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Physicochemical characteristics of polysaccharide conjugates prepared from fresh tea leaves and their improving impaired glucose tolerance

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ABSTRACT

Hot-water extracts were prepared from fresh tea leaves and fractionated by DEAE-cellulose DE-52 column chromatography to yield one unexplored polysaccharide-conjugate fraction TPC-L (tea polysaccharide conjugates). Chemical components, molecular weight and its distribution, water vapor sorption properties, zeta potentials and optical characteristics of TPC-L were investigated. As compared with injured cell group, the two dosages of TPC-L (150 and 300 μg/mL) were discovered to possess remarkably protective effect on human umbilical vein endothelial cells against impairments induced by high glucose in a dose-dependent manner ($p < 0.05$, $p < 0.001$, respectively). Compared with group NC (normal control), the ingestion of 40 mg/kg of TPC-L could significantly reduce blood glucose levels of normal mice ingesting starch, and significant difference of AUC (area under the curve of blood glucose) and ΔAUC ($p < 0.05$, $p < 0.01$) at the postprandial time point of 0.5 and 1.0 h were observed. The three dosages of TPC-L (10, 40 and 160 mg/kg) did not significantly lower postprandial blood glucose levels of normal mice ingesting glucose. TPC-L could improve starch tolerance to prevent impaired glucose tolerance (IGT) from developing into diabetes as well as protective effects on HUVE cells against impairments induced by high glucose It was suggested that TPC-L improved IGT through its capability of inhibition on digestive enzymes.

1. Introduction

The statistics demonstrated that in 2010, 11.6% of Chinese adults were suffering from diabetes mellitus, among which Type 2 diabetes mellitus account for about 90% and easily complicated by cardiovascular and cerebrovascular diseases (Xu et al., 2013). In addition, it is important to notice that the prevalence of prediabetes was estimated to be 50.1% (Xu et al., 2013).

IGT (impaired glucose tolerance) is an intermediate state of metastasis in which the fasting blood glucose level is normal whereas postprandial blood glucose level verges on diabetes mellitus. Although it has not been considered as a disease at the moment, IGT is a main manifestation of prediabetes and a high risk status which could develop into type 2 diabetes mellitus and cardiovascular disease. At present, the percentage of the IGT population transforming to diabetics has reached 8–11% annually in China (Yoon et al., 2006). Therefore, the early intervention on IGT stage can minimize its development into diabetes and reduce the risk of cardiovascular diseases.

Diabetes mellitus, especially type 2 diabetes mellitus, has oxidative damage, since the effectiveness of anti-oxidative factors such as GSH-px, vitamin C, SOD and CAT in the tissues and cells of patients is reduced, suggesting that the damage plays an important role in the

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onset and development of diabetes. The IGT stage occurring before diabetes also accompanies anti-oxidative deficits, and the mutations of mtDNA are ubiquitous in the IGT population. Based on the diversity of these mutations, it is presumed that they are the consequence of IGT rather than the cause and related to the oxidative damage. Therefore, the applications of anti-oxidants such as TPC should be capable to prevent IGT from developing into diabetes and cardiovascular diseases.

In addition to some low-molecular-weight molecules such as tea polyphenols, theanine, theaflavin, etc., the biomacromolecules, tea polysaccharide conjugates, have gained increasing attentions in recent years on account of investigation on several fronts for the anti-oxidative, immunological, and hypoglycemic functions (Chen, Ye, Cheng, Jiang, & Wu, 2009; Lu, Zhao, Sun, Yang, & Yang, 2013; Wang et al., 2000; Zhou, Ding, Wang, & Xie, 1997). The studies have found that the contents of polysaccharide conjugates and polyphenols in summer and autumn tea are higher than those in spring tea (Chen, Ye, Cheng, Jiang, et al., 2009). However, summer and autumn tea are usually inexpensive, and considered as low-grade tea in China owing to poor taste. Therefore, a mass of summer and autumn tea leaves are not picked and utilized because of their low economic values, resulting in a waste of bioresources. Consequently it is of great significance that TPC are prepared from summer and autumn tea.

The objectives of this work were to investigate tea polysaccharides conjugates directly prepared from summer tea leaf (TPC-L), instead of processed tea, and their effects on the IGT of mice and vascular endothelial cells exposed to high glucose. The research results would provide theoretical bases for direct utilization of fresh tea leaves and TPC interposing the IGT population as well as its prevention of diabetes complications such as cardiovascular disease.

2. Materials and methods

2.1. Materials and chemicals

Fresh tea leaves were picked from upper third part of tea trees at the tea garden in the West Lake district, Hangzhou, China. The standard monosaccharides (rhamnose, fucose, arabinose, xylose, mannose, glucose, galactose) were purchased from Merck Co. (Darmstadt, Germany). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Co. (St. Louis, MO). Fetal calf serum (FCS) was provided by Hangzhou Sijiqing Corp. (China). Triethylamine and acetonitrile for chromatography were of HPLC grade from Tedia (Fairfield, USA). The AccQ-Tag Ultra Chemistry Package was obtained from Waters (Milford, Massachusetts). Human umbilical vein endothelial ECV304 cell line was obtained from Xiangya School of Medicine, Central South University. DMEM (Dulbecco's modified Eagle's medium) was purchased from Gina Biomedical Technology Co., Ltd. (China). All other reagents were of analytical grade unless otherwise specified. Male ICR mice (Production License: ZJMA 20080033) which were of SPF grade were purchased from Experimental Animal Center of Zhejiang Province.

