Research Article

Synergistic Hypoglycemic Effect of Pumpkin Polysaccharides, Pueraria Flavonoids and Pu-erh Tea Theabrownin in Type 2 Diabetic Mice through Enhancing the Nrf2/HO-1 Signaling Pathway

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ABSTRACT

In order to investigate the hypoglycemic effects and potential mechanism of Pumpkin Polysaccharides, Pueraria Flavonoids and Pu-erh Tea Theabrownin on diabetes, type 2 diabetes mellitus (T2DM) mice were established and treated with the active components of plants. Results showed that the active components of plants treatment could result in the decrease of blood glucose and possessed the efficacy of insulin resistance alleviation in the diabetic mice. Furthermore, the levels of Nrf2/HO-1 were up-regulated by the active components of plants, which indicated the involvement of the Nrf2/HO-1 signal pathway in the hypoglycemic mechanism. Results suggested that Pumpkin Polysaccharides, Pueraria Flavonoids and Pu-erh Tea Theabrownin could ameliorate the T2DM and combined groups had much more efficient effects than its single groups which might be a promising candidate functional food or medicine for T2DM treatment.

Keywords: Type 2 diabetes; Oxidative stress; Pumpkin polysaccharides; Pueraria flavonoids; Pu-erh tea theabrownin

Introduction

Diabetic mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and mainly affects carbohydrate, lipid, and protein metabolism [1]. The global prevalence of diabetic is increasing rapidly worldwide and this is leading to dramatic increases in complications such as nephropathy, atherosclerosis, nephropathy and cardiovascular diseases. Insulin and oral hypoglycemic agents, including biguanides, sulfonylureas, and thiazolidinediones, are the main treatments for DM and are effective in controlling hyperglycemia, but those drugs also have prominent side effects, such as hypoglycemia, gastrointestinal disturbance, and weight gain. Therefore, there is an urgent need for efficacious alternatives with fewer side effects.

Traditional plant is a promising area of research in diabetes therapy throughout the world. Studies have shown that treatment
with some plant active ingredients has hypoglycemic effects and stimulates β-cell regeneration in addition to other anti-diabetic effects, and the combination of two or more kinds of ingredients usually can achieve better results through different targets [2-4].

Oxidative stress in patients with DM contributes to impairing β-cell function. There are considerable evidences supporting that elevated levels of oxidative stress can potentially impair cellular glucose metabolism via a variety of mechanisms, including redox imbalance and insulin resistance [5,6]. Numerous studies have shown that active ingredients isolated from plants exerted beneficial effects on DM and its complications through protecting against oxidative stress damage or improving insulin sensitivity [7].

Nuclear factor-E2-related factor 2 (Nrf2) has been drawn an increasing attention for their role in protecting tissue injury such as liver disorders. Nrf2 is a transcription factor that is activated by oxidative stress and electrophiles that regulates the expression of numerous detoxifying and antioxidant genes. Studies have shown that Nrf2 protects the liver from xenobiotic toxicity [7,8]. Therefore, the Nrf2-ARE pathway is currently the most important endogenous antioxidant signaling pathway. Nrf2 can induce expression of antioxidant enzymes and phase II detoxifying proteins, such as heme oxygenase-1 (HO-1) by binding to antioxidant responsive elements in the promoters of these genes [9]. Increasing evidence suggests that the elevation of Nrf2-mediated target genes, including HO-1, also promotes cell survival in oxidizing environments via enhancement of free radical metabolism, inhibition of cytokine-mediated inflammation, and recognition of damaged DNA [10].

However, the effects of pumpkin polysaccharides, pueraria flavonoids and pu-erh tea theabrownin supplementation on Nrf2 and HO-1 activation in type 2 diabetic mice are not known. In this study, it has been investigated the effects of diabetic on glucose metabolism and oxidative stress, and to further examine whether this damage could be repaired by these active components of plants. The aim of this work was to provide theoretical basis for developing hypoglycemic health foods.

**Materials and Methods**

**Materials and chemicals**

Pumpkin polysaccharides were extracted from pumpkin according to previous method in our laboratory [11]. Pu-erh theabrownin was obtained from Tianjin Tasy Co., Ltd (Tianjin, China). Pueraria flavonoids were purchased from Ankang Kangyuan Medical Technology Co., Ltd (Beijing, China). Insulin ELISA assay kit were purchased from Beijing Solarbio Technology Co., Ltd. The antibodies to Nrf2, HO-1, β-actin were purchased from Abclonal (Wuhan, China). The secondary antibodies peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated goat anti-mouse IgG were purchased from Solarbio Co., Ltd (Beijing, China). Other laboratory chemicals were obtained from local firms and were of the highest purity.

