4g trisodium citrate precisely and adding double distilled water 100mL, mix solution A and solution B as per 1:1, adjust pH value into 4.4 and filtrate after mixing to obtain filter liquor).After one-week free diet of mouse mentioned above, their fasting blood glucose was tested with blood drawn by tail cutting. Mouse with blood glucose value more than 11.1mmol/L were selected as diabetic mouse [4].

50 diabetic mouse with successful modeling were randomly divided into 5 groups – model group, melbine group, high dose group of procyanidine, middle dose group of procyanidine and low dose group of procyanidine, with 10 mouse in each group.

2. Administration dose and method. Mouse were subject to intragastric (ig) administration for once per day for 8 consecutive weeks from the seventh day of modeling. Normal control group and model control group were subject to ig administration of distilled water as per 10mL/kg weight. Melbine and procyanidine were prepared into appropriate concentration with distilled water. Melbine group was subject to ig administration as per 250mg/kg weight and high, middle and low dose groups of procyanidine were respectively dosed as per 400mg/kg, 200mg/kg and 100mg/kg weight. They were weighed every other week and administration dosage was adjusted according to weight.

3. Observation index and method. The change of physical characteristics of mouse was observed every day during the experiment and mortality rate was recorded. The change of their weight was recorded every two weeks. Blood was drawn by tail cutting of mouse at days 0, 28 and 56 of administration and fasting blood glucose value of mouse was tested with glucometer. After the end of administration, blood was drawn by eyeball extraction of mouse; serum was centrifugalized; contents of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in serum were measured with fully automatic biochemical analyzer; contents of serum insulin (INS) and malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) were measured with kit method.

4. Quantitative index of statistical treatment. Database was established with Excel and data analysis was conducted with SPSS17.0 statistical analysis software. Quantitative data were expressed with $\bar{x}\pm s$. One-way ANOVO variance analysis was conducted on data among groups and t test was conducted for comparison among groups. $P<0.05$ indicates statistical significance.

Result

General condition. During the experiment, mouse in normal control group had a good mental state and normal diet, drinking, feces, pee and activities. Diabetic mouse with successful modeling were dispirited with rough fur and activity reduction and had obvious symptoms of polydipsia, polyphagia, diuresis and weight reduction. After intragastric administration, mouse in melbine group and procyanidine group had symptomatic relief and their general conditions were improved.

Influence of pine bark procyanidine on weight of STZ diabetic mouse

As shown in fig.1, after continuous administration for 2 weeks, diabetic mouse had obvious weight reduction. After continuous administration for 4 weeks, mouse in melbine group and procyanidine group had slow weight reduction and those in model group still had obvious weight reduction. After continuous administration for 6 weeks, mouse in melbine group, high dose group of procyanidine and middle dose group of procyanidine had weight increase and mouse in low dose group had slow weight reduction.

After continuous administration for 8 weeks, mouse in each dose group of pine bark procyanidine had weight increase and the weight of mouse in high and middle dose groups of pine bark procyanidine and that of mouse in model group had significant differences ($P < 0.05$).The result shows that pine bark procyanidine can effectively alleviate emaciation symptom of diabetic mouse.
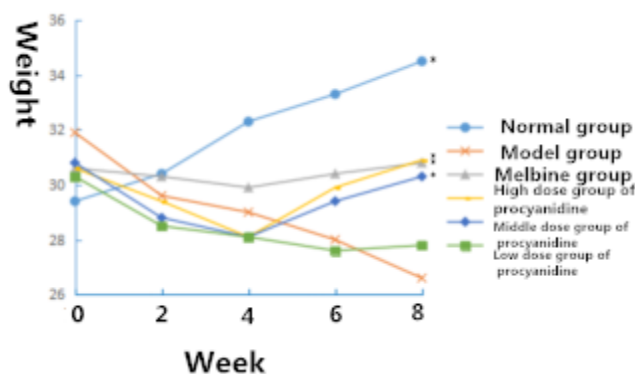

Fig.1. Effect of pine bark procyanidins extract on weight of STZ diabetic mice（x ± s,n=8）

* Compared with model group P＜0.05

Influence of pine bark procyanidine on fasting blood glucose of experimental diabetic mouse

Table 1. Effect of pine bark procyanidins extract on fasting blood glucose in STZ induced diabetic mice（x ± s,n=8）