2.2. Preparation of polysaccharide conjugates from fresh tea leaves

The fresh tea leaves (1000 g) were rinsed, chopped and incubated in 4.0 L of distilled water with continuous stirring at 90 °C for up to 2 h. After passing through a fine gauze, the filtrates were deproteinized with trichloroacetic acid, bleached with H_2O_2 at 25 °C for 30 min and precipitated with 3 volumes of absolute ethanol.

Subsequent dialysis against distilled water for 24 h was performed, and the retentate was collected and freeze-dried to yield crude tea polysaccharide conjugates. An aliquot (4.5 g) of the latter was loaded into an anion-exchange DEAE-cellulose DE-52 column (3.6 cm × 60 cm), and then eluted stepwise with 0–0.8 M NaCl to give rise to a main fractions, i.e., TPC-L. Following collection, dialysis and lyophilization, it was obtained for the subsequent studies.

2.3. Chemical analysis

Total neutral sugars were evaluated via the anthrone-sulfuric acid method, using glucose as standard (Morris, 1948). Total uronic acid was estimated by m-hydroxydiphenyl method using galacturonic acid as standard (Filisetti-Cozzi & Carpita, 1991). The protein content was determined based on the amino acid content in TPC-L. The composition of amino acids in TPC-L was determined. The overall content of amino acids (OCAA) in TPC-L was summarized. Since the average molecular weight of amino acids which make up proteins is 128 Da and that of the homologous residues is 110 Da (Wang, Zhu, & Xu, 2006), the content of protein in TPC is calculated by OCAA × 110/128.

To test the moisture, each sample (16 mg) was placed in evaporation dish, and heated to constant weight at 115 °C.

2.4. Compositions of monosaccharides and amino acids

The samples in which myoinositol was added as the internal standard were hydrolysed in 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h. The hydrolysate was converted into the alditol acetates via facile acetylation with pyridine and acetic anhydride (1:1, v/v) following reduction with $NaBH_4$. Subsequent analysis was performed on a Hewlett-Packard HP 6890 gas chromatograph (Agilent Technologies Inc.) equipped with a fused-silica capillary column DB-225 (60 m × 0.25 mm, id; film thickness, 0.25 μm) and a flame-ionization detector (FID). The temperatures of injector and detector were 250 and 270 °C, respectively. After keeping the temperature at 190 °C for 3 min, it was raised to 230 °C at a heating rate of 4 °C min^{-1}. Helium was used as a carrier gas (1.2 mL/min, flow rate). Prior to the detection of samples, the standard monosaccharides (L-rhamnose, D-galactose, D-fucose, D-glucose, D-mannose, D-xylose and D-arabinose) and myoinositol were assessed for the identification of their respective typical retention times and electron impact profiles according to the identical procedure.

In the case of amino acid constituents (except for tryptophan), solutions of the samples in 6 M HCl were incubated at 110 °C for 2 h. Subsequent pre-column derivatisation of the resulting hydrolysates was performed with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). An Alliance system (Waters Corp., Milford, MA) equipped with 2695 Separation Module, 2996 Photodiode Array Detector, and 2475 Multi λ Fluorescence Detector was used for HPLC analysis, as described previously (Desportes, Charpentier, Duteurtre, Maujean, & Duchirona, 2000). For the separation of compounds, an AccQ-Tag reversed-phase dC18 column (4 μm, 3.9 mm × 150 mm) thermostated at 37 °C was used, and the eluted AQC derivatives were detected by monitoring their fluorescence (λ_{ex} = 250 nm, λ_{em} = 395 nm).

2.5. Determination of molecular weight of TPC-L

The solution of TPC-L in 0.05 mol/L sodium nitrate (3.5 mg/mL) was applied to a Viscotek TDAmax system (Malvern, UK) equipped with a pair of gel-filtration chromatographic column (7.8 mm × 300 mm) of A600M under a constant flow

(0.700 mL/min) of 0.05 mol/L sodium nitrate at 30 °C. The injection volume was 100 μL, and the eluate was monitored by Viscotek 270_{max} detection system (differential viscometer detector and double-angle (right angle/low angle) light scattering detectors) combined with refractive index (RI) detector V3580.

2.6. Zeta potentials and gel electrophoresis

Solution of TPC-L in water (1 mg/mL, pH 7.14) was monitored with a Zetasizer NanoZS (Malvern Instruments, UK) at a temperature of 25 °C and pH of 7.14. The viscosity and dielectric constant of water were estimated to be 79.0 cP and 0.89, respectively. Agarose gel electrophoresis was performed for the detection of nucleic acid in TPC-L (2 mg/mL, pH 7.0) (Sambrook & Russell, 2001).

2.7. Optical analysis

Infrared spectra of TPC-L was recorded on a Nicolet Nexus 670 FT-IR spectrometer with KBr pellets in the range 4000–400 cm^{-1}. CD spectra of TPC-L dissolved in deionised water (100 μg/mL) were recorded with a Jasco J-815 CD spectrophotometer (Chen, Ye, Cheng, Jiang, et al., 2009).

