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# Suppression of diabetes in non-obese diabetic (NOD) mice by oral administration of water-soluble and alkali-soluble polysaccharide conjugates prepared from green tea

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ABSTRACT

Water-soluble tea polysaccharide conjugates (TPC-W) and alkali-soluble tea polysaccharide conjugates (TPC-A) were extracted from green tea by hot and alkali water respectively. Physicochemical properties of TPC-W and TPC-A were analyzed. Non-obese diabetic (NOD) mice were used to evaluate antidiabetic bioactivities of TPC-W and TPC-A. The daily oral administration of 150 mg $kg^{-1}$ TPC-W can significantly decrease the level of blood glucose in NOD mice. The anti-glutamic acid decarboxylase (GAD) antibody level in NOD mice treated with 150 mg $kg^{-1}$ TPC-W decreased 27% ($P < 0.05$). To the end of trial, only 2 out of 10 mice in NOD groups treated with TPC-W or TPC-A exhibited diabetic symptoms compared with model control group, in which 7 of 10 mice developed diabetes. The result of organ index showed that both TPC-W and TPC-A can protect thymus from shriveling to some extent. In sum, our studies demonstrated that both TPC-W and TPC-A can suppress spontaneous diabetes mellitus in NOD mice.


## 1. Introduction

Tea, a daily beverage widely consumed in China and other countries, demonstrates great benefits to human health. In the past decades, much attention has been paid for its bioactivities which include antioxidation (Satoshi & Hara, 1990), anticancer (Isao, 1990; Yang, Wang, Lu, & Picinich, 2009), antimutagenesis (Jain et al., 1989; Kada, Kaneko, Matsuzaki, Matsuzaki, & Hara, 1985; Yen & Chen, 1994) and so on. These activities are associated with its constituents, such as tea polysaccharide conjugates (TPC), tea polyphenols (or catechin), tea pigments, cafeine, and theanine.

TPC, a promising constituent in tea, attracted much attention in the world for its antidiabetic and immunomodulatory activities (Kenichi, Tkuro, & Tadakazu, 1992). It has been reported that TPC showed antidiabetic activity in the mice or rats with hyperglycemia. However, TPC used in previous report actually is the water-soluble one (TPC-W). In our study, we extracted alkali-soluble polysaccharide conjugates (TPC-A) from tea by diluted alkali. TPC-A shows significant difference with TPC-W in the physicochemical properties and bioactivity.

To evaluate antidiabetic activity of TPC, people applied animal model that the mice or rats were induced to hypoglycemia by administration of toxin (streptozotocin or alloxan) in the previous studies. It should be noted that in these models, diabetes can occur even in the absence of functional T and B cells and cannot be reliably transferred to syngenic recipients by the transfer of splenocytes in contrast to the spontaneous animal models such as NOD mice (Arata et al., 1994), can spontaneously develop autoimmune responses mediated by T cells, which is similar to human Type 1 diabetes (Arata et al., 1994). As an ideal animal model, the NOD mice have been widely used in biomedical research to explore the features and mechanism of IDDM since its development. Therefore, we employed NOD mice model to investigate antidiabetic activity of TPC in our study.

To better understand the bioactivity of TPC, we extracted TPC-W and TPC-A from green tea, characterized both of them and evaluated their antidiabetic activity in NOD mice model. The result would help investigate the hypoglycaemic mechanism of TPC and define the health claims related to tea consumption.

*Abbreviations:* NaOH, sodium hydroxide; RT, retention time; ELISA, enzyme-linked immunosorbent assay; B.A., before administration; A.A., after administration.

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## 2. Materials and methods

### 2.1. Materials

Female non-obese diabetic (NOD) mice (19–25 g) and female ICR mice (22–24 g) of SPF grade were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (SLAC, CAS, China). Glutamic acid decarboxylase (GAD) antibody ELISA Kit was purchased from Limi Biotechnology (Beijing, China).

Low-grade green tea was donated by Tea Research Institute of China Academy, Agricultural Sciences (Hangzhou, Zhejiang Province). D-Galacturonic acid (GalA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The protein determination kit was purchased from Biyuntian Co., Ltd. (China). The standard monosaccharides (rhamnose, fucose, arabinose, xylose, mannose, glucose, galactose) were purchased from Merck Co. (Darmstadt, Germany).

