Nutritional Appraisal and Antidiabetic Activity of a Kind of Mixed Plasma Proteolytic Peptide from *Tachpleus tridentatus*

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Abstract: Based on the bioactivities of blood protein, the nutritional appraisal and antidiabetic activity of a kind of mixed plasma proteolytic peptide from *Tachpleus tridentatus* (PPPT) were studied in this work. Results indicated that the amino acid level of PPPT could meet an adult’s FAO/WHO recommended standard; PPPT also manifested inhibitory activity on α-glucosidase with IC₅₀ at 2.62 mg/mL in vitro, and relieved symptoms of type II diabetic mouse in vivo by improving glucose tolerance, increasing insulin secretion by 5.15 mIU/L in the PPPT-4 group and by 4.54 mIU/L in the PPPT-1 group, reducing blood glucose values to nearly normal levels 120 min after intragastric administration, and restoring pathological damage in the thymus. Thus, PPPT can be developed into both a healthcare product and an adjuvant for type II diabetes in the future.

Keywords: *Tachpleus tridentatus*; plasma; peptide; nutritional appraisal; diabetes

1. Introduction

Diabetes is associated with chronic hyperglycemia, inducing hypertension, coronary heart disease, renal failure, disability, and premature death, bringing great pain to patients and their families [1–5]. According to the 10th edition of the International Diabetes Federation (IDF) diabetes overview published in December 2021, 537 million adults in the world are living with diabetes, and one person dies of diabetes every 5 s. China is currently a country with the largest number of diabetes patients and a total population of 140 million, of which type II diabetes patients account for more than 90%; diabetes is obviously becoming one of the major health hazards [6,7]. Proteins, as an ordinary food born with the common characteristic of good biodegradability, compatibility, and biological activity, have been widely used in the food, health food, cosmetics, biomedicine, and manufacturing industries. Recent studies have continued to confirm its therapeutic effects, including antidiabetic, cholesterol-lowering, antihypertensive, anticancer, antimicrobial, and multifunctional aspects [8–12]. Proteins not only serve as nutrients but also perform physiochemical roles—it is inevitable to develop them for the purpose of diabetic therapy. *Tachpleus tridentatus* possesses an amazing variety of innate immune defense systems suitable for hematology research into an excellent biomaterial, but *Tachpleus tridentatus* was upgraded to “endangered (EN)” under the IUCN Red List in 2019, depicting its global declining trend. The blood of *Tachpleus tridentatus* is also restricted to use in China; the decarded plasma in our experiment is from Zhanjiang Bokang Marine Biology Co. Ltd., a manufacturer of *Tachypleus* amebocyte lysate with a blood sampling license, which has actively engaged in the breeding research of *Tachpleus tridentatus* along with universities and scientific research institutes due to a lack of blood resources. Besides essential amino acids such as Thr, Met, Phe, Ile, and Leu in *Tachpleus tridentatus* blood, there are 12 kinds of free amino acids, in which the sum of 5 kinds of amino acids, including Gly, Ile, Asp, Glu, and Leu, accounts for more than 75% [13]. The plasma protein in *Tachpleus tridentatus* is hemocyanin, owning significantly higher research and utilization value for its various bioactivities, which can not only play the roles of antibacterial and antivirus but can...
also inhibit the growth of tumor cells and induce the differentiation of cancer cells; its hydrolysate can be further separated and purified into various functional substances for health products, medicines, and chemical products [14–16]. In this paper, the discarded plasma after extracting amebocytes was adopted for PPPT. The nutritional value of PPPT was first appraised according to the FAO/WHO standard, and PPPT bioactivities related to hyperglycemia diseases were then tested both in vivo and in vitro in order to develop badly needed nutraceuticals and medicines that not only rationally make use of precious resources but also provide a reference for the development of the discarded plasma from Tachpleus tridentatus.

2. Materials and Methods

2.1. Preparation of PPPT

PPPT was prepared according to our authorized patent in 2020 [17]. Tachpleus tridentatus plasma was immersed in constant temperature water at 90 °C for 3 h, and then Tachpleus tridentatus plasma was filtered by suction. The filter residue was washed with distilled water three times, then hydrolyzed with trypsin and centrifuged to obtain a supernatant. Finally, the supernatant was freeze-dried to obtain PPPT. PPPT molecular weight range is 0.5–3.4 KDa, mainly 2.0 KDa.

2.2. Amino Acid Composition Analysis

The amino acid composition of PPPT was determined by S-433D sykam amino acid analyzer at a wavelength of 570 nm and 440 nm according to GB/T5009.124-2003 (the abbreviation for National Standards of the People’s Republic of China is GB, and T5009.124-2003 stands for the document number of <<determination of amino acids in foods>>). The sample processing method was as follows: 1 g of sample was completely dissolved in 6 mL HCl solution (6 µmol/L) and hydrolyzed at 110 °C for 20 h [18].