The ultraviolet–visible (UV) spectra of TPC-L dissolved in deionised water (100 μg/mL) were recorded with a SHIMADZU UV-2550 spectrophotometer (Japan) in the range 700–200 nm.

Chrominance analysis of TPC-L dissolved in deionised water (2.0 and 1.0 mg/mL) were performed with a Minolta Colorimeter (CM-3500d) and the values of L^*, a^* and b^* were determined (Chen, Zhang, et al., 2009).

2.8. Water vapor sorption properties

The water vapor sorption properties of TPC-L were investigated using a dynamic vapor sorption apparatus (DVS intrinsic, Surface Measurement Systems, London, UK). After pre-equilibration, 2.0–5.0 mg of TPC-L was placed onto a precleaned sample pan and then carefully placed on the lop wire in the sample chamber that would be closed afterwards. The experiment conditions on water vapor sorption/desorption were pre-programmed. The temperature was controlled at 25 °C throughout, and the relative humidity (RH) was varied programmatically. When the mass of TPC-L leveled off in the starting RH condition of 0%, the RH was increased to step at 10% increments until reaching 95%, and then decreased in the same ramping rate. The mass variation with the lapse of time dm/dt of 0.00002% min^{-1} was selected to determine the mass equilibrium at each humidity.

2.9. Effect of TPC-L on human umbilical vein endothelial cells exposed to constant high glucose

2.9.1. Endothelial cell culture and treatment

Human umbilical vein endothelial (HUVE) cells were grown in DMEM medium supplemented with 10% FCS (fetal calf serum), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37 °C until desired density of cells is reached. Following further dilution with the culture medium to a final concentration of 5×10^4 cells/mL, the cells were seeded in 96-well plates and incubated for 24 h when they were fused. The cells were allowed to quiesce for another 24 h subsequent to the renewal of culture medium. In the case of cell-based treatments, HUVE cells were incubated with normal glucose (5.5 mM) for 12 h, followed by 12 h incubation with 33 mM of high glucose (cell injury group, CIG). Parallel groups were pretreated with TPC-L (50, 150 and 300 μg/mL) for 12 h before incubation with high glucose. Cells cultured in 5.5 mM of normal glucose or 33 mM of mannitol for 24 h served as normal control (NC) and hypertonic mannitol control (HMC), respectively.

2.9.2. Cell viability

Cytotoxicity was determined by the MTT-based experiment (Mosmann, 1983). A colorimetric assay that measures the ability of viable cells to cleave the yellow tetrazolium salt MTT to purple formazan crystals. In brief, 20 μL of MTT reagent (5 mg/mL) was added to 200 μLof the serum-free culture medium in each cell and after incubating for 4 h, the medium was removed and 150 μL DMSO was added to solubilize the formazan formed by mitochondria reducing in the cell. Absorbance at 490 nm was recorded by a Bio-Rad 680 ELISA plate reader.

2.10. Effect of TPC-L on starch and glucose tolerance of normal mice

Male ICR mice which weighed 24–29 g, were randomly divided into five groups (10 mice in each group). Before the treatment experiment, the mice were fasted for 6 h, and then blood samples were taken from their tail veins for the determination of blood glucose concentration via glucose oxidase kit. Then mice were treated with TPC-L through gavage.

The TPC-L treatments were performed as follows: three TPC-L-treated groups, which were respectively fed with 10, 40 and 160 mg/kg of TPC-L (group TPC-L10, group TPC-L40 and group TPC-L160), a normal control group which were fed with distilled water (group NC) and positive control group which were fed with 100 mg/kg of acarbose (group PC).

Mice in all groups were orally fed with 2.5 g/kg starch at the time 0.5 h after the end of TPC-L treated experiments. The glucose levels in their tail vein blood were measured at the time 0.5, 1, 2 and 3 h after mice ingesting starch, respectively. The area under the curve of blood glucose (AUC) were calculated as follows: $1/4A + 1/2B + 3/4C + D + 1/2E$ (mmol h/l), in which A, B, C, D, E referred to the blood glucose values of mice at different times: the moment before ingesting starch and 0.5 h after ingestion, 1.0 h after ingestion, 2.0 h after ingestion and 3.0 h after ingestion. ΔAUC, namely AUC value added, was given by a formula: $\Delta AUC = AUC - A \times 3$ h.

The experiments of TPC-L effect on glucose tolerance of normal mice were carried out as described above. Mice in all groups were orally ingested 2.5 g/kg of glucose at the time 0.5 h after the end of TPC-L treated experiments. The glucose levels in their tail vein blood were measured at the time 0.5, 1 and 2 h after mice ingesting glucose, respectively. The area under the curve of blood glucose (AUC) were calculated as follows: $1/4A' + 1/2B' + 3/4C' + 1/2D'$ (mmol h/l), in which A', B', C', D' referred to the blood glucose values of mice at different times: the moment before ingesting glucose, 0.5 h after ingestion, 1.0 h after ingestion and 2.0 h after ingestion. ΔAUC, namely AUC value added: $\Delta AUC = AUC - A' \times 2$ h.

These experiments were in agreement with Guidelines on the Care and Use of Animals for Scientific Purposes and Laboratory Animal Permit Management Regulations (Trial).