The equipments used in this study mainly included the blood glucose meter (Beijing Yicheng Biological Electronic Technology Co., Ltd, China), the microplate reader (Bio-Tek, USA), the electrophoresis apparatus (Bio-rad, USA) and the Trans-Blot Cell (Bio-Rad, USA). The biochemical analyses were carried out according to the instructions of the related reagent kits.

**Animals**

Male KM mice (n = 64) weighing 13-15g were purchased from Beijing Military Medical Science Academy Laboratory Animal Center. The animals were housed at the temperature of 22±2°C, humidity of 55±5%, and with a 12/12 h light/dark cycle throughout the experiment. The experimental procedures and protocols were approved by the local legislations for ethics of animal experiments.

**Experimental design**

Briefly, after one week of accommodation, all the mice (except 8 mice set as normal control group) were fed with HFD (high fat diet) (78.8% commercial standard pellet diet, 10% lard, 10% custard powder, 1% cholesterol and 0.2% sodium cholate) with free access to water. After four weeks, the mice were injected intraperitoneally thrice in 72 h with STZ (streptozocin) at the dosage of 35 mg/kg dissolved in normal saline (NS). NS alone was injected to normal control. Blood samples were withdrawn from the tail vein of the overnight fasted mice a week after STZ induction. Hyperglycemia was assessed by measuring fasting blood glucose (FBG) level. The mice with blood glucose higher than 11.0 mmol/L were considered diabetes and included in the study. After FBG determination, the diabetic mice were randomly divided into seven groups with six animals each as (i) DM group: mice were fed a high-fat diet; (ii) PP group: mice were orally gavaged with pumpkin polysaccharides dissolved in distilled water at the doses of 500 mg/kg b.w. per day; (iii) PT group: mice were orally gavaged with Pu-erh tea theabrownin dissolved in distilled water at the doses of 900 mg/kg b.w. per day; (iv) PF group: mice were orally gavaged with pueraria flavonoids dissolved in distilled water at the doses of 500 mg/kg b.w. per day. (v) PP+PF group: mice were orally gavaged with 250 mg/kg pumpkin polysaccharides and 200 mg/kg pueraria flavonoids dissolved in distilled water b.w. per day; (vi) PT+PF group: mice were orally gavaged with 250 mg/kg pumpkin polysaccharides and 450 mg/kg Pu-erh tea theabrownin dissolved in distilled water b.w. per day; (vii) PT+PF group: mice were orally gavaged with 450 mg/kg Pu-erh tea theabrownin and 200 mg/kg pueraria flavonoids dissolved in distilled water b.w. per day.

The test drugs were administered orally once daily using intragastric tube for 4 weeks. During the administration, the normal control was supplied with normal standard diet while other groups were supplied with HFD. All the groups were supplied free access to water.

**Oral glucose tolerance test (OGTT)**

All the mice were subjected to an oral glucose tolerance test when the second day before four weeks after administration of drugs. Briefly, mice were fasted overnight before the test and then orally administrated with glucose (2.5 g/kg b.w.) which was resolved in NS. The blood glucose value was measured via blood obtained from mice tail vein at time of 0, 30, 60, 120 min after glucose administration. The results of OGTT were expressed as AUC over 120 min.

**Biochemical analysis**

For the measurement of biochemical markers, the animals were sacrificed following animal ethical guideline. Blood samples were separated into serum through centrifugation. The sera were immediately stored at −20°C until measurement. After blood sample collection, liver and colon were removed and washed in normal saline.
(NS). In the serum, the blood insulin of all groups was determined by ELISA assay kits. Blood glucose level was determined by the blood glucose meter. The levels of FFA, ROS and MDA were analyzed to evaluate the antioxidant capacity using ELISA kits purchased from Solarbio Co., Ltd..