### 2.2. Preparation of water-soluble and alkali-soluble polysaccharide conjugates from green tea

The isolated polysaccharides from green tea were sequentially extracted with hot water and hot alkaline aqueous solution. The powder of low-grade green tea (250 g) was suspended in 3500 ml of distilled water, and incubated at 90 °C for up to 2 h under the continuous stirring condition. The extract was filtered (the residue for extraction by hot alkali), concentrated by vacuum freeze-dryer, and deproteinized with trichloroacetic acid. After centrifuged (9000 rpm, 5 min), the supernatant was adjusted to pH 7.0 by NaOH, bleached with $H_2O_2$ at 30 °C for 1 h, and then precipitated with 4 volumes of absolute ethanol. Subsequent dialysis against distilled water for 24 h gave rise to the retentate by the membranes with molecular weight cut-off of 7 kDa. The retentate was collected and lyophilized to afford water-soluble tea polysaccharide conjugates (TPC-W). The residue was subsequently incubated at 50 °C for 2 h with 4 L of 1.0% aqueous sodium hydroxide. The extract from the residue was filtered, concentrated, adjusted to pH 9.0 by acetic acid, bleached with $H_2O_2$, precipitated, dialyzed and lyophilized as described above to obtain alkali-soluble tea polysaccharide conjugates (TPC-A).

### 2.3. Physicochemical analysis of tea polysaccharide conjugates

#### 2.3.1. Main component analysis

Neutral sugar contents were measured by 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 contents were determined according to the method of Bradford with Coomassie Brilliant Blue and bovine serum albumin (BSA) as the standard (Bradford, 1976). To test the moisture, samples were placed in evaporation dish, and heated to constant weight at 115 °C.

#### 2.3.2. Monosaccharide and amino acid composition analysis

TPC-W and TPC-A were hydrolyzed respectively in 2 M trifluoroacetic acid at 120 °C for 2 h. The hydrolysate was converted into its respective alditol acetates by reduction with $NaBH_4$ and acetylation with $AC_2O$ (Dai, Zhang, Zhang, & Wang, 2009), which were analyzed by HP 6890 GC (Hewlett-Packard, USA) equipped with a capillary column DB-225 (60 m × 0.25 mm i.d., film thickness 0.25 μm) and a flame-ionization detector (FID). The injector and detector temperatures were 250 and 270 °C, respectively. The column temperature program was set to hold for 3 min at 190 °C, then increase to 230 °C at 4 °C per min. Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The standard monosaccharides with myoinositol as the internal standard) were measured following the same procedure.

TPC-W and TPC-A were respectively dissolved in 6 mol/L HCl and hydrolyzed at 110 °C for 2.0 h. The hydrolysates were derivated and analyzed according to Waters AccQ-Tag pre-column derivatization manual by HPLC using a Waters alliance w2695 system with a fluorescence detector and a AccQ-Tag $NH_2$ column. The fluorescence excitation and emission wavelengths were set at $\lambda_{ex}$250 nm and $\lambda_{em}$395 nm, respectively.

#### 2.3.3. Distribution and determination of molecular weight of homogeneous polysaccharide constituent

Solutions of TPC-W and TPC-A dissolved in 0.1 mol/L sodium nitrate (3.5 mg/ml) were respectively applied to a Waters HPLC system (Allances 2695, Waters, USA) equipped with a gel-filtration chromatographic column (7.8 mm × 300 mm) of OHpak SB-802HQ (Shodex, Japan) in conjunction with OHpak SB-804HQ under a constant flow (0.500 ml/min) of 0.1 mol/L sodium nitrate at 35 °C. The injection volume was 200 μL, and the eluate was monitored by a multi-angle laser photometer (DAWN HELEOS, Wyatt Technology Co., USA) combined with refractive index (RI) detector (Optilab rEX, Wyatt Technology Co., USA).

### 2.4. NOD mice

Female NOD mice and ICR female mice were selected for these experiments. The animals were housed in a SPF animal laboratory of barrier system at 22 ± 1 °C, with a 12 h light–dark cycle, the humidity of 50–70% and low noise (<50 dB). The animals were fed with a complete diet. All the mice were allowed free access to food and filtered water.