2.11. Statistical analysis

All the data were expressed as means ± standard deviation (SD). The comparisons between groups were analyzed by Dunnett-t two-tailed test of one-way ANOVA. All statistical methods were performed by the statistical software Statistical Package for Social Sciences 13.0 (SPSS: Chicago, IL, USA, 2001). Values of $p < 0.05$ and $p < 0.01$ were considered to be significant and very significant respectively.

Table 1
Compositions of monosaccharides and amino acids.

Monosaccharide composition (mol%)	
Rha	21.16
Xyl	1.72
Ara	35.33
Man	3.54
Glc	3.41
Gal	34.84
Composition of amino acids (mg/g)	
Asp	6.54
Glu	13.27
Ser	3.39
Gly	3.95
Arg	1.22
Thr	3.05
Ala	1.82
Tyr	4.43
Cys	13.34
Val	3.40
Ile	1.56
Leu	2.71
Lys	1.92
Phe	6.20
His	1.28

Rha, rhamnose; Xyl, xylose; Ara, arabinose; Man, mannose; Glc, glucose; Gal, galactose.

3. Results

3.1. Contents of chemical components

The contents of neutral sugar, uronic acid and H_2O in TPC-L were 49.68%, 14.10%, and 15.35% respectively. Monosaccharide and amino acid compositions of TPC-L were measured and summarized in Table 1. GC–MS analysis indicated that TPC-L was composed of six monosaccharides, namely rhamnose, xylose, arabinose, mannose, glucose and galactose. The three monosaccharides, that is, monosaccharides, rhamnose, arabinose and galactose, account for more than 90%. A total of 15 essential amino acids were detected in TPC-L and the overall content was 68.06 mg/g. In terms of protein moieties of TPC-L, the content is calculated as $68.06 \times 110/128 = 58.49$ mg/g.

3.2. Determination of molecular weight of homogeneous components in TPC-L

TPC-L contains neutral sugar, uronic acid and protein. HPGPC with a double-angle laser photometer and a refractive index detector can accurately measure the distribution proportion and molecular weight of homogeneous components in TPC-L. TPC-L was found to have a total of four homogeneous components with the molecular weight of 1.16×10^6, 1.96×10^5, 5.68×10^4, and 3.06×10^4 Da in mass proportion of 21.58%, 10.09%, 21.40% and 46.93%, respectively (see in Fig. 1).

3.3. Zeta potentials and gel electrophoresis

Zeta potentials of TPC-L in water (2 mg/mL) were determined to be −20.6 mV, suggesting that the molecules of aqueous solutions present the behavior of fine stability. The result of agarose gel electrophoresis demonstrated that there were no nucleic acids in TPC-L.

3.4. Optical analysis

The IR spectrum of TPC-L revealed main absorption bands at 3409, 2924, 1624, 1424, 1382, 1075 (shown in Fig. 2). The strong absorption band around 3409 cm^{-1} was attributed to stretching vibration of O—H while the band at 2924 cm^{-1} was due to that of C—H. The presence of a broad band at 1624 cm^{-1} was in association with absorbed water, and suggested that TPC-L held a strong affinity for moistures. A combination of two intensive bands at 1424 and 1382 cm^{-1} was characteristic of C—O stretch and C—H or OH bending. The broad band at 1075 cm^{-1} was dominated by the glycosidic linkage ν(C—O—C) and ν(C—O—H) contributions (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000).

The chroma of TPC aqueous solution can be quantitatively measured and analyzed through L^*, a^*, b^* table color system. The values of L^*, a^* and b^* of TPC-L (1.0 mg/mL) were 97.08, −0.25, 5.1 respectively as well as 95.00, −0.36, 10.08 (2.0 mg/mL). In the table color system, L^* is the index of lightness. The value of L represents the brightness with a positive correlation.

The a^* is an index of chrominance indicating red and green. The positive value of a^* means red and negative value for green. The b^* is an index of chrominance indicating yellow and blue. The positive value of b^* means yellow and the negative value for blue. The higher the absolute value of a^* or b^*, the greater the corresponding chroma (Chen, Zhang, et al., 2009). These values manifested that TPC-L aqueous solution showed high brightness and slightly light yellowish green which was in accordance with the experience of the senses.

The UV–vis spectra scanning of TPC-L was carried out in the range of 200–700 nm (see Fig. 3). The spectra of TPC-L displayed a very weak unsmooth absorption curve in the region of 255–360 nm. Because TPC-L possessed no nucleic acid, no characteristic absorption peak at 280 nm and weak light yellowish green of aqueous