**Western blot analyses**

In all groups, the livers were removed from sacrificed mice. Small pieces of the samples in each group of animals were pooled together for Western blot analysis. The cell lysates from liver were prepared in lysis buffer with the ratio of 1:10 (v/v) on ice in the 5 mL tube and solubilized for 1 h at 4°C and centrifuged for 20 min at 12000 × g. The supernatants were collected and protein concentrations were measured with BCA protein assay reagent and then stored at −20°C until further analysis. The proteins of the experimental mice were separated electrophoretically on SDS-polyacrylamide gel. Subsequently, proteins were transferred on to a polyvinylidene difluoride membrane that was blocked overnight with 5% skim milk for 2 h followed by incubation with the primary antibodies anti-Nrf2 and anti-HO-1 at 4°C for overnight. After washing, the membranes were incubated with either HRP conjugated goat antimouse or goat anti rabbit secondary antibody in TBST for 2 h. The desired blots were washed with TBST five times, each 10 min, and were visualized with enhanced chemiluminescence (ECL) detection.

**Statistical analyses**

The values are expressed as the means ± standard deviation (SD) of three replicates. Statistic analysis was carried out by using one-way ANOVA. The differences in mean were calculated using the Duncan’s multiple-range tests for means with 95% confidence limit (p < 0.05).

**Results and Discussion**

**Oral glucose tolerance test**

In recent years, some researchers have already developed the model by feeding the animal with high-fat diet following low-dose STZ, which would closely mimic the metabolic characteristics of human T2DM. Oral glucose tolerance test is a simple and accurate method for detecting insulin resistance in T2DM mice. As shown in Figure 1, the normal control reached its highest value at 30 min and then gradually back to initial level. Compared with diabetic control, the active components of plants groups could efficiently suppress the value peak and the effect of combined groups is better than its single group at 30 min and the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glucose tolerance and the active components of plants treated groups had a lower AUC value than diabetic control. Among treated groups, the combined group of PT and PF showed much more potent glucose tolerance. The area under the curve (AUC) in each group could tell the potent of blood glu
compared with the diabetic control and the effect of combined groups were better than the single groups. PT+PF groups had a much more efficient effect than the single groups (p < 0.05). Insulin resistance is often accompanied by compensatory hyperinsulinemia and hyperglycemia. The results (Table 1) showed that the active components of plants treated groups had lower IR value than diabetic control. These results proved that the active components of plants could ameliorate the insulin resistance caused by T2DM and efficiently alleviate the main syndromes which would be beneficial for the active components of plants study on diabetes.

Effects on oxidative stress in type 2 diabetes mice

T2DM were in oxidative stress status and amount of ROS, FFA and MDA generated. The three kinds of plants extracts can ameliorate oxidative stress in T2DM (Table 2). The combined group was better than the single component comparing the FFA, and the difference between the PP+PF group, polysaccharides group and flavones group was significant; The combined group was better than the single group in the terms of reducing MDA, and the combination of polysaccharides and flavonoids was significantly different from the single group (P < 0.05). In the reduction of ROS content, there was no statistical difference between the combined group and the single group, but the effect was better than the single component. In general, these three active substances could improve oxidative stress levels in T2DM mice, and the combined group was better than the single group. The three active substances may reduce FFA and ROS production from the source of oxidative stress and inhibit the damage of oxygen free radicals to beta cells of the islet and protects the beta cells, then can reduce the blood glucose in diabetic mice.

Western blotting analysis of the levels of protein Nrf2 and HO-1

Recent research has identified Nrf2 as a key transcription factor for combating hepatic oxidative stress [12]. Nuclear factor erythroid 2-related factor 2 controls the antioxidant response element (ARE)-dependent gene regulation in response to oxidative stress. Nrf2 sequestered in the cytoplasm by the cytosolic repressor Kelch-like ECH-associated protein 1 (Keap 1) plays an important role in the maintenance of the cellular redox balance. Keap1 links Nrf2 to the cytoskeleton to retain Nrf2 in the cytoplasm, thereby promoting its degradation. Oxidative stress facilitates Nrf2 to escape Keap1-mediated proteasomal degradation, leading to Nrf2 stabilization, subsequent nuclear translocation, and binding to ARE [13]. From the results displayed in Figure 3(A-C), the expression of Nrf2 in diabetic control was obviously decreased than control groups (p < 0.05). After the treatment of the active components of plants, the level of Nrf2 was better than the single component comparing the FFA, and the difference between the PP+PF group, polysaccharides group and flavones group was significant; The combined group was better than the single group in the terms of reducing MDA, and the combination of polysaccharides and flavonoids was significantly different from the single group (P < 0.05). In the reduction of ROS content, there was no statistical difference between the combined group and the single group, but the effect was better than the single component. In general, these three active substances could improve oxidative stress levels in T2DM mice, and the combined group was better than the single group. The three active substances may reduce FFA and ROS production from the source of oxidative stress and inhibit the damage of oxygen free radicals to beta cells of the islet and protects the beta cells, then can reduce the blood glucose in diabetic mice.

