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Hypoglycemic Effect of the Water Extract of Pu-erh Tea

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ABSTRACT: The water extraction and composition of pu-erh tea, as well as the hypoglycemic effect of the water extract of pu-erh tea (WEPT) in vivo and in vitro, are reported to investigate its hypoglycemic effect on diabetes. High-performance liquid chromatography and colorimetric methods are used to analyze the tea catechins, caffeine, polyphenols, amino acids, and polysaccharides of the WEPT. The effect of the WEPT on glucose uptake by cultured HepG2 cells and the inhibition effect of rat intestinal sucrase, maltase, and porcine pancreatic amylase are determined in vitro. Then, the blood glucose and insulin levels of intragastrically administered WEPT on fasting and oral glucose tolerance test (OGTT) using type 2 diabetic db/db (BKS.Cg-m +/+ Lepr^{db}/J) mice are determined in vivo. The results showed that the WEPT dose-dependently and significantly increased glucose uptake by HepG2 cells and inhibited rat intestinal sucrase, maltase, and porcine pancreatic amylase activity. The WEPT intragastrically given for 4 weeks suppressed the increase in blood insulin and glucose levels of db/db mice fasted overnight. In OGTT, the WEPT improved impaired glucose tolerance and ameliorated retarded insulin response at 60 and 120 min in db/db mice. These results suggest that the WEPT has beneficial effects on glucose homeostasis in type 2 diabetes and in amendment of insulin resistance.

KEYWORDS: *water extract of pu-erh tea, hypoglycemic effect, glucose uptake, α-glucosidase, insulin resistance, db/db mice*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a group of metabolic disorders characterized by hyperglycemia, resulting from resistance to insulin action and/or inadequate insulin secretion. Chronic hyperglycemia is strongly associated with the increased risk of kidney, eye, and nerve complications (microvascular complications), as well as increased risk of stroke, heart disease, and amputations (macrovascular complications), which are responsible for the majority of morbidity and mortality in patients with T2DM.

The overall lowering of glucose is of pivotal importance in the treatment of diabetes, with proven beneficial effects on micro- and macrovascular outcomes.[1] Furthermore, evidence suggests that "glucose variability" may be an independent risk factor for cardiovascular complications in diabetes.[2] The therapeutic challenge lies in the need for intensive glycaemic control and maintenance of glycaemia within a strict normal narrow range. Some studies have suggested that an elevated postprandial glucose (PPG) level is a critical component of diabetes-related vascular complications because PPG is a contributor to both acute glucose fluctuations and chronic sustained hyperglycemia.[3] The German Diabetes Intervention Study showed that controlling PPG has a greater effect on cardiovascular disease and all-cause mortality than controlling fasting plasma glucose (FPG) levels.[4] Studies have also shown that oral antidiabetic agents that target PPG slow and even reverse the progression of carotid intima-media thickening.[5] These results, which have also been confirmed by other studies, suggest that PPG is an independent risk factor for vascular complications in diabetes.[6] Ingestion of highly available carbohydrates will lead to a sharp rise of PPG. Frequent consumption of such foods or high glycemic index foods is suggested to play a causative role in development of type 2 diabetes.[7] Introduction of α-amylase and α-glucosidase inhibitor into the diet has been proposed to be effective in retarding carbohydrate digestion, resulting in reduced PPG.[8] Identifying effective α-amylase and α-glucosidase inhibitor from natural sources can be beneficial in the prevention and treatment of diabetes mellitus.

Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. Generally, tea can be broadly classified according to production method as unfermented tea (green tea), semi-fermented tea (oolong tea), fully fermented tea (black tea), and post-fermented tea (pu-erh tea). Pu-erh tea is a kind of post-fermented tea produced mainly in the Yunnan province of China and widely consumed by the Chinese. The key process of pu-erh tea preparation is secondary fermentation, in which microorganisms play a very important role in producing the taste, color, fragrance, and functional components. During the fermentation process, catechins are oxidized and then polymerized to form bisflavanol, theaflavins (TFs), thearubigins (TRs), and other high-molecular components. Some studies have shown that pu-erh tea and its components have many biological and biochemical effects, such as prevention of cancer and cardiovascular diseases.[9,10] It also

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has antiobesity,[11,12] antibacterial,[13] antioxidative,[14,15] antiviral,[16] and hypolipidemic[17,18] effects.

