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Anti-diabetic effect and mechanism of *Kursi Wufarikun Ziyabit* in L6 rat skeletal muscle cells

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ABSTRACT

Kursi Wufarikun Ziyabit (KWZ) is a traditional prescription that used in folk tea drinking for its health care effect in treatment of type 2 diabetes mellitus (T2DM) in central Asia. However, the underlying mechanism of KWZ in T2DM has not been investigated extensively. This study designed to observe the effect of KWZ on glucose consumption and assess the molecular mechanism on associated proteins in insulin signaling and ER stress pathway in L6 rat skeletal muscle cells. The results showed that, KWZ exhibited proteins of PTP-1B and α-glycosidase inhibitory activity in vitro. No cytotoxicity of KWZ was found on L6 cell line. The best effect of glucose consumption of cells was shown at 6.25 μg/mL after KWZ treatment for 12 h. Expression of PTP-1B protein was inhibited by KWZ in L6 moytubes. PI3K-dependent Akt phosphorylation was found to be activated by KWZ. Moreover, the insulin-mediated induction of IRS-1 and GSK-3 were also activated by KWZ. Western blot results indicated that KWZ significantly improved the levels of ER stress proteins, which reduced the expression of GRP78, enhanced the expression of the PERK, eIF2α and XBP1s. The activation of PERK/eIF2α was likely consequence of GRP78 inhibition, and this might be beneficial for improving the stability of ER and alleviating insulin resistance. These results suggest that KWZ might be serving as the potential drug for the prevention and treatment of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disease, this metabolic disorder characterized by the impairment of insulin secretion from pancreatic beta cells, hyperinsulinemia, glucose intolerance and insulin resistance in peripheral tissues, liver, skeletal muscle, and adipose tissue.[1–3] According to the World Health Organization (WHO), there were 350 million people suffering from diabetes around the world and this may double by 2030.[4] Insulin plays an important role in the regulation of plasma glucose homeostasis, and promotes glucose consumption, glycogenesis and protein synthesis in skeletal muscle tissue through the tyrosine kinase receptor pathway.[5] Impairment of the glucose transport in T2DM skeletal muscle induces insulin resistance and it can lead to many serious complications such as hyperglycemia and hyperlipidemia. The exact causes of these complications remain unknown, but environmental factors and diet may be its primary triggers.[6] Therefore, the prevention and treatment insulin resistance in skeletal muscle by using activity natural compounds and extractions are becoming more and more important in the field of antidiabetic clinical research.

As one of the metabolic diseases, T2DM is an insulin resistant condition in which insulin cannot successfully activate the signaling pathway responsible for the stimulation of glucose metabolism. Protein tyrosine phosphatase-1B (PTP-1B) is a key enzyme, which is a negative regulator of insulin signaling, play a role in the induction of insulin resistance as well as inhibits insulin-stimulated tyrosine phosphorylation of insulin receptor (IR) and

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insulin receptor substrate-1 (IRS-1).[7] IR phosphorylates tyrosine residues of IRS-1, which leads to subsequent activation of PI3K pathway.[8] Akt is a key protein involved in PI3K pathway, its phosphorylation leads to activation of signaling cascades involving PI3K/Akt/GSK-3, which promotes glycogen synthesis and glucose utilization for the metabolic activities in skeletal muscle.[9,10]

Endoplasmic reticulum (ER) is the main site of calcium storage, folding and maturation of secreted and transmembrane proteins.[11] The loss of ER homeostasis, such as expression of mutant unfolded or misfolded proteins, insufficient ER chaperone levels, calcium content, changes of ATP status, and cholesterol accumulation leading to ER stress response and induces the diseases such as obesity, diabetes, atherosclerosis, and cancer.[12,13] However, ER stress response can restore the irregularity of physiological conditions through the activation of PERK (PKR-like ER kinase) -eIF2α (eukaryotic translation initiation factor 2) -ATF4 (activating transcription factor 4) pathway, IRE1 (inositol-requiring 1) -XBP1 (X-box binding protein 1) pathway and ATF6 (activating transcription factor 6) pathway.[14–17] The activation of IRE1/XBP-1, PERK/eIF2α and ATF6 signaling pathways improve the function of insulin β cell through promotes normal function of ER, such as improve the insulin sensitivity, reduced the fasting blood glucose by reducing the gluconeogenesis.[18,19]

In recent years, herbal medicines are considered as the primary sources of new drug research and development, especially for hypoglycemic agents. The studies on the role of natural products in diabetes have been reported more and more, some natural plant extracts had been shown to reduce the blood glucose, blood lipid levels and other biomarkers in type 2 diabetes patients, which is safety for daily consumption.[20–22]