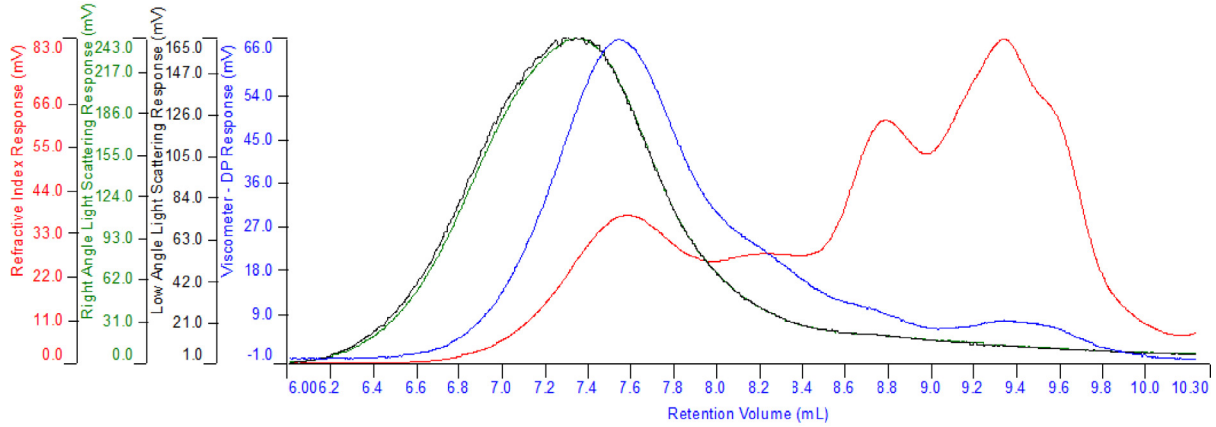

Fig. 1. Elution profiles of TPC-L in GPC, using Viscotek 270_{max} (Malvern, USA).

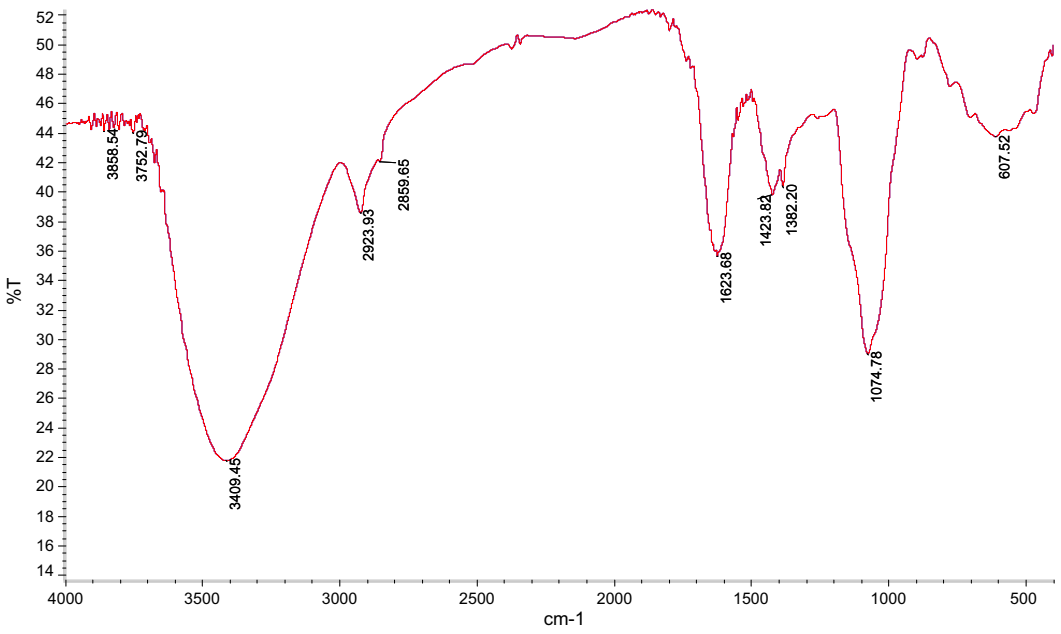

Fig. 2. Infrared spectrum of TPC-L in the range 4000–400 cm^{-1}.

solution, a very weak unsmooth absorption curve in the regions of 250–360 nm was caused by pigments that firmly adsorbed TPC-L.

To get further insight into the physical and chemical character, CD experiment was performed on TPC-L. As depicted in Fig. 4, the CD spectrum of TPC-L revealed negative Cotton effects at 222 nm.

3.5. Water vapor sorption properties

The moisture sorption–desorption isotherms for TPC-L show typical S-shape curve illustrated in Fig. 5. The desorption isotherm is above the corresponding sorption one and exhibits hystere-