2.3. Amino Acid Score (AAS)

PPPT nutritional value was evaluated according to a gram of amino acid per the grading standard model recommended by FAO/WHO in 1993; threonine, valine, methionine + cystine, phenylalanine + tyrosine, isoleucine, leucine, lysine, and tryptophan were equal to 4.0, 5.0, 3.5, 6.0, 4.0, 7.0, 5.5, and 1 g/100 g, respectively, in the model [19], and each amino acid score was calculated by Equation (1):

\[
\text{AAS} = \frac{\text{amino acid content in experimental protein}}{\text{amino acid content in FAO/WHO standard model}} \times 100
\]

2.4. Protein Efficiency Ratio (PER)

According to the equations proposed by Alsemeyer [20], PER was calculated as follows (Equations (2)–(4)):

\[
\text{PER (1)} = -0.684 + 0.456H_{\text{Leu}} - 0.047H_{\text{Pro}}
\]

\[
\text{PER (2)} = -0.468 + 0.454H_{\text{Leu}} - 0.105H_{\text{Tyr}}
\]

\[
\text{PER (3)} = -1.816 + 0.435H_{\text{Met}} + 0.780H_{\text{Leu}} + 0.211H_{\text{His}} - 0.944H_{\text{Tyr}}
\]

where \(H_{\text{Leu}}\) stands for the mass percentage of Leu in protein, analogous to others in the formula.

2.5. Determination of DPPH Free Radical Scavenging Ability

In total, 50 µL PPPT solution and 150 µL DPPH anhydrous ethanol solution (0.15 mmol/L) were added into microtiter plates and evenly mixed before being placed in the dark to react for 30 min, and the mixture absorbance was measured at a wavelength of 517 nm [21]. Regarding vitamin C (Vc) as positive control and pure water as blank control,
all results were obtained from three parallel measurements. The scavenging ratio was calculated using the following Equation (5):

\[
\text{Scavenging ratio (\%)} = \frac{C_0 - (C_1 - C_2)}{C_0} \times 100
\]

where \(C_0\) is the absorbance value of the blank control (only including pure water), \(C_1\) is that of the test samples (only including PPPT), and \(C_2\) is that of the sample control (using anhydrous ethanol instead of DPPH anhydrous ethanol solution).

2.6. Determination of Hydroxyl Radical Scavenging Capacity

Salicylic acid ethanol solution (9 mmol/L), FeSO\(_4\) solution (9 mmol/L), and H\(_2\)O\(_2\) solution (0.024%), 20 \(\mu\)L each, were added to 20 \(\mu\)L PPPT solution into microtiter plates and evenly mixed; subsequently, 120 \(\mu\)L distilled water was added, and the solution was mixed again, incubated in a thermostatic oscillator at 37 °C for 30 min, and the mixture absorbance at a wavelength of 510 nm was determined after incubation. Regarding Vc as positive control and pure water as blank control, all results were obtained from three parallel measurements [22]. The scavenging ratio was calculated using the following Equation (6):

\[
\text{Scavenging ratio (\%)} = \frac{D_0 - (D_1 - D_2)}{D_0} \times 100
\]

where \(D_0\) is the absorbance value of the blank control (pure water instead of PPPT), \(D_1\) is that of the test samples (only including PPPT), and \(D_2\) is that of the sample control (pure water instead of H\(_2\)O\(_2\) solution).

2.7. Total Antioxidant Capacity Test

A total of 20 \(\mu\)L PPPT solution and 180 \(\mu\)L FRAP working fluid was mixed into microtiter plate and incubated at 37 °C for 10 min in a thermostatic oscillator, and the mixed liquid absorbance was measured at a wavelength of 593 nm. Calculation of total antioxidant capacity of PPPT was based on the FeSO\(_4\) standard curve equation. Regarding Vc as positive control and pure water as blank control, all results were obtained from three parallel measurements [23].

2.8. Inhibitory Activity on \(\alpha\)-Glucosidase

All solutions were prepared with PBS solution (0.01 M, pH 7.0). A total of 20 \(\mu\)L PPPT solution and 30 \(\mu\)L enzymic solution was added into microtiter plate, incubated at 37 °C for 10 min in a thermostatic oscillator, then 50 \(\mu\)L pNPG (1 mmol/L) was added as substrate to react for 30 min, and, finally, 100 \(\mu\)L Na\(_2\)CO\(_3\) solution (1 mol/L) was used as stop-buffer to terminate all reactions; the absorbance was measured at a wavelength of 405 nm. Regarding acarbose as positive control, all results were obtained through three parallel measurements [24]. The inhibitory ratio was calculated using the following Equation (7):

\[
\text{Inhibitory ratio (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100
\]

where \(A_0\) stands for the absorbance value of the blank control (only including enzyme), \(A_1\) for that of the test samples (including PPPT and enzyme), and \(A_2\) for that of the sample control (only including PPPT).