Kursi Wufarikun Ziyabit was made of *Geranium collinum* Steph.ex Willd and *Hypericum Scabrum* Linn, as a classic traditional medicine used for the prevention and treatment of diabetes in China, Tajikistan and Mongolia, it was mentioned in Avicenna's "Canon of Medicine".[23] *G.collinum*, recorded in "Chinese pharmacopoeia" (China Pharmacopoeia Committee, 2015), has a good role in treating rheumatism, gout, dysentery, external and internal bleeding, eczema, scabies, tenosynovitis, pruritus, treatment of skin wounds, and many research had proved these effects,[24–26] especially for its excellent activities on hypoglycemic and antioxidant.[27–29]

Hypericum species is recorded in the "kazakh medicine" and widely used in folk and modern medicine.[30] Hypericum species has showed remarkable antiviral (such as inhibited HIV, influenza A, cytomegalovirus, Herpes simplex 1 and 2, Epstein–Barr virus, etc), wound healing, antioxidant, antimicrobial, antifungal, anxiolytic, anticonvulsant, antidepressant activities.[31,32] Some research had provided pharmacological activities of it such as antioxidant, antidiabetic, antimicrobial etc.[33]

In our previous study, the effective part of *Kursi Wufarikun Ziyabit* was named as KWZ, the best proportion and extraction condition were optimized, and the main chemical components of it were analyzed.[34] In this study, in order to present the traditional application value of herbal medicine in the prevention and treatment of diabetes, the effect and underlying pharmacological mechanism of KWZ were investigated in L6 rat skeletal muscle cells.

2. Materials and methods

2.1. Materials and reagents

Root of G. *collinum* and aerial parts of H. *scabrum* were collected from Takob village of the Republic of Tajikistan (38.5357500 N, 68.7790500 E, and 2000 m above sea level, Tajikistan). The plants were identified by Professor Yusuf Nuraliev from Avicena's Institute of Medicine and Pharmacology of the Republic of Tajikistan. Voucher specimens (Barcode: G. *collinum* WY01053, H. *Scabrum* WY01054) were deposited at the Herbarium of the Key Laboratory of Plant Resources and Chemistry of Arid Zone, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences.

All of the solvents used for extract were of analytical grade (Baishi Chemical Co. Ltd., Tianjin, P. R. China), and water was double distilled. PTP-1B (human, recombinant) was expressed and purified in our laboratory, and stored in a −80 °C. P-nitrophenyl phosphate (pNPP) and α-Glucosidase, 4-N-trophenyl-a-D-glucopyranoside (pNPG) were purchased from Sigma Aldrich Co., LLC (St. Louis, Missouri, USA). L6 rat skeletal myoblasts were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Fetal bovine serum (FBS), Bovine serum albumin (BSA), antibiotics and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco1 Life Technologies (Carlsbad, CA, USA). Specific antibodies to PTP1B, GRP78 β-actin, phospho-Akt^{473}Ser, phospho-GSK-3β, phospho-AMPK, phospho-IRS-1, phospho-IER1α, phosphor-PERK, phospho-e1F2α, XBP1s, Akt, GSK-3β, AMPK, IRS-1, IER1α, PERK and e1F2α were obtained from Cell Signaling Technology (Danvers, MA, USA). Human recombinant insulin was obtained from Sigma Chemical Corp. (St. Louis, MO, USA). Electrophoresis reagents, including Bis-Tris gels, running buffer, and poly (vinylidene fluoride) (PVDF) membrane were obtained from Invitrogen (Carlsbad, CA, USA). The Berberine standard compounds were purchased from Beijing Century AokeBiology Research Co., Ltd. (Beijing, China) and the purity were higher than 98%.

2.2. Preparation of KWZ

The *Kursi Wufarikun Ziya*bit (KWZ) (root of *G. collinum* and aerial parts of *H. Scabrum* were mix with the ratio of 7:3) were prepared according to the article.[34] Extraction and purification of effective parts dried using freeze-drying (FDU-2100; Eyela, Tokyo, Japan) at −80 °C for 36 h. The dried matter was powdered, weighed, and packed in zip pack bags, stored at 4 °C for further study.

2.3. PTP-1B and α-Glycosidase assays

The PTP-1B and α-glycosidase test were conducted according to a method reported in the articles[35,36] with a slight modification. The PTP-1B and α-glucosidase test were carried out in 96 microporous plates. The total volumes of PTP-1B and α-glucosidase test were 200 μL and 100 μL, respectively. The absorbance values were measured by Spectra Max MD5 Microplate Reader (Molecular Devices, USA) at 405 nm, with the system without enzyme solution as a blank. The inhibition rate (IR) was calculated according the following formula: Q3

$$IR(\%) = \frac{OD_{405blank} - OD_{405Sample}}{OD_{405blank}} \times 100$$

2.4. Cell culture and differentiation assays

L6 myoblasts cells are cultured according to conventional cell culture. The induction differentiation is conducted according to the experimental methods reported by Tang.[5]

2.5. Establishment of insulin resistance model in L6 rat skeletal muscle cells

According to the methods of Huang[37] and Bailey,[38] the differentiated cells were inoculated in the 96-well board as 8×10^3/well, After L6 cells covered the board, the cells were pretreated with high glucose (25 mmol/L) DMEM and 100 nmol/L insulin for 24 h, and

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