Group	Dose (mg/kg)	Blood glucose (mmol/L)		
		Day 0	Day 28	Day 56
Normal	–	5.27±0.44	5.35±0.42	5.08±0.30△
Model	–	24.07±5.75	23.40±5.64	24.56±5.87▲
Melbine	250	20.8±5.82	19.49±5.49	17.50±5.47△▲
High dose of procyanidine	400	20.86±5.64	20.46±6.03	14.73±4.15△▲
Middle dose of procyanidine	200	20.90±5.50	20.15±5.15	18.54±4.58△▲
Low dose of procyanidine	100	19.99±5.13	21.21±4.27	20.66±3.69▲

△Compared to model group, P＜0.05; ▲Compared to normal group, P＜0.05.

As shown in table 1, compared to normal group, fasting blood glucose value of mouse in model group increased significantly (P<0.05), indicating successful modeling of diabetic mouse. After continuous administration for 28 days, fasting blood glucose of mouse in melbine group, high dose group of pine bark procyanidine and middle dose group of pine bark procyanidine decreased, which had no significance compared to model group (P>0.05).After administration for 56 days, fasting blood glucose of mouse in melbine group, high dose group of pine bark procyanidine and middle dose group of pine bark procyanidine decreased obviously, which had significant differences compared to model group (P<0.05); fasting blood glucose in low dose group did not reduce obviously and had no significance (P>0.05).High dose group of pine bark procyanidine had better effect of reduction of fasting blood glucose than melbine control group. The result shows that high-dose pine bark procyanidine has significant function of blood glucose reduction and such effect is relevant to drug dose.

Influence of pine bark procyanidine on INS of experimental diabetic mouse

As shown in fig.2, compared to normal control group, the content of serum insulin of mouse in model control group decreased significantly (P<0.01);

INS content of mouse in melbine group and high dose group of pine bark procyanidine increased significantly compared to model group (P<0.01); INS content of mouse in middle dose group of pine bark procyanidine increased obviously compared to model group (P<0.05); INS content of mouse in low dose group of pine bark procyanidine increased compared to model group but had no significant differences. The result shows that blood glucose reduction function of pine bark procyanidine is relevant to the increase of serum insulin content.
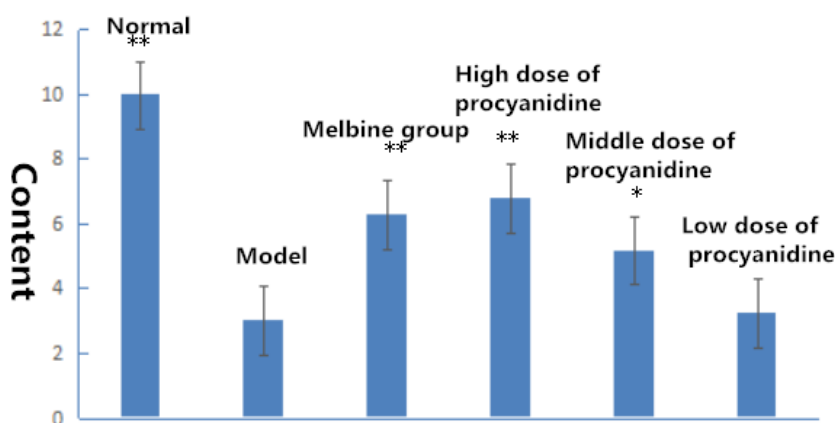

Fig.2. Effect of pine bark procyanidins extract on INS in STZ induced diabetic mice（x ± s,n=8）

*Compared to model group, P<0.05; **Compared to normal group, P<0.01.

Influence of pine bark procyanidine on blood lipid of experimental diabetic mouse

For the result, see table 2. High dose group and middle dose group of pine bark procyanidine can obviously reduce TG and TC level of diabetic mouse (P<0.05) but HDL-C and LDL-C had no significant differences compared to model group (P > 0.05).Low dose group of pine bark procyanidine and model group had no significant differences (P>0.05).