An effective means to control the biological network that underlies diseases is needed to treat complex diseases, such as diabetes or cancer. However, monotherapy acting on a single target is often limited in its therapeutic effect because of the inherent robustness and redundance of biological networks to external perturbations and changes.[19] Multi-target therapeutics is often more effective compared to monotherapies for complex diseases, such as diabetes. In general, herbal medicines are complex mixtures of different active compounds that often act in a synergistic fashion and exert their full beneficial effects as total extracts. In this study, the water extraction and composition of pu-erh tea are presented. The glucose uptake promotion effect is explored in vitro to discuss the influence of the water extract of pu-erh tea (WEPT) on the hepatic glucose metabolism that mainly affects FPG. The inhibition effects of sucrase, maltase, and amylase are performed to study the potency of the WEPT in retarding carbohydrate digestion and reducing PPG in vitro. To determine the relationship between FPG, PPG, and insulin values with the WEPT treatment, an oral glucose tolerance test (OGTT) was performed on obese and diabetic db/db (BKS.Cg-m +/+ Lepr^{db}/J) mice in vivo. The experiments are expected to be valuable in further understanding the potential uses of pu-erh tea for the development of therapeutic and preventive agents for diabetes.

MATERIALS AND METHODS

Reagents. Caffeine (CAF), (−)-epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (±)-catechin (DL-C), (−)-epicatechin (EC), (−)-epicatechin 3-gallate (ECG), (−)-gallocatechin 3-gallate (GCG), α-amylase (EC 3.2.1.1), and acarbose were purchased from Sigma-Aldrich. 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was obtained from Molecular Probes. HepG2 cells [from American Type Culture Collection (ATCC)] were grown in high-glucose Dulbecco's modified Eagle's medium (DMEM) (purchased from Hyclone) containing 10% fetal bovine serum (FBS) (obtained from Sijiqing Biological Engineering Materials, Hangzhou, China). All animals were purchased from Model Animal Research Center of Nanjing University.

Sampling. The pu-erh tea sample used was Yunnan pu-erh 2008 provided by Bio-Resources Innovative Development Office of the People's Government of Yunnan Province. Preliminary assays were conducted to establish the appropriate amount of samples for analysis and ensure sample homogeneity and representativeness. Each of the tea samples (100 g) was cut into small pieces and soaked twice in boiled distilled water (1 and 0.2 L of each bulk; 45 and 35 min each time). After the leaves were separated through filtration, whole extracts were concentrated and freeze-dried. The freeze-dried extracts were then mixed, ground, and passed through a 40-mesh sieve for later experiments.

High-Performance Liquid Chromatography (HPLC) Analysis of Tea Catechins and Caffeine. HPLC (Shimadzu, Japan) was performed to identify catechins and caffeine. The chromatographic conditions were as follows: Shim-pack ODS C18 column (4.6 × 150 mm, 5 μm); mobile phase composed of mobile phase A, double-distilled water; mobile phase B, DMF/MeOH/HAC (40:2:1.5); flow rate, 1.1 mL/min; and quantification of catechins and caffeine at 278 nm.

Measurements of Polyphenols, Amino Acids, and Polysaccharides of Extracts. The content of the extracts of the total tea polyphenols was determined using the Folin−Dennis method.[20] The total polyphenol content was determined at 760 nm by comparison to a standard curve prepared using tannic acid as a standard reference.

Total amino acids were estimated spectrophotometrically by the ninhydrin assay at a wavelength of 570 nm.[21]

The anthrone colorimetric method[22] was used to assay tea polysaccharide, and the absorbance was recorded using a spectrophotometer at a wavelength of 620 nm. Standard solutions of glucose were tested in the same manner to obtain a calibration curve.

Assay of Sucrase, Maltase, and Amylase Activity.[23] Sucrase and maltase activity was assayed according to the method described by Matsuo and Izumori.[23] Briefly, four male Wistar rats of 180−240 g of body weight were fasted 12 h before use. The rats were killed by cervical dislocation, and a segment (40−45 cm) of jejunum was quickly excised and rinsed with ice-cold saline solution (0.9% NaCl).

The mucosal surface was removed by gently scraping with a microscope slide. All of the collected mucosa from the four rats were homogenized together with a 9-fold volume of cold saline solution (0.9% NaCl) and centrifuged at 4 °C for 30 min at 12 000 rpm, and then the supernatant was used as a crude enzyme solution to assay the activity of maltase and sucrase. Maltose and sucrose were used as substrates of maltase and sucrase, respectively. The released D-glucose was determined colorimetrically using the glucose oxidase methods diagnostic kits (Nanjing Jiancheng Bioengineering Institute).

The porcine pancreatic amylase was determined using soluble starch as substrates. The procedure for determining the enzymatic activity of α-amylase was aligned with the protocol of the Sigma-Aldrich enzymatic assay of α-amylase (EC 3.2.1.1). The 50% inhibition concentration (IC_{50}) was obtained from the linear regression curve of the concentrations and inhibitory rates.