2.9. Inhibitory Activity on Lipase

A 10 \(\mu\)L lipase solution (0.5 mg/mL) and a 10 \(\mu\)L PPPT solution were added into 170 \(\mu\)L Tris-HCl buffer (0.05 mol/L, pH 8.0), preheated at 37 °C for 15 min in a constant temperature oscillator, then poured into 10 \(\mu\)L substrate (0.01 mol/L) to react at the same temperature for 15 min; immediately, the absorbance was measured at a 405 nm wavelength
with an enzyme-labeling measuring instrument [25]. Regarding orlistat as positive control, the inhibitory ratio was calculated using the following Equation (8):

\[
\text{Inhibitory ratio (\%)} = \frac{B_0 - (B_1 - B_2)}{B_0} \times 100
\]  

(8)

where \( B_0 \) stands for the absorbance value of the blank control (including substrate and enzyme), \( B_1 \) for that of the test samples (including PPPT, substrate, and enzyme), and \( B_2 \) for that of the sample control (only including PPPT).

2.10. Establishment of Type II Diabetic Model Mice

About 4-week-old healthy Kunming female mice, weighing 18–22 g, SPF grade, were housed under standardized conditions in a room on a 12 h light/dark cycle with available food and water. Mice were randomly allocated into two groups a week later, 10 mice to the normal group (feeding on an ordinary diet) and the others to the model group (fed on a high-fat, high-sugar diet). After 3 weeks, mice in the model group were fasted overnight before being injected with 50 mg/kg·BW streptozotocin (2%, STZ), and continually maintained on a high-fat and high-sugar diet. Normal group was injected with phosphate buffer at the same dosage and fed on an ordinary diet. Seven days later, after fasting for 5 h, blood was taken out via the mouse tail vein, and fasting plasma glucose was measured by a blood glucose tester. Mice with more than 11.1 mmol/L of blood glucose concentration were finally selected as the type II diabetic mice [26].

2.11. Animal Grouping

Mice were randomly divided into 5 groups, 10 mice per group, including a normal group, a diabetic model group (model group), a positive drug control group (drug group), a PPPT-1 group, and a PPPT-4 group, in which all mice were fed with 10 g ordinary diet every day for 21 consecutive days. In addition, the drug group was orally administered with metformin at dosage of 100 mg/kg·BW once a day; similarly, PPPT-1 group was orally administered with PPPT at dosage of 100 mg/kg·BW, while PPPT-4 group with PPPT at dosage of 400 mg/kg·BW.

2.12. Test of Fasting Serum Insulin (FINS)

FINS was measured by INS kit. On the last day of the experiment, each mouse was subjected to fasting for 8 h, but still watered; blood was collected from the mouse tail vein and centrifugated for 15 min at 10,000 rpm under 4 °C freezing conditions, and supernatant was isolated for FINS test.

2.13. Test of Immune Organ Index

Mice were executed by way of the dislocated cervical vertebrae when the experiment was over. All of thymus, spleen, liver, and kidney were excised from mice. Thymus, spleen, liver, and kidney indices were calculated as follows (Equations (9)–(12)):

\[
\text{Thymus index} = \frac{\text{thymus weight (mg)} \times 1000}{\text{BW (g)}}
\]  

(9)

\[
\text{Spleen index} = \frac{\text{spleen weight (mg)} \times 1000}{\text{BW (g)}}
\]  

(10)

\[
\text{Liver index} = \frac{\text{liver weight (mg)} \times 1000}{\text{BW (g)}}
\]  

(11)

\[
\text{Kidney index} = \frac{\text{kidney weight (mg)} \times 1000}{\text{BW (g)}}
\]  

(12)

2.14. Test of Mice Fasting Blood Glucose and Oral Glucose Tolerance Test

To determine fasting blood glucose, all mice had to be on an empty stomach for 6 h. Then, the tail vein blood was drawn to measure fasting blood glucose by a blood glucose meter; fasting blood glucose (FBG) levels were measured one time per week during the 3-week experiment. Oral glucose tolerance test (OGTT) was performed on the last day
before ending the experiment. The measured blood glucose value at the last time was regarded as 0 h blood sugar value, then PPPT groups were intragastrically supplied with different concentrations of PPPT. Normal group and model group were given the same dose of phosphate buffer solvent, and mice in drug group were treated with metformin at a dosage of 4 mg/kg-BW. After 20 min, all mice were treated with administration of 2.0 g/kg-BW glucose; blood glucose levels were respectively measured five times during the 2 h after glucose loading. The area under the curve (AUC) of blood glucose was calculated based on the blood glucose curve: \[ AUC = 0.25 \times (0 \text{ h blood glucose value} + 0.5 \text{ h blood glucose value} + 3 \times 2.0 \text{ h blood glucose value}) \].