### 2.5. NOD mice grouped and treated by TPC-W and TPC-A

12-week-old female NOD mice which weighed 19–25 g and had no diabetic symptoms, according to blood glucose levels and body weight, were randomly and averagely divided into groups ($n$ = 10): group TPC-W-50, TPC-W-150, TPC-A-50 and TPC-A-150 NOD mice, which refered to mice fed 50, 150 mg $kg^{-1}$ of TPC-W or TPC-A daily correspondingly once a day for 10 weeks running. 10 NOD mice and 10 female ICR mice, fed 10 ml $kg^{-1}$ of physiological saline daily regarded as model control group (group MC) and normal control group (group NC) respectively.

### 2.6. Assessment of diabetic symptoms

The mice were weighed and the blood was taken from tail vain to determine fasting blood glucose values by OneTouch® Ultra glucometer (Johnson, USA) once a week. Diabetes was defined by a blood glucose level exceeding 10.0 mmol/L on two consecutive weekly measurements. 24 h after the last administration of TPC-W or TPC-A, mice blood was drawn and serum was separated by centrifugation (3000 rpm, 10 min). The concentration of anti-GAD antibody in serum was quantified using an ELISA method. Observation ended at the age of 22 weeks. Then the mice were sacrificed for measuring the weights and organ indexes of spleen and thymus.

### 2.7. Statistical analysis

Except the data of cumulative incidence, all other data was expressed as means ± standard deviation (SD). Comparison of cumulative incidence of diabetes between treated and model control group was analyzed using the $\chi^2$ test, and other comparisons between groups were analyzed using Student's $t$-test. All statistical methods were performed by the statistical software Statistical Package for Social Sciences 13.0 (SPSS, Chicago, IL). Values of $P < 0.05$

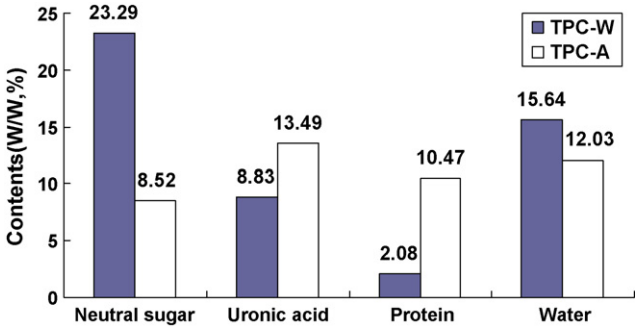


**Fig. 1.** Levels of chemical composition in TPC-W and TPC-A (numerical values (%) represent the percentage composition).

were considered to be significant and values of $P < 0.01$ very significant.

## 3. Results

### 3.1. Chemical analysis of TPC-W and TPC-A

TPC-W, an offwhite and loose powder, was isolated from extracts of green tea after sequential deproteinization, neutralization, decolorization, concentration, and precipitation with ethanol. After filtration, the TPC-A, a canary yellow and loose powder, was further isolated from the extracts of the residues by hot alkali aqueous solution. Fig. 1 showed partial chemical composition of TPC-W and TPC-A. There is high content of moisture in both TPC-W (15.64%) and TPC-A (12.03%). Compared with TPC-W, TPC-A contained more protein and uronic acid, and less neutral sugar.

GC–MS analysis indicated that TPC-W and TPC-A were both composed of seven monosaccharides, namely rhamnose, fucose, arabinose, xylose, mannose, glucose and galactose with molar ratios of 8.74:4.69:29.04:0.42:7.11:14.10:35.89 and 13.81:1.43:36.07:5.24:4.89:6.28:32.27 respectively (shown in Figs. 2–4). Arabinose and galactose were dominant monosaccharide ingredients in TPC-W and TPC-A. There is difference in monosaccharide composition of TPC among different tea resources and isolation procedures. In our preliminary study, if using monosaccharide mixtures with molar ratios in TPC as standard for calibration, the value of neutral sugar contents in TPC was as 140–180% as the one using glucose as the standard. Regarding that