Glucose Uptake Assay.[24] HepG2 cells was obtained from ATCC and cultured in DMEM. HepG2 cells were plated into 96-well tissue culture plates. After the cells reached 80−90% confluence, the medium was replaced by DMEM containing various concentrations of the WEPT (0.01, 0.03, and 0.1 g/L), rosiglitazone (10 μmol/L, used as a positive reference compound), or acarbose (10 μmol/L, used as a α-glucosidase positive reference compound) added to the wells. After 24 h of treatment, the medium was removed and all cells were incubated at 100 μM 2-NBDG in phosphate-buffered saline (PBS) for 15 min and then washed 5 times with additional PBS to remove excess 2-NBDG. Fluorescence in the cells was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a DTX880 multifunction counter (Beckman Co., Ltd.).

Mouse Experiments. Animal husbandry, care, and experimental procedures were conducted in compliance with the "Principles of Laboratory Animal Care" National Institutes of Health (NIH) guidelines, as approved by the Institutional Animal Care and Use Committee (IACUC). Mice homozygous for the diabetes spontaneous mutation (BKS.Cg-m +/+ Lepr^{db}/J) were used for in vivo studies. A total of 48 obese and diabetic db/db (BKS.Cg-m +/+ Lepr^{db}/J) male mice were used for in vivo studies, and a total of 8 age-matched non-diabetic mice (C57BLKS/J) were used as controls. All of the animals were housed at 22 ± 2 °C and 55 ± 5% relative humidity, with a light/dark cycle of 12 h. Food and water were given ad libitum. From 8 weeks of age, different doses of the WEPT (100, 200, and 400 mg/kg of body weight per day) were administered by intragastric rout once a day for 28 days. Rosiglitazone (5 mg/kg of body weight per day) and acarbose (10 mg/kg of body weight per day) were used as positive controls. Age-matched non-diabetic mice (C57BLKS/J) were used as controls.

Blood Collection. The fasting blood was collected from rats after a 12 h overnight fast. Place the rat in a plexiglass tube and gently wash the side of the tail over the vein with gauze soaked with 75% ethanol. In the blood glucose determination, puncture the vein using a needle held perpendicular (90° angle) to the tail and collect the blood directly into a blood glucose strip. In the blood insulin determination, insert a blood collection needle parallel with the tail into the vein until a flash of blood is seen and use a evacuated blood collection tube to collect the blood.

OGTT. After a 12 h overnight fast on day 28, db/db mice were given different doses of the WEPT, rosiglitazone, acarbose, or distilled water intragastrically; 0.5 h later, glucose (2 g/kg of body weight) solution was intragastrically administered to the animals. Blood samples were collected sequentially from the tail vein before and 60, 120, and 180 min after glucose administration.

Measurement of Blood Glucose and Insulin. Blood glucose from the tail vein was measured using an Accu-Chek comfort glucometer (Roche Diagnostics, Basel, Switzerland). The insulin concentration in the serum was measured by commercially available enzyme-linked immunosorbant assays (ELISAs; American Laboratory Products Company, Ltd.). During the experimental period, the fasting blood glucose concentrations were measured on days 0, 7, 14, 21, and 28.

Statistical Analysis. Data were shown as the mean ± standard deviation (SD). Differences between individual groups were analyzed by one-way analysis of variation (ANOVA), followed by Dunett's test and least significant difference (LSD) test. A difference with a p value of <0.05 was considered significant.

RESULTS

Catechins and Caffeine Contents. The representative HPLC patterns of the WEPT are illustrated in Figure 1.

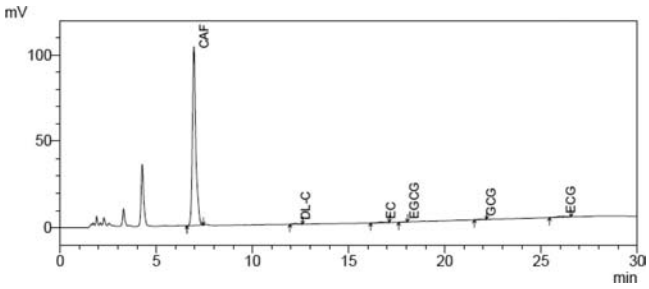

Figure 1. HPLC chromatogram of the WEPT (UV signal at 278 nm): CAF, caffeine; DL-C, (±)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin gallate; GCG, gallocatechin-gallate; and ECG, (−)-epicatechin gallate.

Caffeine and six major catechin forms, namely, (−)-epigallocatechin (EGC), (±)-catechin (DL-C), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), gallocatechin-gallate (GCG), and (−)-epicatechin gallate (ECG), were detected (Table 1), but EGC was not detected in the WEPT.

Contents of Regular Ingredients in the WEPT. Active ingredients, including tea polyphenols, free amino acids, catechins, and polysaccharides, were analyzed and are shown in Table 2.