2.15. Test of TG, TC, HDL-C, and LDL-C Concentration

Hyperlipidemia (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by ELISA kit. Each mouse was subjected to fasting but watering for 8 h on the last day of the experiment. Blood was collected from the tail vein and centrifugated for 15 min at 10,000 rpm under 4 °C, and supernatant was isolated and used for determining the concentration of TG, TC, HDL-C, and LDL-C.

2.16. Microscopical Observation of the Pancreatic Tissue

Sections of mouse pancreas were prepared for microscopical pathological observation according to GDMLAC/SOP-PAT24 <<Microscopical observation of the pathological sections>> (the abbreviation for Guangdong Medical Laboratory Animal Center is GDMLAC), and SOP-PAT24 stands for the document number of <<Microscopical observation of the pathological sections>>).

2.17. Statistical Analyses

All experimental data are presented as mean ± standard deviation. Data were analyzed by SPSS 19.0 software, and \( t \)-test was used to evaluate differences; \( p < 0.05 \) stands for significant difference, and \( p < 0.01 \) for extremely significant difference.

3. Results

3.1. Amino Acid Analysis and Nutritional Evaluation of PPPT

Amino acid composition analysis is a classical method for protein analysis and is widely applied in food and medical science research. The amino acid profile in terms of grams of amino acid per 100 g of protein is presented in Table 1. PPPT shows its total amino acid content is 27.83 g/100 g after acid hydrolysis, and there are several possibilities for the residue nonamino acid content: (1) Some amino acids in PPPT could not be analyzed due to no standard samples. (2) There may be some kind of amino acid whose content is very high, and we have not yet analyzed it, such as taurine. (3) Our current analytical method was acid hydrolysis, in which some amino acids were destroyed and were not analyzed, such as tryptophan. (4) In addition, the hydrolyzed amino acid should be below the measurement limit of the Kjeldahl nitrogen analyzer because the Kjeldahl nitrogen method also analyzes nonprotein nitrogen. On the other hand, cysteine and methionine could not be measured accurately due to partial oxidation, and some amino acids were too small to be measured. It can be observed that the cystine content was the highest (3.42 g/100 g) among the amino acid composition of PPPT, followed by glutamate (3.41 g/100 g). PPPT fundamentally meets the adult FAO/WHO recommendation model but does not conform to the child FAO/WHO recommendation model (FAO/WHO 1973) [27] (Figure 1 and Table 1). It is well known that cystine assists in the formation of skin, protecting cells from copper poisoning and increasing the metabolic detoxification function; it is also helpful in improving insulin supply, promoting cell oxidation and reduction, making liver function vigorous, causing leukocyte proliferation, and preventing bacteria growth. On the other hand, glutamate absorbed by the human body easily relieves ammonia’s toxic effect in the metabolic process, preventing and treating hepatic coma, which is also used as the energy material of brain
tissue to maintain brain function; glutamate mainly exists in the form of glutamine in the human body and is essential for cell proliferation [28]. Thus, analysis of amino acid composition showed that PPPT is a kind of functional plasma proteolytic peptide with better nutritional value.

Table 1. Amino acid composition of PPPT (total amino acid content is 27.83 g/100 g).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Content (g/100 g)</th>
<th>Content (g/100 g) in FAO/WHO Recommended Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Child (2–5 Years)</td>
</tr>
<tr>
<td>Essential amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>1.14</td>
<td>3.4</td>
</tr>
<tr>
<td>Valine</td>
<td>1.68</td>
<td>3.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Methionine + cystine</td>
<td>4.27</td>
<td>2.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + tyrosine</td>
<td>1.94</td>
<td>6.3</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.31</td>
<td>2.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.06</td>
<td>6.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.33</td>
<td>5.8</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.25</td>
<td>1.9</td>
</tr>
<tr>
<td>Nonessential amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>3.41</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Composition of amino acids of PPPT.
According to the chemical properties, amino acids can be divided into nonpolar amino acids, polar neutral amino acids, acidic amino acids, basic amino acids, sulfur-containing amino acids, and aromatic amino acids. Amino acid classification of PPPT showed that the content of nonpolar amino acid was relatively high at 8.83 g/100 g, followed by acidic amino acid (6.51 g/100 g); the content of aromatic amino acid (1.94 g/100 g) ranked last (Table 2). The existing literature shows that nonpolar amino acids play an important role in the activities of bioactive peptides according to the structure–activity relationship of bioactive peptides [29]; our results also indicated that PPPT may be an ideal raw material for preparing bioactive peptides.