Table 2: Determination of FFA, MDA, ROS of mice (x ±SD n=6).

<table>
<thead>
<tr>
<th>groups</th>
<th>FFA(μmol/L)</th>
<th>MDA(nmol/L)</th>
<th>ROS(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.38±0.01</td>
<td>6.07±0.19</td>
<td>510.3±1.96</td>
</tr>
<tr>
<td>DM</td>
<td>0.99±0.09</td>
<td>11.5±0.18</td>
<td>590.0±10.33</td>
</tr>
<tr>
<td>PP</td>
<td>0.54±0.02</td>
<td>7.25±0.29</td>
<td>534.5±20.69</td>
</tr>
<tr>
<td>PT</td>
<td>0.42±0.01</td>
<td>9.64±0.09</td>
<td>543.4±8.90</td>
</tr>
<tr>
<td>PF</td>
<td>0.51±0.04</td>
<td>7.11±0.23</td>
<td>531.2±6.47</td>
</tr>
<tr>
<td>PP+PF</td>
<td>0.39±0.04</td>
<td>6.71±0.23</td>
<td>528.4±5.54</td>
</tr>
<tr>
<td>PP+PT</td>
<td>0.49±0.03</td>
<td>7.14±0.02</td>
<td>536.9±1.66</td>
</tr>
<tr>
<td>PT+PF</td>
<td>0.41±0.04</td>
<td>7.09±0.18</td>
<td>530.6±0.43</td>
</tr>
</tbody>
</table>

Values are means ± standard error of the mean. Data points with different superscripts are significantly different at the level of p < 0.05 by Fisher’s multiple comparison test.

Figure 3: The effect on AUG of glucose tolerance in each group mice. Values are means ± standard error of the mean. Data points with different superscripts are significantly different at the level of p < 0.05 by Fisher’s multiple comparison test.
in diabetic mice was notably increased compared with the diabetic control \((p < 0.05)\). For the expression of Nrf2 on liver, PP+PF groups had a much more efficient effect than PP group \((p < 0.05)\), PT+PF group had a much more efficient effect than PT group \((p < 0.05)\). The increased expression of Nrf2 would be helpful for activation of the antioxidant enzymes, which are regulated by Nrf2, resulting in the decrease of blood glucose \((p < 0.05)\). After the treatment of the active components of plants, the level of Nrf2 in diabetic mice was notably increased compared with the diabetic control \((p < 0.05)\). For the expression of HO-1 on liver, PP+PF group had a much more efficient effect than PP group \((p < 0.05)\), PT+PF group had a much more efficient effect than PT group \((p < 0.05)\).

The present study indicated that the active components of plants might be a potential therapeutic agent through activating the Nrf2/HO-1 signal pathway.

**Conclusion**

The hypoglycemic activities of Pumpkin Polysaccharides, Pueraria Flavonoids and Pu-erh Tea Theabrownin on high fat diet and streptozotocin-induced insulin resistant diabetic mice and its potential mechanism through Nrf2/HO-1 signal pathway was firstly investigated in this study. Results showed that the active components of plants treatment had more potent glucose tolerance and could result in the decrease of blood glucose \((p < 0.05)\). The active components of plants also possessed the efficacy of insulin resistance alleviation and exerted cytoprotective action in T2DM mice. In addition, the active components of plants could up-regulate the levels of the critical proteins in Nrf2/HO-1 signal pathway. Results suggested that Pumpkin Polysaccharides, Pueraria Flavonoids and Pu-erh Tea Theabrownin could ameliorate the T2MD and combined groups had a much more efficient effect than its single groups \((p < 0.05)\). It might be a promising candidate for functional food for T2DM treatment. The results will be helpful to the understanding of its action in vivo and give a new direction for studies on the active components of plants related products.

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**Conflict of Interest**

None

**References**