

Influence of Tea Polyphenols on the Formation of Advanced Glycation End Products (AGEs) *in vitro* and *in vivo*

Shanli Peng¹, Genyi Zhang^{1,2,*}

¹School of Food Science and Technology, Jiangnan University, Wuxi, China

²State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China

*Corresponding author: genyiz@gmail.com

Received July 22, 2014; Revised August 09, 2014; Accepted August 17, 2014

Abstract The effects of tea polyphenols (TP) on the formation of advanced glycation end products (AGEs) were investigated using glutamic acid - glucose and BSA - glucose model systems at 90°C and 37°C, respectively. The spectral characteristics of glycation products were measured. Additionally, a type 1 diabetes mellitus (DM) animal model of Kunming mice by injecting streptozocin (STZ) was used to study the formation of AGEs *in vivo*. Tea polyphenols (TP) were given to mice at a dose of 200mg/kg bodyweight continuously for 8 weeks. Mice were then sacrificed and the glycosylated hemoglobin (GHbA1c) and fluorescence of AGEs in serum were measured to illustrate the effects of TP on protein glycation *in vivo*. The *in vitro* experiment results showed that the production of AGEs was decreased by TP in a dose-dependent manner. TP addition reduced the accumulation of GHbA1c and AGEs in DM mice and relieved the symptoms of diabetic nephropathy (DN), which is an important indicator of diabetic complications caused by AGEs. Thus, TP might be used as an important ingredient in dietary approaches for intervention of diabetes and improved health.

Keywords: tea polyphenols, advanced glycation end products (AGEs), diabetes mellitus, antioxidation

Cite This Article: Shanli Peng, and Genyi Zhang, "Influence of Tea Polyphenols on the Formation of Advanced Glycation End Products (AGEs) *in vitro* and *in vivo*." *Journal of Food and Nutrition Research*, vol. 2, no. 8 (2014): 524-531. doi: 10.12691/jfnr-2-8-15.

1. Introduction

As the increase of diabetic prevalence, preventing or delaying the occurrence and progression of diabetic complications is becoming important to the management of diabetes. Although numerous factors contributing to the development of diabetic complications have been proposed, advanced glycation end products (AGEs) which have a wide range of chemical, cellular, and tissue effects through changes in electrostatic property, solubility, and conformation have been receiving much attention in recent studies [1,2,3,4]. AGEs are irreversible end-products of protein glycation reaction, known collectively as Maillard or nonenzymatic reactions with the production of free radicals [5,6]. The accumulation of AGEs in the body leads to structural and functional impairments of proteins contributing to the age-related increase in brown color, fluorescence and insolubilization of lens crystallins and the gradual crosslinking and decrease in elasticity of connective tissue collagens [6,7,8]. AGEs also interact with specific receptors and proteins to influence the expression of growth factors and cytokines, including TGF- β 1 and CTGF, thereby regulating the growth and proliferation of the various types of renal cells. There is emerging evidence that protein glycation is implicated in

the aging process, and the pathogenesis of diabetes mellitus (DM). In addition, correlations between tissue AGEs concentrations and the severity of some chronic diseases such as cardiovascular disease (CVD) and diabetic complications (retinopathy, neuropathy, nephropathy and atherosclerosis) and Alzheimer's disease have been demonstrated [3,4]. Therefore, inhibition of AGEs formation is considered to be one promising approach for the prevention and treatment of diabetic complications.

In consideration of the significance of oxidative stress to protein modification [5], a supplement of antioxidants and/or radical-scavengers to prevent this event might be a strategy for preventing diabetic complications. This hypothesis has been supported by the clinical results which indicated that the development of Type 2 diabetes and complications may be reduced by the intake of antioxidants in diets [9]. Tea polyphenols (TP), a group of phytochemicals with antioxidative effect, are believed to be one of the physiologically active agents in tea. It has been reported that the main active components of TP are catechins, known to have important biological and pharmacological properties attributed to their antioxidant properties [10,11,12,13]. Some studies showed TP could scavenge free radicals [10,14], increase the activity of antioxidant enzymes, inhibit lipid peroxidation (LP), and reduce oxidative stress [15], suggesting that TP may combat AGEs formation and diabetic complications.