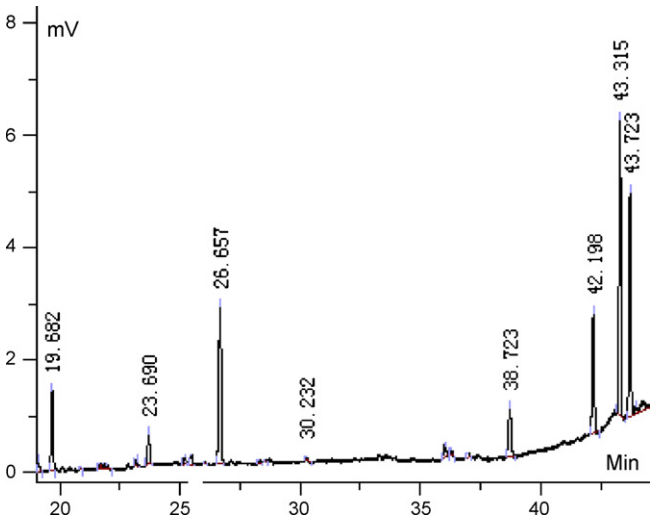


**Fig. 3.** Gas chromatogram of monose compositions of water-soluble tea polysaccharide conjugates (TPC-W).

factor, the exact content of tea polysaccharide and binding protein in either TPC-W or TPC-A is more than 50% after removing of moisture content. Considering the consistence with the result reported before, we still used glucose as the standard for calibration. Owing to complexity of the polysaccharide composition, the accurate determination of saccharide content in TPC is still a challenge which remains to be elucidated.

Amino acid analysis showed that the protein portion of TPC-W and TPC-A consisted of 16 amino acids (mg $g^{-1}$): Asp 3.46, Ser 1.54, Glu 4.54, Gly 3.23, Arg 1.59, Thr 0.94, Ala 1.21, Pro 0.83, Val 0.90, Lys 1.89, Ile 0.46, Leu 0.88, Phe 0.51, His 0.42, Tyr 0.47, Met 0.04 and Asp 18.49, Ser 7.65, Glu 20.45, Gly 11.30, Arg 8.17, Thr 6.75, Ala 9.23, Pro 7.86, Val 9.96, Lys 6.43, Ile 7.40, Leu 11.49, Phe 7.26, His 0.27, Tyr 4.22, Met 0.07 respectively.

### 3.2. Distribution and molecular weight of homogeneous components in TPC-W and TPC-A

TPC-W and TPC-A contain neutral sugar, uronic acid and protein. Their behaviors in high performance gel permeation chromatography (HPGPC) differed tremendously from glucan or dextran, which usually were used as standards for the determination of molecular

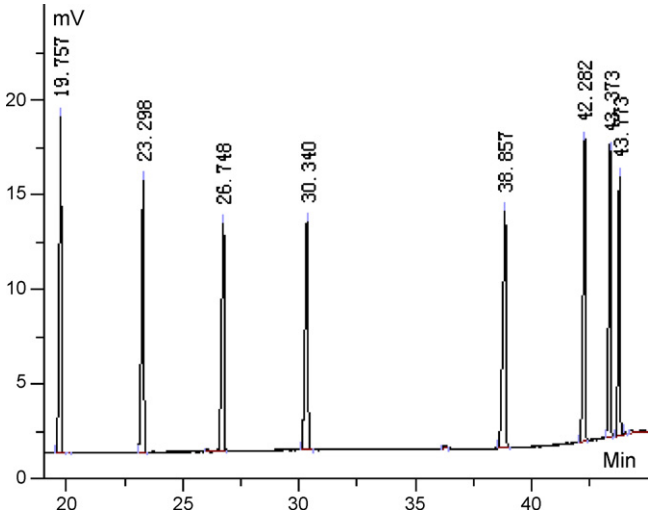


**Fig. 2.** Gas chromatograms of mixture standards (sort by time: rhamnose, fucose, arabinose, xylose, mannose, glucose, galactose, internal standard).