Table 2. Amino acid classification of PPPT.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Content (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolar amino acid</td>
<td>8.83</td>
</tr>
<tr>
<td>Polar and neutral amino acid</td>
<td>4.38</td>
</tr>
<tr>
<td>Acidic amino acid</td>
<td>6.51</td>
</tr>
<tr>
<td>Basic amino acid</td>
<td>4.7</td>
</tr>
<tr>
<td>Sulfur-containing amino acid</td>
<td>4.27</td>
</tr>
<tr>
<td>Aromatic amino acid</td>
<td>1.94</td>
</tr>
</tbody>
</table>

The content of amino acids in protein, especially the content of essential amino acids, is an important index of protein quality. WHO/UN (in 1973) suggested that the ratio of essential amino acids to total amino acids (E/T) in high-quality protein was necessarily over 36%. The E/T of PPPT was 11.73% lower than the WHO/UN recommended pattern, its amino acids score (AAS) was 24.2, and the first, second, third, and fourth limiting amino acids of PPPT were lysine, threonine, leucine, and isoleucine, respectively. Although PPPT had a low E/T value (11.73%) and AAS (24.2), its essential amino acid level is still similar to an adult’s FAO/WHO recommended level. The protein efficiency ratio (PER) is often used as an evaluating index of protein utilization after absorption, and it is generally believed that protein is highly nutritious when PER is more than 2. All PER1, PER2, and PER3 of PPPT were more than 2, also equivalent to that of casein (PER 2.85) and egg protein (PER 3.92), indicating that PPPT has a similar utilization rate of protein with casein and egg protein. (Table 3).

Table 3. Nutritional evaluation of PPPT.

<table>
<thead>
<tr>
<th>Index</th>
<th>PPPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential amino acids (E/T)%</td>
<td>11.73</td>
</tr>
<tr>
<td>Amino acid score (AAS)</td>
<td>24.2</td>
</tr>
<tr>
<td>First restrictive amino acid and score/%</td>
<td>Lysine (24.2)</td>
</tr>
<tr>
<td>Second restrictive amino acid and score/%</td>
<td>Threonine (28.5)</td>
</tr>
<tr>
<td>Third restrictive amino acid and score/%</td>
<td>Leucine (29.4)</td>
</tr>
<tr>
<td>Fourth restrictive amino acid and score/%</td>
<td>Isoleucine (32.8)</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)1</td>
<td>2.54</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)2</td>
<td>2.64</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)3</td>
<td>4.18</td>
</tr>
</tbody>
</table>

3.2. Antioxidant Activities In Vitro

DPPH and HO· scavenging activities and the total antioxidant ability of PPPT were assayed (Figure 2).

DPPH scavenging assay is widely used to evaluate antioxidant ability. PPPT was able to convert DPPH free radicals to yellow diphenyl-picrylhydrazine in a concentration-dependent manner with an IC₅₀ value of 1.085 mg/mL. The scavenging rate to DPPH free radicals also reached a maximum value of 28.9% at a PPPT maximum concentration of 0.50 mg/mL in the experimental range (Figure 2A).
Inhibition against α-glucosidase in vitro

Figure 3. Inhibitory activity against α-glucosidase in vitro.

The inhibitory effect of PPPT on lipase varied with its concentration. The inhibitory rate tended to be stable when the concentration reached a certain value. The quadratic regression equation on the correlation between the lipase inhibitory rate and PPPT concentration could be gained and expressed as $Y = -0.1097X^2 + 0.3398X + 0.0322$, $R^2 = 0.9673$, accordingly obtaining an IC$_{50}$ of 1.0325 mg/mL. Therefore, PPPT could inhibit lipase activity (Figure 4).

HO, as the most reactive oxygen free radical, can react with all biomacromolecules and destroy cells. The HO scavenging activity of PPPT in the range of 0.04–0.08 mg/mL increased in accordance with a rise in PPPT concentration, and its clearance rate reached a peak value of 13.3% at 0.08 mg/mL; the scavenging rate was still not more than 16% in the subsequent concentration range (Figure 2B).

Total antioxidant activity assay is usually performed to determine the antioxidant capacities of natural products. It accounts for the meterage of the TPTZ–Fe (II) complex generated by a reduction in the TPTZ–Fe (III) complex; a higher FRAP value indicates a stronger ferric reducing antioxidant power of samples. PPPT showed that the total antioxidant capacity was obviously lower than that of Vc and FRAP values gradually increased (Figure 2C).

Results indicated that PPPT possessed certain antioxidant activities in a concentration-dependent manner.