Sucrase, Maltase, and Amylase Inhibition Effects of the WEPT. In this study, α-glucosidase inhibition assays for the WEPT were conducted using rat small intestinal sucrase and maltase in vitro. The WEPT showed inhibitory effects on rat intestinal sucrase, maltase, and porcine pancreatic amylase, as shown in Table 3, but less potent compared to acarbose (IC_{50} = 4.35 ± 0.59, 6.63 ± 0.70, and 64.19 ± 6.77 μmol/L for sucrase, maltase, and amylase, respectively).

Glucose Uptake Promotion Effect of the WEPT. The glucose uptake of HepG2 cells treated with the WEPT and positive reference rosiglitazone and acarbose was also determined to evaluate the hypoglycemic effects of the WEPT (Figure 2). The results show that the WEPT (0.03 and 0.1 mg/mL) and rosiglitazone (10 μmol/L) but not acarbose (10 μmol/L) significantly promoted the glucose uptake of HepG2 cells compared to the vehicle control 0.5% H_2O (p < 0.05).

Table 2. Contents of Regular Ingredients in the WEPT[a]

group	polyphenols (%)	amino acid (%)	catechin (%)	tea polysaccharide (%)
WEPT	30.79 ± 0.43	1.06 ± 0.01	1.20 ± 0.02	1.21 ± 0.01

[a]The percentage represents per 100 g of WEPT. Each value represents the mean ± SD (n = 3).

Effect of the WEPT on the Body Weight of Diabetic db/db Mice. The body weights of the group of db/db mice were higher than those in the control group (p < 0.01), as shown in Table 4. The body weights of the db/db mice given 200 and 400 mg/kg of WEPT were lower than that of the type 2 diabetic db/db mice (p < 0.05 or 0.01) on day 28. For the positive control, the body weights of db/db mice given acarbose and rosiglitazone were lower than those of the model db/db mice (p < 0.01) on day 28.

Effect of the WEPT on the Fasting Blood Glucose of Diabetic db/db Mice. The fasting blood glucose levels of the group of db/db mice were higher than those in the control group (p < 0.01), as shown in Table 5. The fasting blood glucose levels of db/db mice given 400 mg/kg of WEPT were lower than those of the type 2 diabetic db/db mice (p < 0.05 or 0.01) on days 21 and 28, the same as 200 mg/kg on day 28 (p < 0.05). For the positive control, the fasting blood glucose levels of db/db mice given acarbose and rosiglitazone were lower than those of the model db/db mice (p < 0.05 or 0.01) from day 14 to day 28.

Effect of the WEPT on the Fasting Blood Insulin Level of Diabetic db/db Mice. The fasting blood insulin levels of the group of db/db mice were higher than those in the control group (p < 0.01), as shown in Figure 3. The fasting blood insulin levels of db/db mice given 400 mg/kg of WEPT were lower than those of type 2 diabetic db/db mice (p < 0.05) on day 28. For the positive control, the fasting blood insulin levels of db/db mice given acarbose were not affected significantly, whereas those of the rosiglitazone group were lower than those of the model db/db mice group (p < 0.01) on day 28.

Effect of the WEPT on the OGTT of Diabetic db/db Mice. *Effect of the Blood Glucose.* The increased post-OGTT blood glucose values of the group of db/db mice were higher than those in the control group (p < 0.01), as shown in Table 6. The increased blood glucose values of db/db mice given 400 mg/kg of WEPT were lower than those of the type 2 diabetic db/db mice group (p < 0.05) at 1 and 3 h post-OGTT. For the positive control, the increased blood glucose values of db/db mice given acarbose and rosiglitazone were lower than those of the model db/db mice (p < 0.05 or 0.01) at 1, 2, and 3 h post-OGTT.

Effect of the Blood Insulin. The post-OGTT blood insulin level of db/db mice did not increase significantly compared with control, whereas the blood insulin level of db/db mice

Table 1. Contents of Five Major Catechin Forms and Caffeine Determination in the WEPT by HPLC[a]

CAF (%)	DL-C (%)	EC (%)	EGCG (%)	GCG (%)	ECG (%)
8.51 ± 0.15	0.57 ± 0.01	0.23 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.15 ± 0.00

[a]The percentage represents per 100 g of WEPT. Each value represents the mean ± SD (n = 3). CAF, caffeine; DL-C, (±)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin gallate; GCG, gallocatechin-gallate; and ECG, (−)-epicatechin gallate.