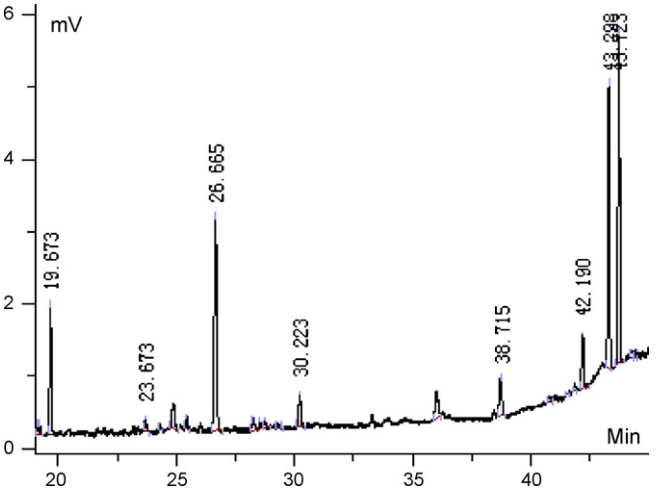


**Fig. 4.** Gas chromatogram of monose compositions of alkali-soluble tea polysaccharide conjugates (TPC-A).

**Table 1**
Effect of TPC-W and TPC-A on blood glucose in NOD mice (mmol/L, $\overline{X} \pm s$, $n = 10$).

| Group/dosage | NC (10 ml kg$^{-1}$) | MC (10 ml kg$^{-1}$) | TPC-W-50 (50 mg kg$^{-1}$) | TPC-W-150 (150 mg kg$^{-1}$) | TPC-A-50 (50 mg kg$^{-1}$) | TPC-A-150 (150 mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| B.A. | 5.03 ± 1.08 | 4.02 ± 0.53 | 3.88 ± 0.39 | 3.78 ± 0.44 | 3.84 ± 0.57 | 3.80 ± 0.64 |
| A.A. | | | | | | |
| 1 | 5.45 ± 0.76 | 4.90 ± 1.06 | 4.00 ± 0.55 | 4.45 ± 0.53 | 4.30 ± 1.01 | 4.34 ± 1.16 |
| 2 | 4.85 ± 0.81 | 3.90 ± 0.69 | 4.78 ± 0.69 | 4.60 ± 0.59 | 4.68 ± 0.48 | 4.34 ± 1.09 |
| 3 | 6.51 ± 0.44 | 9.24 ± 7.59 | 5.62 ± 0.73 | 5.93 ± 0.50 | 7.27 ± 4.63 | 5.94 ± 0.94 |
| 4 | 5.87 ± 0.50 | 11.60 ± 10.31 | 6.45 ± 1.23 | 5.96 ± 0.38 | 8.18 ± 6.77 | 6.44 ± 1.78 |
| 5 | 5.75 ± 0.49 | 12.14 ± 10.26 | 5.94 ± 0.87 | 5.78 ± 0.72 | 8.64 ± 7.12 | 8.15 ± 8.14 |
| 6 | 6.58 ± 0.45 | 13.45 ± 10.74 | 6.27 ± 2.36 | 5.64 ± 0.64* | 7.69 ± 7.63 | 9.74 ± 9.13 |
| 7 | 6.73 ± 0.54 | 13.05 ± 9.35Δ | 7.27 ± 3.55 | 6.10 ± 0.51* | 8.18 ± 6.81 | 9.77 ± 8.62 |
| 8 | 6.30 ± 0.59 | 16.42 ± 11.51Δ | 8.87 ± 7.89 | 6.22 ± 0.62* | 8.72 ± 5.85 | 9.63 ± 8.96 |
| 9 | 6.62 ± 0.46 | 18.76 ± 11.12ΔΔ | 9.56 ± 6.70* | 8.20 ± 1.97** | 10.24 ± 7.50 | 11.28 ± 9.81 |
| 10 | 7.13 ± 0.66 | 18.92 ± 11.76ΔΔ | 9.9 ± 8.32 | 7.16 ± 2.71** | 9.86 ± 8.24 | 10.78 ± 9.88 |

*Note*: Compared with normal control, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with model control, $^{*}P < 0.05$, $^{**}P < 0.01$.

**Table 2**
Effect of TPC-W and TPC-A on the incidence of diabetes in NOD mice ($n = 10$).

| Group/dosage | NC (10 ml kg$^{-1}$) | MC (10 ml kg$^{-1}$) | TPC-W-50 (50 mg kg$^{-1}$) | TPC-W-150 (150 mg kg$^{-1}$) | TPC-A-50 (50 mg kg$^{-1}$) | TPC-A-150 (150 mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| B.A. | 0 | 0 | 0 | 0 | 0 | 0 |
| A.A. | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 2 | 0 | 0 | 1 | 0 |
| 4 | 0 | 2 | 0 | 0 | 1 | 1 |
| 5 | 0 | 3Δ | 0* | 0* | 1 | 1 |
| 6 | 0 | 4Δ | 1 | 0* | 1 | 2 |
| 7 | 0 | 5ΔΔ | 1* | 0** | 1* | 2 |
| 8 | 0 | 6ΔΔ | 1* | 0** | 2* | 2* |
| 9 | 0 | 7ΔΔ | 2* | 2* | 2* | 2* |
| 10 | 0 | 7ΔΔ | 2* | 2* | 2* | 2* |