Table 2. Effect of pine bark procyanidins extract on blood lipid in STZ induced diabetic mice（x ± s,n=8）

Group	Dose (mg/kg)	TG （mmol/L）	TC （mmol/L）	HDL-C （mmol/L）	LDL-C （mmol/L）
Normal	–	1.17±0.26△	2.64±0.32△	3.05±0.45△	0.84±0.10△
Model	–	2.32±0.18▲	4.29±0.34▲	2.23±0.34▲	1.22±0.21▲
Melbine	250	1.36±0.13△	3.63±0.55△▲	3.02±0.31△	0.87±0.13△
High dose of procyanidine	400	1.22±0.28△	3.19±0.90△▲	2.64±58▲	0.95±0.33
Middle dose of procyanidine	200	1.73±0.42△▲	3.02±0.56△	2.16±0.56▲	1.13±0.51
Low dose of procyanidine	100	1.99±0.31▲	3.37±0.32▲	2.01±0.39▲	1.24±0.57▲

△Compared to model group, P＜0.05; ▲Compared to normal group, P＜0.05.

Influence of pine bark procyanidine on SOD and MDA of experimental diabetic mouse

As shown in table 3, compared to normal group, SOD activity of mouse in model group decreased significantly (P＜0.05) and its MDA content increased significantly (P＜0.05), indicating that mouse in model group have lower anti-oxidation ability than mouse in normal group.After administration, SOD activity of mouse in melbine group, high dose group and middle dose group of pine bark procyanidine increased significantly compared to model group (P<0.05) and MDA content decreased significantly (P<0.05). High dose group and middle dose group had equivalent effect of reducing MDA content and low dose group and model group had no significant differences (P＞0.05).The result shows that pine bark procyanidine of high and middle dose can improve antioxidation ability of diabetic mouse.

Table 3. Effects of pine bark procyanidins extract on SOD、MDA in STZ induced diabetic mice（x ± s,n=8）

Group	Dose (mg/kg)	SOD （μ/ml）	MDA （ng/ml）
Normal	–	1.90±0.33△	11.53±4.73△
Model	–	1.07±0.27▲	20.47±4.08▲
Melbine	250	2.00±0.64△	12.40±1.08△
High dose of procyanidine	400	1.78±0.40△	16.60±3.49△▲
Middle dose of procyanidine	200	1.46±0.16△▲	16.35±3.72△▲
Low dose of procyanidine	100	1.33±0.22▲	20.14±4.54▲

△Compared to model group, P＜0.05; ▲Compared to normal group, P＜0.05.

Discussion

Intraperitoneal injection of STZ is a common method for establishing diabetic model. In this experiment, compared to normal group, blood glucose value of diabetic mouse increased significantly and their serum INS reduced. Mouse had obvious symptoms of polydipsia, polyphagia, diuresis and weight reduction. The result shows successful modeling of diabetic mouse.

In the pathogenetic process of STZ-induced diabetic model, oxygen radical damages islet cells selectively, thus making insulin synthesis and secretion reduce and blood glucose increase gradually and finally forming typical diabetic mouse. Continuous hyperglycemia, its concomitant hyperlipidemia and abnormal lipids metabolism are main pathological basis of chronic vascular

complication of diabetes. Therefore, controlling blood glucose and reducing blood glucose level has important clinical significance for the occurrence and development of diabetic complication. This study shows that pine bark procyanidins extract can greatly reduce blood glucose of diabetic mouse and intervene in the occurrence and development of diabetes. High and middle doses have significant effect. The function of pine bark procyanidins extract might be relevant to its strong antioxidant activity: it can resist the function of β cytotoxicity caused by oxygen radical effectively, repair oxidative damage of pancreatic tissue and adjust immunity of the organism. We will further discuss relevant mechanism.

The experimental result shows pine bark procyanidine can improve general symptoms of diabetes of mouse and alleviate weight reduction of diabetic mouse. After administration for 4 weeks, fasting blood glucose of mouse in high and middle dose groups of pine bark procyanidine reduced but had no significance compared to model group. After administration for 8 weeks, high and middle doses of pine bark procyanidine obviously reduced blood glucose of diabetic mouse. This result shows that pine bark procyanidine might play the role of blood gluecose reduction after administration for 4 weeks.

Anti-diabetic medicines used clinically mainly improve patients' symptoms and alleviate pathological changes of the organism by reducing blood glucose. However, they often have side effects such as hepatorenal function damage and gastrointestinal reaction. Comparatively speaking, pine bark procyanidins extract is completely extracted from natural plant and characterized by high efficiency, low toxicity and high bioavailability. It is expected to become a new safe medicine for diabetes prevention and clinical treatment.

Acknowledgement

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