3.3. Inhibitory Activities against Enzymes In Vitro

PPPT inhibitory activity on α-glucosidase showed a dose-dependent effect, with an IC$_{50}$ value of 2.62 mg/mL in the experimental range; the strongest inhibitory rate reached 60.5% at a concentration of 3.2 mg/mL (Figure 3).
Inhibition against α-glucosidase in vitro

![Inhibition against lipase in vitro](image)

**Figure 3. Inhibitory activity on lipase in vitro.**

3.4. Effect of PPPT on Insulin Levels in Serum from Type II Diabetic Mice

Mouse insulin levels were measured according to the instructions in the INS kit. Taking the concentration of standard substance as an abscissa and the corresponding OD$_{450}$ value as an ordinate, a standard curve was drawn, and the linear equation was as follows: $y = 0.2205x + 0.2265$ ($R^2 = 0.9991$), which indicated that the insulin level was well linear and within the standard concentration range.

Insulin concentration was calculated based on the measured OD$_{450}$ value of mouse serum; a standard curve was drawn, and the insulin level in mouse serum was also obtained. PPPT could improve insulin secretion in type II diabetic mice, and insulin levels in the PPPT groups were significantly higher than those in the model group, respectively increasing by 5.15 mIU/L (PPPT-4) and 4.54 mIU/L (PPPT-1) (Figure 5).

![Insulin level in serum](image)

**Figure 5. Effect of PPPT on insulin level in serum from type II diabetic mice.**

3.5. Effect of PPPT on the Immune Organ Index of Type II Diabetic Mice

The thymus, as the central immune organ, plays an important role in the immune response. Various thymic hormones, such as thymosin, are produced in the thymus, and these hormones efficiently induce functional maturation of cells and, finally, regulate immune function. The spleen, as the biggest peripheral immune organ, contains many lymphocytes and macrophages that take part in immune reactions. The spleen index and thymus index were adopted to reflect the positive effect of PPPT on the immune system; PPPT was helpful in the recovery of organs of the immune system due to the spleen index and thymus index in PPPT groups increasing to different degrees (Table 4).
Table 4. Effect of PPPT on organ index in type II diabetic mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thymus Index (g/kg)</th>
<th>Spleen Index (g/kg)</th>
<th>Liver Index (g/kg)</th>
<th>Kidney Index (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>2.98 ± 1.38</td>
<td>4.22 ± 1.18</td>
<td>45.58 ± 3.27</td>
<td>13.06 ± 1.35</td>
</tr>
<tr>
<td>Model group</td>
<td>0.62 ± 0.14 A</td>
<td>2.19 ± 0.09 A</td>
<td>72.83 ± 4.91 A</td>
<td>21.69 ± 1.06 A</td>
</tr>
<tr>
<td>Medicine group</td>
<td>2.57 ± 0.11 B</td>
<td>4.13 ± 1.42 B</td>
<td>48.15 ± 2.85 AB</td>
<td>13.87 ± 1.52 B</td>
</tr>
<tr>
<td>PPPT-4 group</td>
<td>2.21 ± 0.53 aB</td>
<td>4.07 ± 0.82 aB</td>
<td>50.98 ± 3.37 ABC</td>
<td>14.25 ± 1.94 B</td>
</tr>
<tr>
<td>PPPT-1 group</td>
<td>1.10 ± 0.70 ABC</td>
<td>3.86 ± 0.94 aB</td>
<td>56.06 ± 4.18 ABC</td>
<td>19.14 ± 2.04 ABC</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean ± SD; "A" represents that the difference is extremely significant compared with the normal group (p < 0.01); "B" represents that the difference is extremely significant compared with the model group (p < 0.01); "C" represents that the difference is extremely significant compared with the medicine group (p < 0.01); "a" represents that the difference is significant compared with the normal group (p < 0.05); "b" represents that the difference is significant compared with the model group (p < 0.05); "c" represents that the difference is significant compared with the medicine group (p < 0.05). The same below.

An increase in the liver index and kidney index is associated with a variety of severe diseases resulting in hyperemia, edema, hyperplasia, hypertrophy, and so on; conversely, a decrease shows atrophy and anaplasia of those organs [30]. Compared with the model group, the liver index and kidney index of mice in both the PPPT-4 group and medicine group were close to that of mice in the normal group, but the mice in the PPPT-1 group were significantly different from the normal group and better than the model group. Thus, PPPT could improve the function of the liver and kidney in type II diabetic mice in a dose-dependent manner (Table 4).

