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Anti-diabetic effects of shubat in type 2 diabetic rats induced by combination of high-glucose-fat diet and low-dose streptozotocin

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ABSTRACT

Ethnopharmacological relevance: Shubat, probiotic fermented camel milk, has been used both as a drink with ethnic flavor and a medicine among Kazakh population for diabetic patients. Kazakh people have lower diabetic prevalence and impaired fasting glucose (IFG) than do other ethnic groups living in Xinjiang China, which might be related to the beneficial properties of shubat. We therefore prepared shubat in laboratory and tested anti-diabetic activity and evaluated its possible hypolipidemic and renoprotective effects in type 2 diabetic rats.

Materials and methods: Type 2 diabetic rats were induced by an administration of high-glucose-fat diet for 6 weeks and an intraperitoneal injection of streptozotocin (STZ, 30 mg/kg). Diabetic rats were divided randomly into four groups and treated for 28 days with sitagliptin (30 mg/kg) or shubat (6.97×10^6 lactic acid bacteria + 2.20×10^4 yeasts) CFU/mL, (6.97×10^7 lactic acid bacteria + 2.20×10^5 yeasts) CFU/mL and (6.97×10^8 lactic acid bacteria + 2.20×10^6 yeasts) CFU/mL. In addition, a normal control group and a diabetic control group were used for comparison. All drugs were given orally once daily 10 mL/kg for 4 weeks. Fasting blood glucose (FBG) and body weight (BW) were measured before treatment and every week thereafter. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), serum creatinine (SCr), blood urea nitrogen (BUN), C-peptide, glycated hemoglobin (HbAlc), glucagon-like peptide-1 (GLP-1) levels and pancreas tissue sections were tested after 4 weeks.

Results: Shubat demonstrated positive hypoglycemic activity on FBG, HbAlc, C-peptide and GLP-1 levels, high dose shubat decreased FBG ($P < 0.01$) and HbAlc ($P < 0.05$), increased C-peptide ($P < 0.05$) and GLP-1 ($P < 0.01$), decreased serum TC, TG, LDL-c ($P < 0.05$), increased HDL-c ($P < 0.01$), and improved the reduction of body weight as well as decreased SCr and BUN levels ($P < 0.01$) compared to diabetic controls. Histological analysis showed shubat protected the function of islets of type 2 diabetic rats.

Conclusion: The results of this study indicate that shubat has significant hypoglycemic potential in T2D rats and may modulate lipid metabolism and protect renal function in the type 2 diabetic condition, which might be related to various probiotics acting through promoting the release of GLP-1 and improving the function of β-cells.

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1. Introduction

The global prevalence of diabetes is currently about 171 million and predicted to reach 366 million by the end of 2030, according to the World Health Organization (WHO) report (Shaw et al., 2010; Wild et al., 2004). The estimated prevalence of diabetes in Chinese adults was 11.6% and the prevalence of prediabetes was 50.1% (Xu et al., 2013). Diabetes mellitus (DM) is the most common chronic disease characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. Type 2 diabetes mellitus (T2DM) is a complicated metabolic disorder and associated with long-term damage, dysfunction, and failure of different organs, and thus it is currently one of the most burdensome chronic diseases in the world (American Diabetes Association, 2011; Constantino et al., 2013). Therefore, the effective control of blood glucose is the key to preventing or reversing diabetic complications and improving the quality of life for T2DM patients. Recently, there has been growing evidence that functional foods and their bioactive compounds may be used as complementary treatment for T2DM (Mirmiran et al., 2014).

Abbreviations: FBG, fasting blood glucose; BW, body weight; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; HbAlc, glycated hemoglobin; GLP-1, glucagon-like peptide-1; SCr, serum creatinine; BUN, blood urea nitrogen; DPP-IV, dipeptidyl peptidase-IV; IFG, impaired fasting glucose; IGT, impaired glucose tolerance

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Shubat, probiotic fermented camel milk, has been used both as a drink and a medicine in Kazakh for a thousand years, mainly a supplementary medication for patients with diabetes, tuberculosis, gastrointestinal ulcers and chronic hepatitis (Guliziya and Xin-Hua, 2007). Kazakh populations have lower diabetic prevalence and impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) both in children and adults than other ethnic groups living in Xinjiang China (Yan et al., 2005; Wang et al., 2010; Li et al., 2012; Zhang et al., 2013; Yan et al., 2014), which might be related to the daily use of fermented products, such as yogurt, fermented mare's milk and fermented camel milk.

Our team speculated that a fermented product enriched with probiotics might be an effective strategy to improve glycemic control and reduce the prevalence of type 2 diabetic (T2D) patients. In our previous work, we identified and isolated lactic acid bacteria and yeasts from traditionally fermented camel milk by Kazakh population in Xinjiang (Latipa et al., 2014). In this study, we prepared shubat containing 10 lactic acid bacteria and four yeasts in laboratory as well as evaluated its hypoglycemic activity in T2D rat models induced by an administration high-glucose-fat diet and low-dose of streptozotocin.