*Note*: Compared with normal control, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with model control, $^{*}P < 0.05$, $^{**}P < 0.01$.

weight of polysaccharide. HPGPC with a multi-angle laser photometer and a refractive index detector can accurately measure the distribution proportion and molecular weight of homogeneous components in TPC-W and TPC-A. TPC-W was found to have a total of three homogeneous components with the molecular weight of $4.55 \times 10^6$, $4.85 \times 10^4$ and $6.62 \times 10^3$ Da in mass proportion of 37.38%, 6.54% and 56.07% respectively. Further extraction of the tea residue by hot alkali aqueous solution gave rise to TPC-A, which consisted of four homogeneous components with the molecular weight of $4.94 \times 10^6$ (68.57%), $6.77 \times 10^4$ (2.86%), $1.12 \times 10^4$ (11.43%) and $4.13 \times 10^3$ (17.14%) Da respectively.

### 3.3. Effect of TPC-W and TPC-A on blood glucose in NOD mice

The effect of TPC-W and TPC-A on blood glucose in NOD mice is shown in Table 1. Blood glucose of model control mice (group MC) increased suddenly at age of 15 weeks and was higher than that of normal control mice (group NC) at the age of 19–22 weeks ($P < 0.05$ or $P < 0.01$). TPC-W and TPC-A slowed down the exaltation of blood glucose in NOD mice compared with group MC.

The level of blood glucose in NOD mice (group TPC-W-150) orally treated with high dose of TPC-W decreased significantly ($P < 0.05$ or $P < 0.01$). The value was even less than the half of group MC at the age of 17–22 weeks. At the end of the trial (age of 22 weeks), in group MC, the range of 6 mice was 14.0–33.3 mmol/L, while that of 3 mice was 5.8–8.1 mmol/L and one mouse was dead. In group NC, that of 10 mice was 6.1–8.4 mmol/L. In group TPC-W-150, that of 9 mice was 5.3–8.7 mmol/L, while the value of the other 1 mouse was 14.2 mmol/L. In group TPC-W-50, that of 8 mice was 5.4–8.7 mmol/L, while the value of the other 2 mice was 17.8 and 30.6 mmol/L respectively. In group TPC-A-150, the range of 8 mice was 4.8–6.8 mmol/L while the value of the other 2 mice was 24.0 and 33.3 mmol/L, respectively. In group TPC-A-50, the range of 8 mice was 5.4–8.7 mmol/L, while the value of the other 2 mice was 17.8 and 30.6 mmol/L respectively. In the treatment of diabetes, TPC-W showed dose response and better efficacy than TPC-A.

### 3.4. Effect of TPC-W and TPC-A on the incidence of diabetes in NOD mice

The incidences of diabetes are shown in Table 2 as cumulative numbers in each group. At the third week of the trial, two mice showed diabetic symptoms in model control group (group MC). The incidence of diabetes in group MC increased significantly ($P < 0.05$ or $P < 0.01$) during the fifth to tenth weeks of the trial and reached 70% at the end. TPC-W and TPC-A could reduce the incidence of diabetes. Till 18 weeks of age of NOD mice, only one mouse in group TPC-W-50, had an onset of diabetes ($P < 0.05$). None of mice in the group TPC-W-150 showed diabetic symptoms before age of 20 weeks ($P < 0.05$ or $P < 0.01$), whereas group TPC-A-50 and TPC-

**Table 3**
Effect of TPC-W and TPC-A on organ index in NOD mice (g kg$^{-1}$, $\overline{X} \pm s$, $n = 10$).

| Group/dosage | NC (10 ml kg$^{-1}$) | MC (10 ml kg$^{-1}$) | TPC-W-50 (50 mg kg$^{-1}$) | TPC-W-150 (150 mg kg$^{-1}$) | TPC-A-50 (50 mg kg$^{-1}$) | TPC-A-150 (150 mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| Spleen | 3.957 ± 0.643 | 3.932 ± 0.338 | 3.506 ± 0.372 | 3.732 ± 0.387 | 3.973 ± 0.282 | 3.684 ± 0.299 |
| Thymus | 2.386 ± 0.870 | 1.493 ± 0.223ΔΔ | 1.534 ± 0.352 | 1.845 ± 0.257** | 1.749 ± 0.415 | 1.644 ± 0.354 |

*Note*: Compared with normal control, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with model control, $^{*}P < 0.05$, $^{**}P < 0.01$.