3.6. Effect of PPPT on Glucose Tolerance of Type II Diabetic Mice

The change in blood glucose in type II diabetic mice within 120 min after intragastric administration is displayed (Table 5). The blood glucose value of mice increased to the highest level after 30 min, and then began to decrease, and reduced to proximate to the initial values 120 min later in all groups except the model group. Blood glucose values in both the medicine group and PPPT groups had a significant difference compared with the model group (p < 0.01), showing the preferably inhibitory effect of PPPT on blood glucose rise in type II diabetic mice and its improvement in glucose tolerance. PPPT effectively regulated the blood glucose of type II diabetic mice, and AUC in the PPPT groups (15.45 and 17.60 mmol/L/min) were under that in the model group (21.77 mmol/L/min) (Figure 6).

Figure 6. Effect of PPPT on type II diabetic mice. (A) Glucose concentration in serum within 120 min; (B) Area under the curve (AUC).
Table 5. Effect of PPPT on glucose tolerance of type II diabetic mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Concentration (mmol/L)</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td></td>
<td>3.95 ± 0.84</td>
<td>8.13 ± 1.07</td>
<td>13.54 ± 1.27</td>
<td>11.32 ± 1.18</td>
<td>5.35 ± 0.93</td>
<td>4.05 ± 0.77</td>
</tr>
<tr>
<td>Model group</td>
<td></td>
<td>15.70 ± 0.31 A</td>
<td>23.40 ± 0.99 A</td>
<td>25.31 ± 1.14 A</td>
<td>22.90 ± 0.87 A</td>
<td>21.40 ± 0.82 A</td>
<td>18.31 ± 0.75 A</td>
</tr>
<tr>
<td>Medicine group</td>
<td></td>
<td>7.35 ± 0.53 ABC</td>
<td>13.19 ± 2.01 ABC</td>
<td>16.15 ± 1.47 ABC</td>
<td>14.42 ± 1.22 ABC</td>
<td>9.58 ± 1.06 ABC</td>
<td>7.33 ± 0.81 ABC</td>
</tr>
<tr>
<td>PPPT-4 group</td>
<td></td>
<td>9.12 ± 0.65 ABC</td>
<td>15.5 ± 0.72 ABC</td>
<td>19.7 ± 1.34 ABC</td>
<td>18.4 ± 0.82 ABC</td>
<td>14.24 ± 0.92 ABC</td>
<td>8.75 ± 1.05 ABC</td>
</tr>
<tr>
<td>PPPT-1 group</td>
<td></td>
<td>10.8 ± 1.14 ABC</td>
<td>17.9 ± 0.68 ABC</td>
<td>21.8 ± 1.05 ABC</td>
<td>19.6 ± 0.83 ABC</td>
<td>17.11 ± 0.67 ABC</td>
<td>11.37 ± 1.20 ABC</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean ± SD; “A” represents that the difference is extremely significant compared with the normal group (p < 0.01); “B” represents that the difference is extremely significant compared with the model group (p < 0.01); “C” represents that the difference is extremely significant compared with the medicine group (p < 0.01); “c” represents that the difference is significant compared with the medicine group (p < 0.05).

3.7. Effect of PPPT on TG, TC, LDL-C, and HDL-C of Type II Diabetic Mice

The TG level of serum in the model group was 1.79 mmol/L (>1.71), indicating that the type II diabetic mice had slight hypertriglyceridemia. PPPT also made TG levels decrease by 28.5% (PPPT-4) and 26.3% (PPPT-1), and there were significant differences in the TC levels of serum between the PPPT group and model group (p < 0.01), which respectively decreased by 23.7% (PPPT-4) and 20.6% (PPPT-1). Thus, PPPT could effectively regulate blood lipid metabolism in type II diabetic mice. HDL-C, as a carrier of cholesterol alcohol, is considered negatively related to atherosclerosis, and serum HDL-C levels respectively increased by 23.4% in the PPPT-4 group and by 20.4% in the PPPT-1 group; the PPPT effect on HDL-C level was similar to the medicine group. On the contrary, high LDL-C levels easily cause atherosclerosis, and PPPT obviously demonstrated an improved lipid metabolism ability. Above all, the effect of each group on blood lipid regulation in descending order was as follows: normal group > medicine group > PPPT-4 group > PPPT-1 group > model group (Table 6 and Figure 7).