Table 3. In Vitro Inhibitory Effects of Various Concentrations of the WEPT on Rat Intestinal Sucrase, Maltase, and Porcine Pancreatic Amylase[a]

	concentration (g/L)	sucrase	maltase	amylase
inhibition ratio	0.1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	0.3	0.09 ± 0.01	0.12 ± 0.03	0.07 ± 0.02
	1	0.27 ± 0.07	0.29 ± 0.04	0.21 ± 0.03
	3	0.46 ± 0.02	0.49 ± 0.03	0.36 ± 0.03
	10	0.84 ± 0.02	0.85 ± 0.01	0.73 ± 0.03
IC_{50} (g/L)		3.47 ± 0.36	3.14 ± 0.38	5.13 ± 0.61

[a]Each value represents the mean ± SD (n = 3). IC_{50} is the 50% inhibition concentration obtained from the linear regression curve of the concentrations and inhibitory rates.

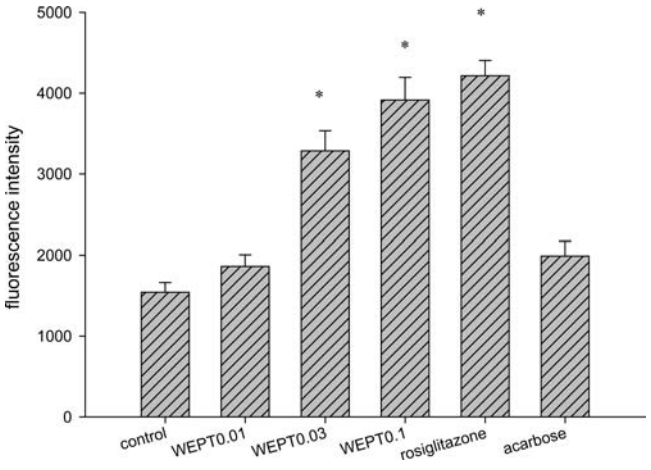

Figure 2. Effects of various concentrations of the WEPT (g/L), rosiglitazone (10 μmol/L), and acarbose (10 μmol/L) on the glucose uptake of HepG2 cells (values are the mean ± SD; n = 12). (*) p < 0.05% versus the control.

given 200 and 400 mg/kg of WEPT and rosiglitazone increased significantly compared with db/db mice (p < 0.05 or 0.01) at 1 h post-OGTT. The blood insulin level of db/db mice that were administered acarbose was not affected significantly compared with db/db mice, as shown in Table 7.

DISCUSSION

Diabetes mellitus is one of the primary threats to human health because of its increasing prevalence, chronic course, and disabling complications. Control of postprandial hyperglycemia is important in the treatment of diabetes. α-Glucosidases are glycoside hydrolases found on the luminal surface of enterocytes containing maltase/glucoamylase and sucrase/isomaltase activity. Targeting mainly postprandial hyperglycemia, α-glucosidase inhibitors favorably affect several cardiovascular risk factors, such as obesity, hypertension, and high glycaemic variability with little to no risk for hypoglycemia.[25] Intensive efforts have been exerted to search for effective and safe α-glucosidase inhibitors in natural materials to develop physiological and functional food for the prevention and cure of diabetes. Koh et al. investigated the ability of green, oolong, and black teas in inhibiting human salivary α-amylase and mammalian α-glucosidase and found that the inhibitory profiles of teas are correlated to their major polyphenol content, TFs, and catechins.[7] Li et al. indicated that two components, EGCG and ECG, are potent α-glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*) inhibitors found in pu-erh tea, with IC_{50} lower than that of acarbose, a commercial α-glucosidase inhibitor.[26] α-Glucosidase from the *S. cerevisiae* assay uses *p*-nitrophenyl-α-D-glucopyranoside (PNPG) that is hydrolyzed by α-glucosidase into *p*-nitrophenyl (PNP). The rate of the reaction is directly proportional to the enzyme activity. This method is a satisfactory and efficient screening test, but the commercial α-glucosidase inhibitor acarbose is less efficient with this method. Toshima et al. also prescribed that catechins are the main factors responsible for maltase inhibition in LG tea, followed by TFs, theasinensins, strictinin, and 1,6-digalloylglucose.[27] In this study, the WEPT showed inhibitory effects on rat intestinal sucrase, maltase, and porcine pancreatic amylase in vitro. The blood glucose levels of db/db mice given 400 mg/kg of WEPT were lower than those of the diabetes model db/db mice group (p < 0.05) at 1 and 3 h post-OGTT. These results provide evidence that the WEPT as an effective α-glucosidase inhibitor could reduce postprandial hyperglycemia and may play a significant role in the prevention and treatment of T2DM and pre-diabetic states. Many studies indicated that the health-promoting actions of the tea consumption could be a summation effect of all of the ingredients present in them, especially the polyphenols. The digestive enzyme inhibitory potency of the WEPT could be mainly contributed by its polyphenol contents according to