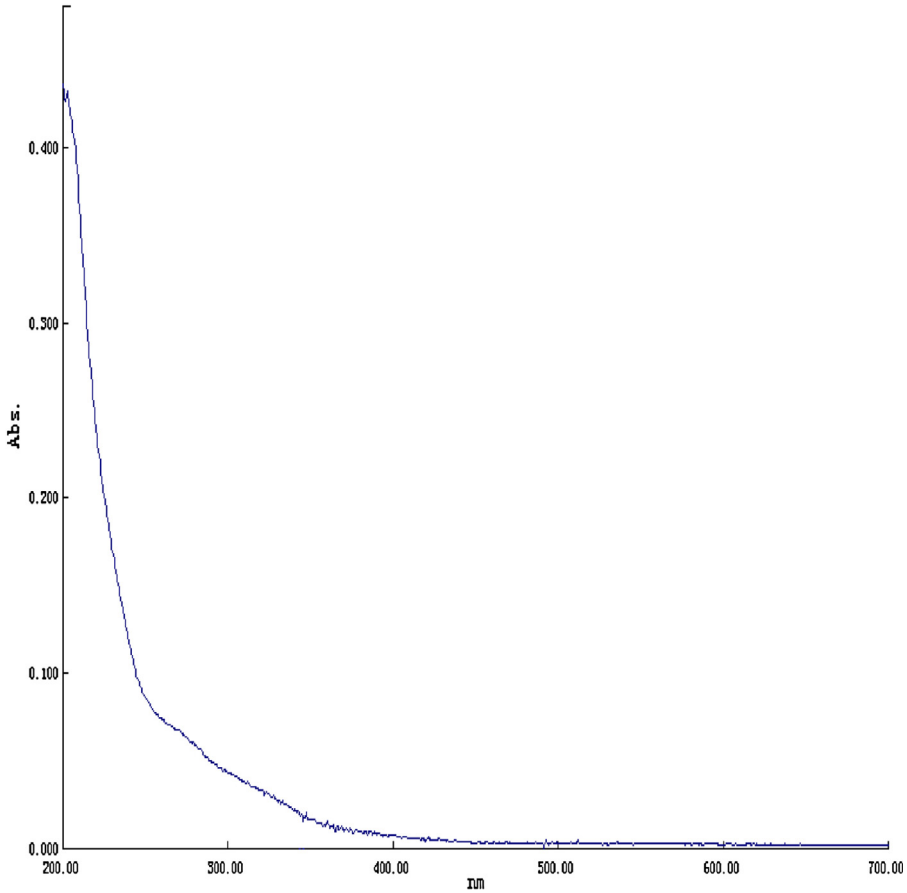

Fig. 3. UV–vis spectra of TPC-L. The spectra were measured in deionized water.

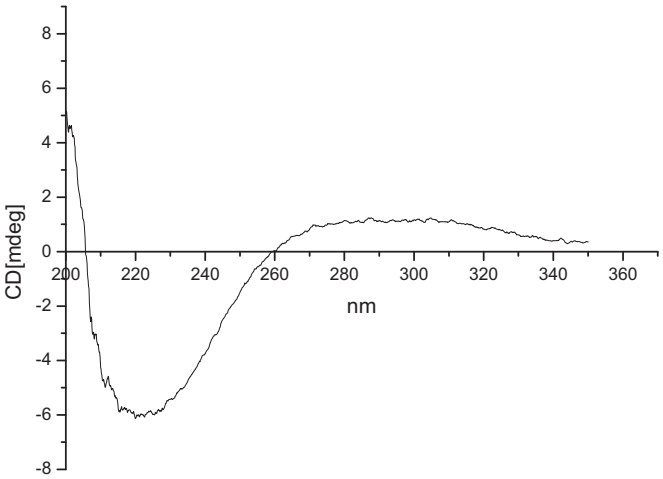

Fig. 4. CD spectra of TPC-L. The spectra were measured in deionized water.

sis between 0 and 80% RH. These results indicated that the desorption–sorption isotherms are of type II (Lowell, Shields, Thomas, & Thommes, 2010). From 0 to 60% RH in water vapor, TPC-L moisture absorbed not more than 15% of its dry weight (14.53%). From 70% to 95% RH, the moisture sorption capacity of TPC-L grew up quickly with uptake from 16.60% to 43.94% of its dry weight. These results showed that TPC-L had strong water retention, and easier to be stored in humidity conditions of less than 60% RH due to its lesser moisture absorption.

3.6. Effect of TPC-L on high glucose-induced loss of HUVE cells viability

After cultured with normal glucose (5.5 mM) in 96-well plate for 12 h, exposure of vascular endothelial cells to 33 mM of high glucose (cell injured group, CIG) for 12 h led to a significant decrease by 28% vs. normal control (NC) in cell viability ($p < 0.01$, Fig. 6). As illustrated in Fig. 6, a concomitant loss of cell viability was not observed in cells that were incubated with 33 mM of mannitol. TPC-L was found to inhibit high glucose-induced HUVE cells loss in a dose-dependent manner, and more interestingly, exhibited a

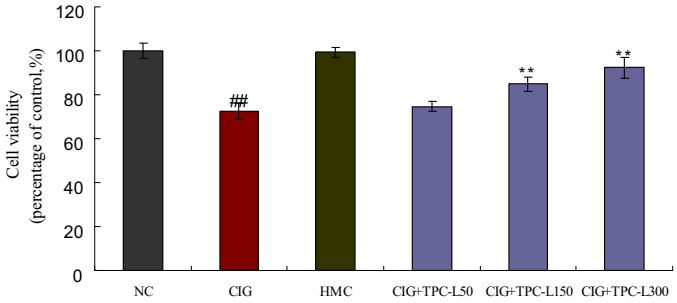

Fig. 6. Effects of TPC-L on high glucose-induced loss of cell viability. HUVE cells pretreated with the indicated concentrations of TPC-L (numerical values embedded in bars, μg/mL) were exposed to high glucose (33 mM) for 12 h. Mean ± SEM of 8 experiments. NC, normal control. HMC, hypertonic mannitol control. CIG, cell injury group. $^{\#\#}p < 0.01$ vs. NC; $^{*}p < 0.05$ vs. CIG; $^{**}p < 0.01$ vs. CIG.

significantly protective effect starting from the concentration of 150 μg/mL ($p < 0.01$).

3.7. Effect of TPC-L on starch and glucose tolerance of normal mice

After food intake, postprandial blood glucose of normal mice reached peak levels. Among the three doses, 40 mg/kg of TPC-L could significantly reduce blood glucose levels of normal mice ingesting starch at the postprandial time 0.5 and 1.0 h, and led to the significant difference of AUC and ΔAUC ($p < 0.05$, $p < 0.01$) compared with group NC (shown in Table 2 and Fig. 7).