In this connection, to further our understanding of the action of tea polyphenols, an *in vitro* study in comparison with an *in vivo* study using diabetic animals was conducted to evaluate the effect of TP on the formation of AGEs under different conditions and to unravel the critical points for the improvement of the *in vivo* efficacy of TP on the formation of AGEs that is beneficial to the prevention of diabetic complications.

2. Materials and Methods

2.1. Chemicals and Reagents

Tea polyphenols (TP) was purchased from Raldshg Chemicals Co., Ltd (Rizhao, China), with a total tea catechin content of ~99%. D-glucose (Glc), glutamic acid (Glu), potassium persulfate ($K_2S_2O_8$) and sodium azide (NaN_3) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Bovine serum albumin (BSA) (Fraction V, Essentially Fatty Acid Free) were provided by Shanghai Sangon Biological Engineering Technology & services Co., Ltd. (Shanghai, China). The four major catechin standard substances (EC, EGC, ECG and EGCG), streptozocin (STZ), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (CH_3CN), methanol (MeOH), all of HPLC grade, were purchased from Sinopharm Chemical Reagent Co. Ltd. All other chemicals used were of analytical grade.

2.2. Analysis of Composition and Antioxidant Activity of TP

The concentration of catechins in TP used was estimated using high performance liquid chromatography (HPLC). The liquid chromatographic system included an Agilent 1100 HPLC chromatograph equipped with a quaternary pump, a diode array detector and an autosampler, and a Hypersil BDS C18 (250mm×4.6mm, 5 μ m) column maintained at room temperature. The mobile phase consists of 0.05% (v/v) phosphoric acid in methanol and water (5:95, v/v; eluent A) and of 0.05 phosphoric acid in methanol and water (80:20, v/v; eluent B). The gradient program was operated as follows: 10-50% B (15min), 50-80% B (1min), 80% B maintained for 3min, 80-10% B (0.5min). Simultaneous monitoring was performed at 280nm at the flow rate of 0.8 mL/min.

Total antioxidant activity of TP was determined using DPPH decolorization assay with slight modification [16,17]. DPPH solution in methanol (0.1 mmol/L) was prepared and used fresh for each test. An amount of 1 mL of TP solution was reacted with 2 mL of DPPH solution and absorbance was recorded within 8 min at 517 nm. The activity was expressed as the concentration of sample necessary to give a 50% reduction in the original absorbance (IC_{50} value).

2.3. In Vitro Glycation of Glutamic/Glucose and BSA/Glucose

The assay is used to evaluate the ability of TP to inhibit the glucose-mediated amino acid and protein glycation *in vitro*. The procedure was performed as described by Farrar [18] and [19] with slight modifications. Briefly, 0.5 mol/L glutamic acid and D-glucose were dissolved in distilled water and mixed with different concentrations of TP. These mixtures were incubated in a water bath at 90°C for 7 hours. For protein glycation, BSA (bovine serum albumin, fraction V, 40 mg/ml) was nonenzymatically glycosylated by incubation under sterile conditions in 100 mmol/L phosphate buffer (pH 7.4) containing 0.02% sodium azide in the presence of 0.8 mol/L glucose and TP [<10 mmol/L to diminish the fluorescence signal from TP itself [20]]. The tested compounds were replaced with 1 ml of potassium phosphate buffer (100 mmol/L, pH 7.4) in the control. All reagents and samples were sterilized by filtration, and the mixture were covered with nitrogen and incubated for one month at 37°C. All tests were operated in triplicate under sterile and dark condition.

2.4. Detection of Quinones and Brown Polymers

Detection of quinones and brown polymers formation during glycation was followed by measuring the increase in absorbance at 280 nm, which was found to be an optimum absorbance of quinones as determined by methylbenzoquinone [21], and formation of brown polymers was determined by the increase in absorbance at 420 nm with SHIMADZU UV-1800 UV/Vis diode array spectro photometer. The relative fluorescence intensity of the glycosylated BSA was measured at an excitation wavelength of 350 nm and emission wavelengths ranging from 370 nm to 550 nm versus an unincubated blank containing the protein, glucose, and inhibitors, using a HITACHI 650-60 spectrofluorometer [20,22]. Samples at different reaction time periods were diluted with distilled water immediately to prevent fluorescence self-absorption. The inhibition capability by different concentrations of TP on glycation = $[1 - (\text{fluorescence or absorbance of the test group} / \text{fluorescence or absorbance of the control group})] * 100\%$.