Table 6. Effect of PPPT on TG, TC, LDL-C, and HDL-C of type II diabetic mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Lipids Concentration (mmol/L)</th>
<th>TG</th>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td></td>
<td>1.13 ± 0.16</td>
<td>2.95 ± 0.22</td>
<td>0.56 ± 0.07</td>
<td>2.50 ± 0.23</td>
</tr>
<tr>
<td>Model group</td>
<td></td>
<td>1.79 ± 0.14 A</td>
<td>4.51 ± 0.17 A</td>
<td>2.62 ± 0.18 A</td>
<td>1.62 ± 0.14 A</td>
</tr>
<tr>
<td>Medicine group</td>
<td></td>
<td>1.25 ± 0.10 B</td>
<td>3.20 ± 0.26 abc</td>
<td>1.11 ± 0.09 ab</td>
<td>2.19 ± 0.31 AB</td>
</tr>
<tr>
<td>PPPT-4</td>
<td></td>
<td>1.28 ± 0.12 abc</td>
<td>3.44 ± 0.34 AB</td>
<td>1.27 ± 0.11 AB</td>
<td>1.95 ± 0.15 AB</td>
</tr>
<tr>
<td>PPPT-1</td>
<td></td>
<td>1.32 ± 0.15 AB</td>
<td>3.58 ± 0.24 ABc</td>
<td>1.31 ± 0.10 Abc</td>
<td>1.87 ± 0.11 Abc</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean ± SD; “A” represents that the difference is extremely significant compared with the normal group (p < 0.01); “B” represents that the difference is extremely significant compared with the model group (p < 0.01); “a” represents that the difference is significant compared with the normal group (p < 0.05); “b” represents that the difference is significant compared with the model group (p < 0.05); “c” represents that the difference is significant compared with the medicine group (p < 0.05).

3.8. Effect of PPPT on Repair of Pancreas Histopathological Lesion of Type II Diabetic Mice

Compared with the normal group, no pancreas islet existed in the model group, small lobes of the pancreas became smaller, many acinus cells degranulated, there was swelling and necrosis, and enhanced basophilia. However, the pathological damage in the PPPT groups was restored to a certain degree, especially in the PPPT-4 group, the effect of which was almost the same as in the medicine group: the pancreas began to resurrect, accompanied by a number of islets rising, the function of producing insulin restoring, the degenerating necrosis of adenocyte improving, and interstitial hyperemia edema alleviating (Figure 8).
Figure 7. Effect of PPPT on TG, TC, LDL-C, and HDL-C of type II diabetic mice.

Figure 8. Effect of PPPT on pancreatic tissue morphology of type II diabetes mice (HE. × 400): (A) normal group; (B) model group; (C) metformin; (D) PPPT-4 group; (E) PPPT-1 group.

4. Discussion and Conclusions

PPPT almost meets an adult’s FAO/WHO recommendation standard, is easily utilized after absorption due to more than two PER values, and can be regarded as a nutritious protein.

More and more studies have shown that oxidative stress is closely related to both diabetes and the occurrence and development of its complications [31,32]. Free radicals produced can destroy islet cells by all means, increasing insulin resistance, causing insufficient insulin secretion, and raising the concentration of blood sugar, leading to lipid peroxidation. In particular, the hydroxyl radical (OH), regarded as a highly effective oxidant, is considered the most active and harmful [33]. PPPT showed antioxygenic properties by effectively eliminating the hydroxyl radical (OH) and DPPH, thereby blocking peroxidation so that β-cells in islet cells were protected and diabetes symptoms were finally improved. However, the specific mechanism of action needs to be further proved.

PPPT displayed inhibitory activity on α-glucosidase and a positive effect on fasting blood glucose and the oral glucose tolerance of type II diabetic mice. As we know, the mechanism of an α-glucosidase inhibitor is to competitively inhibit α-glucosidase located in the small intestine [34], resulting in slowing starch decomposition, glucose absorption, and postprandial hyperglycemia; PPPT, as an α-glucosidase inhibitor, is helpful for treating diabetes. Diabetes is also related to abnormal lipid metabolism [35]. PPPT could restrain lipase activity so that fats were not hydrolyzed into free fatty acids, and single acylglycerol, TG, TC, and LDL-C in serum could be accordingly adjusted to a basically normal level, accompanied by a better HDL-C level.

The reduced indices of the liver and kidney and the increased indices of the thymus and spleen showed a better effect of PPPT on immune function and overall diabetic symptoms [36]. The morphology of pancreatic tissue in type II diabetic mice as well indicated in the PPPT groups, especially in the PPPT-4 group, made a number of islets rise...
and adenocyte necrosis and interstitial hyperemia edema alleviate—the pancreas began to be resurrected, so as to normalize insulin secretion.

In summary, PPPT improved the symptoms of type II diabetic mice. All results in this work provide a theoretical basis for PPPT development into healthcare products and natural antidiabetic drugs, but the specific mechanism needs further study.

**Author Contributions:** Conceptualization, X.K. and C.D.; methodology, C.D.; formal analysis, G.Z.; investigation, G.Z.; resources, X.K. and C.D.; data curation, Y.W.; writing—original draft preparation, C.D.; writing—review and editing, G.Z. and C.D.; project administration, X.K.; funding acquisition, X.K. and C.D. All authors have read and agreed to the published version of the manuscript.

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