**Table 4**
Effect of TPC-W and TPC-A on OD value of anti-GAD antibody in NOD mice ($n = 10$).

| Group/dosage | NC (10 ml kg$^{-1}$) | MC (10 ml kg$^{-1}$) | TPC-W-50 (50 mg kg$^{-1}$) | TPC-W-150 (150 mg kg$^{-1}$) | TPC-A-50 (50 mg kg$^{-1}$) | TPC-A-150 (150 mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| OD value | 0.429 ± 0.132 | 1.557 ± 0.176ΔΔ | 1.386 ± 0.613 | 1.129 ± 0.376* | 1.260 ± 0.538 | 1.417 ± 0.469 |

*Note*: Compared with normal control, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with model control, $^{*}P < 0.05$, $^{**}P < 0.01$.

A-150 developed diabetes as early as at the age of 15 or 16 weeks. To the end of the trial, only 2 NOD mice in each group treated with TPC had a diabetes onset. After biostatical analysis, the cumulative incidences showed significant difference ($P < 0.05$ or $P < 0.01$). The result of trial demonstrated that diabetes was inhibited in NOD mice by the treatment of TPC-W and TPC-A.

### 3.5. Effect of TPC-W and TPC-A on organ index in NOD mice

Compared with the group NC mice, the group MC mice had lower thymus index (Table 3). However, there was little difference between their spleen indexes. It indicated that the spleens of NOD mice grew normally but their thymuses shriveled.

In the group of NOD mice treated with TPC-W or TPC-A, the thymus index increased to some extent among which that of the TPC-W-150 group increased significantly ($P < 0.01$). In contrast, there was no significant difference in the spleen index between treated and untreated NOD mice ($P > 0.05$). These results indicated that both TPC-W and TPC-A could prevent the thymus from shriveling to some extent.

### 3.6. Effect of TPC-W and TPC-A on OD value of anti-GAD antibody in NOD mice

Many studies found that immune responses to GAD (GAD65) were pivotal in the development of IDDM (Delovitch & Singh, 1997; Nepom, Quinn, Sercarz, & Wilson, 2003). At the end of the trail (the age of 22 weeks), we measured the OD value of serum anti-GAD antibody of mice in each group (10 mice per group). The OD value of serum anti-GAD antibody in the mice of group NC, MC, TPC-W-150, TPC-W-50, TPC-A-150, TPC-A-50 ranged 0.28–0.59, 1.39–1.80, 0.74–1.79, 0.58–2.32, 0.46–1.84 and 0.39–1.73 respectively.

The level of anti-GAD antibody in serum of NOD mice was analyzed in Table 4. OD value of anti-GAD antibodies of model control mice (group MC) was higher than that of normal control group ($P < 0.01$). In contrast to the model control mice (group MC), TPC-W and TPC-A reduced the level of anti-GAD antibody in serum of NOD mice. The average content of serum anti-GAD antibody in group TPC-W-150 mice decreased 27% and showed significant difference from control groups ($P < 0.05$). In NOD mice and human, GAD in pancreatic β cells switches on the earliest proliferative response of T cells. The anti-GAD (GAD65) response preceded other related autoimmune responses (Yoon et al., 1999; Endl et al., 1997). Our data indicated that TPC, especially TPC-W, can suppress the GAD-reactive T cells and consequent autoimmune responses.

### 3.7. Effect of TPC-W and TPC-A on body weight of NOD mice

Compared with the weight of normal control mice (group NC), that of model control mice (group MC) was significantly low ($P < 0.01$) (shown in Table 5). At the beginning of the treatment trial, the average body weight of normal control mice was 28.77 g, whereas that of untreated and treated NOD mice ranged from 21 to 22 g.

To the end of trial, the mice in normal control group got an average weight gain of ~7.4 g (26%). However, the mice in group MC and TPC-treated group only got a weight gain ranging from 2 to 3 g.There was no significant difference between group MC and TPC-treated group. These data indicated that TPC-A and TPC-W could significantly inhibit the gain of body weight in NOD mice.

## 4. Discussion

In this study, we confirmed that oral administration of TPC-W or TPC-A prevented NOD mice from the development of autoimmune diabetes. TPC-W had a dose-dependent effect on blood glucose, thymus index, and the level of anti-GAD antibodies. The cumulative incidences of NOD mice treated with TPC-A were equal to those of NOD mice treated with TPC-W. However, regarding the effect of delaying diabetes onset, TPC-W had better potential than TPC-A. There was little difference in molecular weight between TPC-W and TPC-A, so the high level of saccharide content in tea polysaccharide conjugates may lead to high efficiency for lowering blood glucose, which has not be verified yet.