The 10 mg/kg of TPC-L could decrease the postprandial blood glucose level of normal mice ingesting starch at the postprandial time 0.5 h and 1.0. The reductions of both 0.5 h postprandial blood glucose ($p < 0.01$) and of ΔAUC revealed significant difference ($p < 0.01$, $p < 0.05$, respectively).

As shown in Table 3, these three dosages of TPC-L did not significantly lower postprandial blood glucose levels of normal mice ingesting glucose after 0.5, 1 and 2 h.

It has been reported that tea polysaccharide conjugates have a strong inhibiting effect on amylase, intestinal sucrase and maltase in vitro (Ding, He, & Jie, 2005; He, Xue, Wei, & Jiang, 2007). This inhibition might prevent carbohydrate polymers such as starch from being degraded into glucose in living body, and thereby prevent

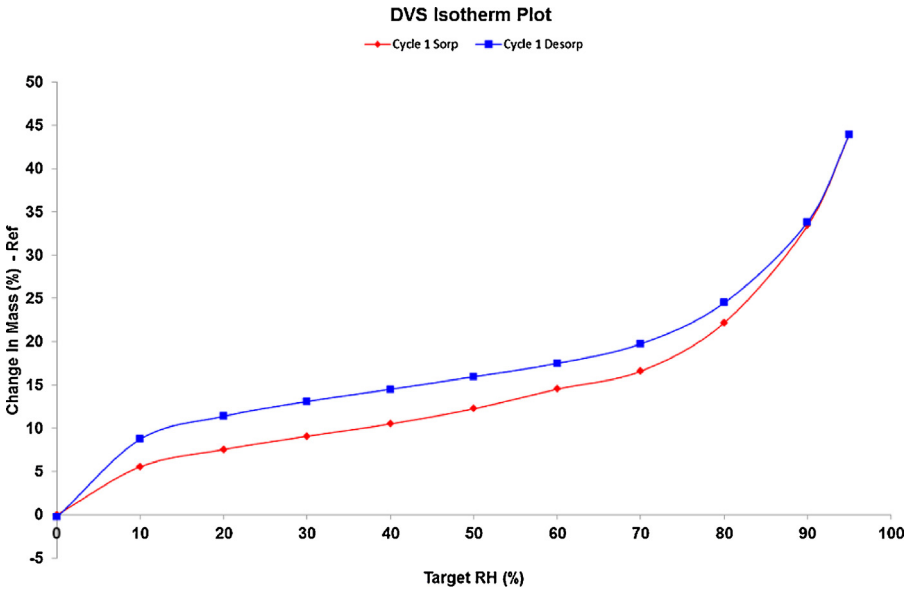

Fig. 5. Water sorption/desorption isotherms obtained by using dynamic vapor sorption (DVS) meter (25 °C, 0–95% RH).

Table 2
Effect of TPC-L on starch tolerance of normal mice (mmol/l, $n = 10$).

Groups	Dosage (mg/kg)	FBG	BG level after mice ingesting starch					
			0.5 h	1 h	2 h	3 h	AUC	ΔAUC
NC	–	5.01 ± 0.71	10.32 ± 1.00	11.45 ± 0.72	8.37 ± 0.64	6.87 ± 0.73	26.8 ± 1.8	11.8 ± 1.1
PC	100	4.97 ± 0.67	6.40 ± 0.64**	7.18 ± 0.66**	6.53 ± 0.55**	6.36 ± 0.44	19.5 ± 1.5**	4.6 ± 1.6**
TPC-L160	160	4.97 ± 0.97	9.79 ± 0.80	10.98 ± 0.84	8.66 ± 0.97	6.32 ± 0.84	26.2 ± 2.3	11.3 ± 1.4
TPC-L40	40	4.95 ± 0.73	9.10 ± 0.72**	10.39 ± 0.91**	7.87 ± 0.85	6.19 ± 0.96	24.6 ± 1.8*	9.7 ± 1.7**
TPC-L10	10	4.95 ± 0.95	8.85 ± 1.12**	10.56 ± 1.17	8.59 ± 0.90	6.42 ± 0.87	25.4 ± 2.7	10.5 ± 0.9*

FBG, fasting blood glucose; BG, blood glucose
* Compared with normal control (group NC), $p < 0.05$.
** Compared with normal control (group NC), $p < 0.01$.