Table 4. Effect of the WEPT on the Body Weights of Fasted Diabetic db/db Mice[a]

	body weights (g)				
groups	day 0	day 7	day 14	day 21	day 28
control	17.1 ± 1.1[b]	18.2 ± 1.3[b]	19.7 ± 1.3[b]	21.4 ± 1.4[b]	22.4 ± 1.0[b]
db/db	27.4 ± 1.4	29.2 ± 1.3	31.2 ± 1.2	33.1 ± 0.8	35.6 ± 0.8
100 mg/kg of WEPT	27.4 ± 1.4	29.1 ± 1.0	30.9 ± 1.5	32.4 ± 1.2	34.1 ± 1.1
200 mg/kg of WEPT	27.6 ± 1.1	29.6 ± 1.0	31.4 ± 1.0	32.3 ± 1.2	32.1 ± 1.4[c]
400 mg/kg of WEPT	27.5 ± 1.0	29.3 ± 1.3	30.6 ± 1.5	31.1 ± 1.4	30.3 ± 0.8[b]
10 mg/kg of acarbose	27.6 ± 1.0	29.6 ± 1.0	30.8 ± 1.2	31.0 ± 1.4	30.8 ± 1.7[b]
5 mg/kg of rosiglitazone	27.4 ± 1.4	29.3 ± 1.4	29.7 ± 1.2	29.2 ± 0.9	28.7 ± 1.6[b]

[a]Values are the mean ± SD; n = 8. [b]p < 0.01 versus the db/db group. [c]p <0.05 versus the db/db group.

Table 5. Effect of the WEPT on the Fasting Blood Glucose of Diabetic db/db Mice[a]

groups	fasting blood glucose (mol/L)				
	day 0	day 7	day 14	day 21	day 28
control	5.20 ± 0.43[b]	5.25 ± 0.45[b]	5.26 ± 0.38[b]	5.27 ± 0.48[b]	5.23 ± 0.43[b]
db/db	23.63 ± 2.20	27.42 ± 2.40	28.96 ± 1.95	30.39 ± 2.13	31.35 ± 2.28
100 mg/kg of WEPT	22.65 ± 3.19	26.54 ± 3.16	28.29 ± 3.33	28.46 ± 2.71	29.14 ± 4.51
200 mg/kg of WEPT	22.90 ± 2.22	26.37 ± 2.09	28.07 ± 1.68	27.67 ± 1.58	24.99 ± 1.42[c]
400 mg/kg of WEPT	23.11 ± 2.58	26.87 ± 2.53	27.45 ± 2.27	25.61 ± 3.37[c]	22.60 ± 2.87[b]
10 mg/kg of acarbose	22.60 ± 2.53	25.48 ± 1.68	22.33 ± 2.33[c]	20.74 ± 2.91[b]	19.28 ± 2.30[b]
5 mg/kg of rosiglitazone	22.67 ± 3.15	25.46 ± 3.20	23.31 ± 2.34[c]	20.62 ± 2.55[b]	18.52 ± 3.15[b]

[a]Values are the mean ± SD; $n = 8$. [b]$p < 0.01$ versus the db/db group. [c]$p < 0.05$ versus the db/db group.

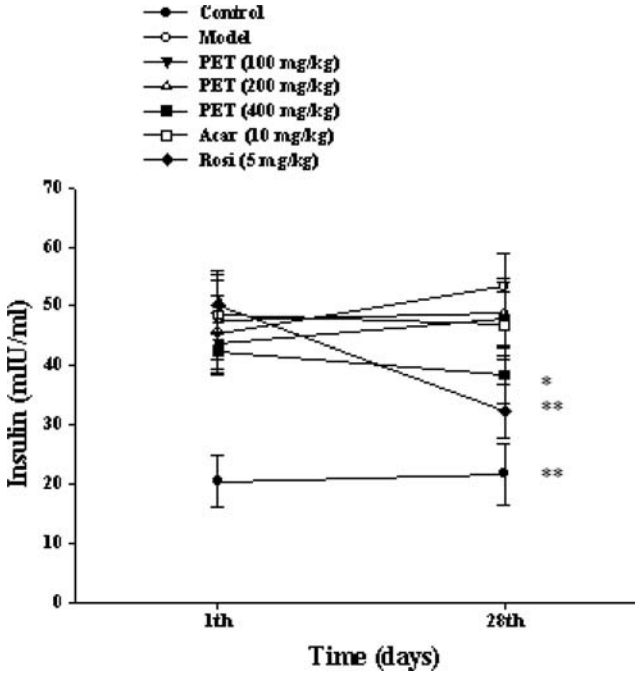

Figure 3. Effect of the WEPT on the fasting insulin level of diabetic db/db mice (values are the mean ± SD; $n = 8$). (*) $p < 0.05$ and (**) $p < 0.01$ versus the db/db model group.