GAD (GAD65) is the first cell antigen that induces the proliferative response of T cells prior to other responses during autoimmune process in NOD mice as well as in the peripheral blood of recent-onset IDDM patients (Arata et al., 1994; Baekkeskov et al., 1990; Kaufman et al., 1993). These autoimmune responses are directly linked to the induction of β cell damage through lymphocytic infiltration into the islets. We selected anti-GAD antibody as an indicator to compare autoimmune responses between control and treated mice. The assay of anti-GAD antibody demonstrated that TPC treatment may suppress the anti-GAD response to protect

**Table 5**
Effect of TPC-W and TPC-A on body weight in NOD mice (g, $\overline{X} \pm s$, $n = 10$).

| Group/dosage | NC (10 ml kg$^{-1}$) | MC (10 ml kg$^{-1}$) | TPC-W-50 (50 mg kg$^{-1}$) | TPC-W-150 (150 mg kg$^{-1}$) | TPC-A-50 (50 mg kg$^{-1}$) | TPC-A-150 (150 mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| B.A. | 28.77 ± 1.96 | 21.52 ± 1.78ΔΔ | 21.55 ± 2.10 | 21.25 ± 2.11 | 21.67 ± 1.87 | 21.89 ± 1.87 |
| A.A. | | | | | | |
| 1 | 30.78 ± 1.94 | 22.70 ± 1.21ΔΔ | 21.80 ± 2.17 | 21.65 ± 1.75 | 21.64 ± 1.66 | 21.61 ± 1.25 |
| 2 | 31.07 ± 2.41 | 22.60 ± 1.34ΔΔ | 21.87 ± 1.77 | 21.85 ± 1.49 | 21.91 ± 1.46 | 22.23 ± 1.11 |
| 3 | 33.15 ± 2.46 | 22.99 ± 1.55ΔΔ | 22.90 ± 1.52 | 22.94 ± 1.81 | 22.79 ± 1.74 | 23.05 ± 1.22 |
| 4 | 34.23 ± 2.87 | 23.72 ± 1.59ΔΔ | 22.74 ± 1.50 | 22.74 ± 1.37 | 22.46 ± 1.51 | 22.87 ± 1.39 |
| 5 | 34.85 ± 3.27 | 23.58 ± 2.12ΔΔ | 21.91 ± 1.46* | 23.01 ± 1.19 | 22.46 ± 1.78 | 22.76 ± 1.26 |
| 6 | 36.19 ± 3.43 | 24.46 ± 1.73ΔΔ | 22.91 ± 1.87 | 23.15 ± 1.79 | 22.74 ± 1.76* | 23.13 ± 1.15 |
| 7 | 35.25 ± 3.04 | 24.75 ± 1.20ΔΔ | 22.77 ± 1.88* | 22.97 ± 1.46** | 22.32 ± 1.38** | 23.41 ± 1.14* |
| 8 | 35.63 ± 2.87 | 24.55 ± 1.11ΔΔ | 23.21 ± 1.78 | 23.39 ± 1.37* | 23.28 ± 1.31* | 23.51 ± 1.49 |
| 9 | 35.65 ± 2.85 | 26.65 ± 2.89ΔΔ | 23.80 ± 1.85 | 24.09 ± 1.48 | 22.92 ± 1.90 | 23.49 ± 1.09 |
| 10 | 36.17 ± 2.84 | 24.38 ± 2.73ΔΔ | 24.27 ± 1.62 | 23.85 ± 1.44 | 23.49 ± 1.72 | 24.13 ± 1.06 |

*Note*: Compared with normal control, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with model control, $^{*}P < 0.05$, $^{**}P < 0.01$.

NOD mice against diabetes. In sum, our studies demonstrated that both TPC-W and TPC-A showed great potential in suppressing the autoimmune response.

To our knowledge, tea needs to be soaked in hot water before people drinking it. Some components (polysaccharide conjugates, etc) extracted by hot water from tea will be taken in the body. Our results demonstrated that TPC has great potential in protecting against diabetes and will be a promising supplement to human health. However, the effective components in TPC and exact mechanism by which TPC suppress diabetes in NOD mice remain to be elucidated.

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