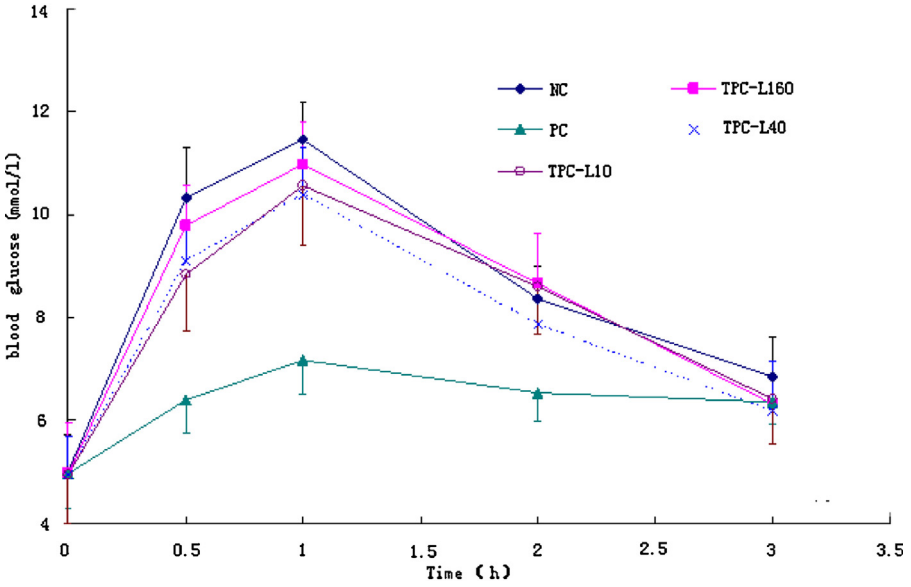

Fig. 7. Effect of TPC-L on blood glucose level of mice ingesting starch.

Table 3
Effect of TPC-L on glucose tolerance of normal mice (mmol/l, $n = 10$).

Groups	Dosage (g/kg)	FBG	BG level after mice ingesting glucose (mmol/l)				
			0.5 h	1 h	2 h	AUC	ΔAUC
NC		5.33 ± 0.74	10.68 ± 1.12	8.39 ± 0.60	7.17 ± 0.54	16.6 ± 1.1	5.9 ± 1.1
PC	100	5.32 ± 0.46	11.33 ± 1.32	8.96 ± 0.89	7.13 ± 0.76	17.3 ± 1.5	6.6 ± 1.2
	160	5.29 ± 0.47	11.03 ± 1.36	8.30 ± 1.14	6.86 ± 042	16.5 ± 1.5	5.9 ± 1.6
TPC-L	40	5.33 ± 0.73	10.50 ± 1.35	8.35 ± 1.47	6.77 ± 0.76	16.2 ± 2.1	5.6 ± 2.0
	10	5.33 ± 0.57	10.47 ± 0.75	8.35 ± 0.34	6.91 ± 0.53	16.3 ± 0.7	5.6 ± 0.9

FBG, fasting blood glucose; BG, blood glucose
*Compared with normal control (group NC), $p < 0.05$.
**Compared with normal control (group NC), $p < 0.01$.

blood glucose to ascend. We verified this inhibition in vivo through the comparative trial of mice uptake of starch and glucose.

It was suggested that TPC-L could improve impaired glucose tolerance of normal mice through its capability of inhibiting these digestive enzymes, nevertheless the inhibitory effect of TPC-L may have an optimal dose.

4. Discussion

This study shows that fresh tea leaves can be directly applied as raw materials to prepare tea polysaccharide conjugates of high-activity, in stead of processed tea. The direct utilization of fresh tea leaves can reduce material cost and save energy consumption.

According to the determination on molecular weight distribution, tea polysaccharide conjugates prepared from unprocessed fresh tea leaves are composed of both high and low molecular weights. Diverse tea processing technologies that produce six kinds of traditional Chinese tea such as green tea, white tea, yellow tea, black tea, oolong tea, puerh tea might have various effects on the polysaccharides conjugates especially in terms of molecular weight distribution.

The CD spectra of protein possess a strong signal and high sensitivity in the far-UV circular dichroism region, i.e. 200–240 nm. Tea polysaccharide conjugates (TPC) are heteropolysaccharides combined with a small quantity of protein. Therefore, CD can be used a spectral probe to analyze solution conformation of TPC and effective means of quality control and testing in the preparation process of polysaccharide (Chen, Ye, Cheng, Jiang, et al., 2009). The CD spectra of TPC-L would enrich the database of carbohydrates.

The characterization by DVS provided many parameters that could reflect material properties. It showed that TPC-L has strong capability of moisture adsorption that surpasses those of microcrystalline cellulose, chitosan as previously reported (Agrawal, Manek, Kolling, & Neau, 2004; Driemeier, Mendes, & Oliveira, 2012).

The mortality risk of cardiovascular disease in type 2 diabetics does not hide in the duration of diabetes, but the prediabetes, namely IGT stage. The results of this study demonstrated that TPC-L could improve starch tolerance thereby to prevent IGT from developing into diabetes as well as protective effects on HUVE cells against impairments induced by high glucose in a dose-dependent manner.

As is well-known, IGT can be converted to diabetes and cardiovascular disease. The endothelial dysfunction is the initial stage of cardiovascular disease, and high levels of blood glucose can cause excessive oxygen free radicals that engender toxic effects on endothelial cells through oxidative stress production. In general, these reactive oxygen species (ROS) can be scavenged by a variety of intra- and extra cellular mechanisms, but the latter in a situation of oxidative stress is insufficient to cope with the excessive generation of ROS. Significant preventions of TPC-L on HUVE cell loss in the presence of high glucose relates to its potential scavenging potency of ROS.

Though many researches on the anti-oxidation of TPC have been carried out (Nie & Xie, 2011; Xiao, Huo, Jiang, & Yang, 2011), the application of TPC to interposing the IGT population has just begun. TPC will be used as a supplementary nutrient or medicine, and their effect, mechanism, dosage need to be further studied.

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