some studies.[7,26] Polyphenols interact with the enzyme primarily via noncovalent interactions, namely, hydrogen-bonding and $\pi-\pi$ interactions with digestive enzymes.[7]

T2DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. Insulin enables muscle, fat, liver, and other body cells to uptake glucose from the blood and use glucose as energy. When a person has T2DM, either no or low glucose uptake into these cells occurs, which results in high glucose levels in the blood. Chronic amelioration of insulin resistance would preserve pancreatic β cell function and delay or prevent the onset of T2DM. Zhang et al. showed that EGCG can inhibit dexamethasone-induced insulin resistance and improve insulin-stimulated glucose uptake by increasing GLUT4 translocation to the plasma membrane in rat L6 cells.[28] Cao et al. showed that green tea extract can increase gene expression in glucose uptake and the insulin-signaling pathway in rats fed with a fructose-rich diet.[29] In the earliest stages of T2DM, the major defect involves the inability of insulin to promote glucose uptake and storage as glycogen. Accordingly, impaired insulin action at the liver causes fasting hyperglycemia.[30] In this study, the glucose uptake of HepG2 cells treated with varied concentrations of the WEPT was determined to evaluate the hypoglycemic effects of the WEPT in vitro. The results showed that the WEPT (0.03 and 0.1 g/L) and rosiglitazone (10 μmol/L) but not acarbose (10 μmol/L) significantly promoted glucose uptake of HepG2 cells compared to the control (vehicle, 0.5% H_2O; $p < 0.05$; Figure 2). From the in vivo study, the fasting blood glucose levels of db/db mice given 200 and 400 mg/kg of WEPT and the fasting blood insulin levels of db/db mice given 400 mg/kg of WEPT were lower than those of the T2DM model db/db mice on day 28. The same result was obtained for rosiglitazone (5 mg/kg). The fasting blood insulin levels of db/db mice given acarbose were not affected significantly. The determinants of elevated fasting glucose and 2 h plasma glucose levels in OGTT differ. Raised hepatic glucose output and a defect in early insulin secretion are characteristic of the fasting hyperglycemia, and peripheral insulin resistance is most characteristic of the latter. From our study, only the 400 mg/kg of WEPT suppressed the increased post-load glucose levels on the OGTT, although both 200 and 400 mg/kg of WEPT decreased fasting glucose levels. The amelioration of impaired insulin action at the liver and the defect in early insulin secretion could be contributed to the effect of the lower dose (200 mg/kg) WEPT on fasting glucose

Table 6. Blood Glucose Effect of the WEPT on the OGTT of Diabetic db/db Mice[a]

groups	blood glucose pre-OGTT (mol/L)	increased blood glucose value post-OGTT (mol/L)		
		1 h	2 h	3 h
control	5.23 ± 0.43[b]	1.90 ± 0.41[b]	0.66 ± 0.19[b]	0.30 ± 0.13[b]
db/db	31.35 ± 2.28	5.82 ± 1.71	3.79 ± 1.32	1.96 ± 0.94
100 mg/kg of WEPT	29.14 ± 4.51	5.11 ± 1.32	3.32 ± 1.79	1.75 ± 0.82
200 mg/kg of WEPT	24.99 ± 1.42[c]	3.92 ± 1.30	2.51 ± 1.22	1.34 ± 0.78
400 mg/kg of WEPT	22.60 ± 2.87[b]	3.12 ± 1.06[c]	2.21 ± 1.04	0.92 ± 0.36[c]
10 mg/kg of acarbose	19.28 ± 2.30[b]	2.72 ± 1.36[c]	1.65 ± 0.90[c]	0.69 ± 0.40[b]
5 mg/kg of rosiglitazone	18.52 ± 3.15[b]	2.95 ± 1.16[c]	1.78 ± 1.06[c]	0.41 ± 0.36[b]

[a]Values are the mean ± SD; $n = 8$. [b]$p < 0.01$ versus the db/db group. [c]$p < 0.05$ versus the db/db group.