2. Materials and methods

2.1. Preparation of shubat

Fresh camel milk samples were collected from healthy domestic camels in Chang-ji Xinjiang, skimmed by centrifuging at 3000*g* for 20 min, homogenized under the pressure of 1.5–1.7 Mpa and pasteurized (pasteurization temperature is 95 °C, kept for 5–10 min), then cooled to about 30 °C. Shubat was prepared by inoculating 10 lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactococcus lactis*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Lactobacillus rhamnosus*, *Lactobacillus mucosae*, *Lactobacillus par*, *Lactobacillus paracasei subsp.tolerans*, and *Lactobacillus pentosus*) and four yeasts (*Kluyveromyces marxianus*, *Pichia membranifaciens*, *Candida ethanolica*, and *Issatchenkia orientalis*) and incubating at 37 °C for 12 h (Yadav et al., 2007).

2.2. Chemicals and reagents

STZ was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium pentobarbital was purchased from Merck & Co., (Germany). Sitagliptin was purchased from Merck Sharp & Dohme (Australia) Pty Ltd., (Hangzhou, China). GLP-1 active ELISA kit was purchased from EMD Millipore Corporation (Billerica, MA, USA). Both rat C-peptide ELISA kit and rat glycated hemoglobin ELISA kit were purchased from Cusabio Biotech Co., Ltd. (Wuhan, China).

2.3. Animals and treatment

Male Wistar rats, weighing 160–200 g, specific pathogen free (SPF), were provided by the Experimental Animal Center of Xinjiang Medical University, China (Certificate number: SCXK2011-0004), and caged with free access to water. After a week of adaptive feeding, the diabetic models were developed using a high-glucose-fat diet for 6 weeks and the normal control groups were given regular diet. The high-glucose-fat diet consisted of 67% standard laboratory chow, 2.5% cholesterol, 0.5% sodium cholate, 10% lard and 15% carbohydrate, which were provided by Experimental Animal Center of Xinjiang Medical University, China. Following 6 weeks of dietary intervention, the diabetic group was injected with low dose of STZ (30 mg/kg, dissolved in 0.1 M sodium citrate buffer, pH 4.5), while the normal control group with vehicle citrate buffer in a dose volume of 1 mL/kg, both intraperitoneally (ip). Fasting blood glucose was measured three days and a week after the injection (Zhang et al., 2013). The rats with fasting blood glucose levels above 11.1 mmol/L were considered T2D. The T2D rats were fed on the high-glucose-fat diet for another a week and, then all the rats were randomly divided into 6 groups with 10 in normal group and 12 in diabetic group as below: Group 1 (normal control, NC), was treated with saline in a matched volume; Group 2 (diabetic control, DM) had diabetic rats treated with pasteurized camel milk in a matched volume; Group 3 (positive control) had diabetic rats administered with sitagliptin 30 mg/kg; Group 4, Group 5 and Group 6 were treated with shubat (6.97×10^6 lactic acid bacteria + 2.20×10^4 yeasts) CFU/mL, (6.97×10^7 lactic acid bacteria + 2.20×10^5 yeasts) CFU/mL and (6.97×10^8 lactic acid bacteria + 2.20×10^6 yeasts) CFU/mL, respectively (low, medium and high doses). All drugs were given orally once daily 10 mL/kg for 4 weeks. All animals received care in compliance with the Chinese Convention on Animal Care, and the study was approved by the Institutional Ethics Committee of Xinjiang Medical University.

2.4. Measurement of fasting blood glucose and body weight

Fasting blood glucose (FBG) and body weight were monitored before treatment once a week throughout the experimental period. FBG was measured after 12 h fasting by one-touch glucose auto analyzer (ACCU-CHEK Advantage II Test Strips; Roche Diagnostics, Mannheim, Germany) between 8:30 am and 9:30 am from the tail vein. The second drop of blood was used for testing after cleaning the tail vein by ethyl alcohol cotton swab and removing the first drop of blood.

2.5. Collection of blood and tissue samples

At the end of experiments, all rats were fasted for 12 h, weighed and then animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (Merck & Co.). The animals were continually monitored until total loss of consciousness was reached, as indicated by a total lack of response after a foot pinch. Blood samples were collected from the abdominal aorta, allowed to clot on ice and subsequently subjected to centrifugation (3500 rpm at 4 °C for 10 min), where after serum and plasma aliquots were stored at −80 °C for further analysis. Glycated hemoglobin, C-peptide and GLP-1 were tested with ELISA kits according to the manufacturer's recommendations. The serum TC, TG, LDL-c, HDL-c, Cr and BUN levels were examined by an automatic biochemical analyzer (BS-120, Shenzhen Mindray High-Tech Co., Ltd. China). The kidneys were dissected, rinsed with cold isotonic saline and then weighed. Kidney hypertrophy index was estimated by comparing the wet weight of the kidney to the body weight. (KI – kidney weight/body weight). Histopathological analyses were performed by way of optical microscopy on paraffin material of the pancreas. Pancreas tissue sections were fixed in 10% buffered formalin and immediately histological preparations were made. An amount of 5 μm thick sections were cut and stained with haematoxylene and eosin (HE) for histopathological examination (Luna, 1960; Xu et al., 2014).

2.6. Statistical analysis

All the data were expressed as mean ± S.E.M. Significant difference between control and experimental groups were assessed by Student's *t*-test. A probability level of less than 0.05 was considered significant.

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