Table 7. Insulin Effect of the WEPT on the OGTT of Diabetic db/db Mice[a]

groups	insulin pre-OGTT (mIU/mL)	increased insulin value post-OGTT (mIU/mL)		
		1 h	2 h	3 h
control	21.78 ± 5.24[b]	20.14 ± 5.32[b]	7.36. ± 2.01	1.17 ± 0.57
db/db	53.31 ± 5.49	7.76 ± 1.03	9.28 ± 3.02	2.16 ± 0.65
100 mg/kg of WEPT	47.78 ± 6.18	8.43 ± 2.33	10.21 ± 3.19	2.70 ± 1.12
200 mg/kg of WEPT	48.89 ± 5.77	12.15 ± 3.10[c]	8.79 ± 2.24	1.87 ± 0.61
400 mg/kg of WEPT	38.56 ± 4.88[c]	16.61 ± 2.76[b]	6.21 ± 1.04	1.72 ± 0.54
10 mg/kg of acarbose	46.78 ± 5.65	9.97 ± 1.36	10.65 ± 2.90	1.69 ± 1.01
5 mg/kg of rosiglitazone	32.48 ± 4.52[b]	19.25 ± 5.18[b]	6.37. ± 1.06	1.41 ± 0.76

[a]Values are the mean ± SD; $n = 8$. [b]$p < 0.01$ versus the db/db group. [c]$p < 0.05$ versus the db/db group.

levels. The hypoglycemic molecular mechanism of active compounds in the WEPT needs further investigation.

In accordance with the ratio of the human body surface area, the rat dose was 100, 200, and 400 mg/kg of WEPT per day and the adult dose was calculated as 15, 30, and 60 mg/kg of WEPT per day. Consider a human with a body weight of 60 kg, the adult dose was 0.9, 1.8, and 3.6 g of WEPT per day. The phase I trial of oral green tea extract (GTE) in adult patients with solid tumors[31] and the phase II trial of GTE in patients with high-risk oral premalignant lesions[32] showed that a dose of 1.0 g/m^2 3 times daily (equivalent to 5.2 g of GTE per day) can be taken safely for at least 6 months. According to these studies, oral WEPT at the doses that we studied can be taken safely.

The processing of pu-erh tea is quite different from that of black tea, although both are fermented teas. During pu-erh tea processing, the first step is sun fixation and rolling, which arrests enzymatic oxidation in the leaf. The second step is wet piling by prolonged bacterial and fungal fermentation in a warm humid environment under controlled conditions, which typically takes from 0.5 to 1 year. The bacterial and fungal cultures in the fermenting piles consist of multiple strains of *Aspergillus* spp., *Penicillium* spp., yeasts, and a wide range of other microflora. During the fermentation process, the polyphenols in the tea leaves may be partially transformed to TFs, TRs, and other oligomers by the enzymes produced by the microbes. In comparison to GTE, the WEPT contains less polyphenols but a little more caffeine. Studies showed that coffee exerts a suppressive effect on hyperglycemia by improving insulin sensitivity partly because of caffeine.[33] Anderson et al. showed that TFs and EGCG enhance insulin with the greatest activity in rat epididymal adipocytes.[34] Cameron et al. found that three TFs (theaflavin 3-*O*-gallate, theaflavin 3′-*O*-gallate, and theaflavin 3.3′-di-*O*-gallate) and TRs are novel mimics of insulin/IGF-1 action on mammalian FOXO1a and PEPCK. Moreover, evidence that the effects on this pathway of EGCG depend upon its ability to be converted into these larger structures was provided.[35] The catechin, caffeine, TF, and TR contents in the WEPT may partially contribute to improve insulin resistance. More investigations should be conducted to define the components responsible for the effect.

According to the National Health and Nutrition Examination Survey (NHANES), from 2003 to 2006, 31.9% of children and adolescents were overweight or obese.[36] Adolescent obesity has been attributed to a variety of factors, one of which is beverage consumption. Project EAT-II (Eating Among Teens; a 5 year longitudinal study) indicates that intake of soda and sugar-sweetened beverages, as well as alcohol, increased significantly, whereas significant secular decreases were observed in fruit juice and coffee/tea intake among teenagers.[37] The onset of type 2 diabetes is due primarily to lifestyle factors and genetics. Pu-erh tea is a popular beverage in southwestern China and south Asian countries. In traditional Chinese medicine, pu-erh tea is believed to invigorate the "spleen" and inhibit "dampness". Pu-erh tea is a healthy beverage that helps regulate weight and cholesterol.[11,12] Chinese culture also believes that pu-erh tea counteracts the unpleasant effects of heavy alcohol consumption. In this study, the WEPT exhibited α-glucosidase inhibition and glucose uptake promotion in HepG2 cells in vitro. From the in vivo study, the WEPT reduced both fasting and postprandial hyperglycemia significantly and ameliorated insulin resistance of T2DM db/db mice. Regular consumption of pu-erh tea can contribute to the prevention of the progress of hyperglycemia and insulin resistance of T2DM with regard to the amelioration of metabolism dysfunction. With the composition of active molecules with a wide variety of physiological and pharmacological functions, the powerful effect of pu-erh tea on metabolism is important to public health because the benefits that it provides can be achieved without drastic changes in the lifestyle of modern people. Thus, regular pu-erh tea consumption is proposed as a strategy for the regulation of energy balance and metabolism.

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Notes

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