2.5. Induction of Diabetic Animal Model and Treatment

Male KM mice (20 ± 2 g) were purchased from Shanghai SLAC Slac laboratory animal Company. The mice were housed in a segregated air-conditioned room at 25°C with a lighting schedule of 12 h light and 12 h dark. Mice were provided with a basal diet (purchased from Shanghai SLAC Slac laboratory animal Company) and free access to drinking water. The mice were allowed to acclimate for 1 week before the commencement of the study and fasted for 12 h prior to the induction of diabetes. STZ freshly prepared in buffer solution (0.1 mol/L sodium citrate and 0.1 mol/L citric acid, pH 4.2), was immediately injected intraperitoneally with a single dose of 150 mg/(kg, body-weight) [23]. 72 hours after STZ injection, mice with blood glucose levels greater than 11.1 mmol/L and displaying diuresis, polydipsia and weight reduction were considered to have type 1 diabetes.

Mice were randomly allocated into the following groups (six mice in each group): control group; normal

mice as control; DM group: STZ-induced diabetic mice; intervention group: diabetic mice received TP at the dose of 200mg/(kg.bw) by oral administration while normal saline (NS) were given to DM and normol animals. After 8 weeks of treatment, mice were fasted overnight, blood was collected and immediately centrifuged for 10 min at 3000 rpm at 4°C to obtain serum (stored at -70°C), then the animals were sacrificed by cervical dislocation, kidney tissues were excised from the animals for the pathological histology by hematoxylin and eosin (HE) stain. Other parts of the kidney were removed promptly and stored at -70°C.

All mice were allowed free access to their original diets and water in the duration of the study. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Jiangnan University (Wuxi, China). All experimental animals were overseen and approved by the Animal Care and Use Committee of our Institute before and during experiments.

2.6. Measurement of Indexes Related to AGEs

Blood samples were obtained from the tail vein of the overnight fasted mice and their glucose levels were tested by blood glucose test strips. Serum was obtained from blood samples after centrifugation and analyzed for concentrations of glycolated hemoglobin (GHbA1c) and relative fluorescence intensity of glycated protein. GHbA1c concentration was quantified using an ELISA kit (R&D systems). In an effort to further characterize effects of TP on the metabolic responses of STZ-treated mice, the concentration of creatinine (Cr) and urea nitrogen (UN) were determined using commercial available kits diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China) according to their manuals. Kidneys were weighted, homogenized and centrifuged for 10 min at 3000 rpm at 4°C. The supernatant was immediately used for the assays of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) level and total antioxidant capacity (T-AOC) according to the instructions of their corresponding kits respectively.

2.7. Statistical Analysis

Experimental data of *in vitro* glycation were expressed as the mean \pm standard deviation (S.D.), and standard error (S.E.) of the mean was used for *in vivo* experimental results. Statistical analysis was performed with one-way analysis of variance (oneway-ANOVA) in SPSS 19.0 for windows (SPSS, Inc., Chicago, IL, USA). Differences were considered significant at $p < 0.05$. Correlations between parameters were obtained by Pearson correlation coefficient in bivariate correlations.

3. Results and Discussion

3.1. Composition and Antioxidant Activity of TP

The tea polyphenols used were separated effectively using HPLC (seen Figure 1). The major active

components are four catechins, including (-) epigallocatechin-3-gallate (EGCG), (-) epicatechin-3-gallate (ECG), (-) epigallocatechin (EGC) and (-) epicatechin (EC), EGCG being the most abundant (67.79%), ECG next, EC the least. The IC₅₀ values of TP used on DPPH radical scavenging activities were 2.56 μ g/mL, which is consistent with the green tea constituents and high antioxidant capacity of these plant chemicals [24].

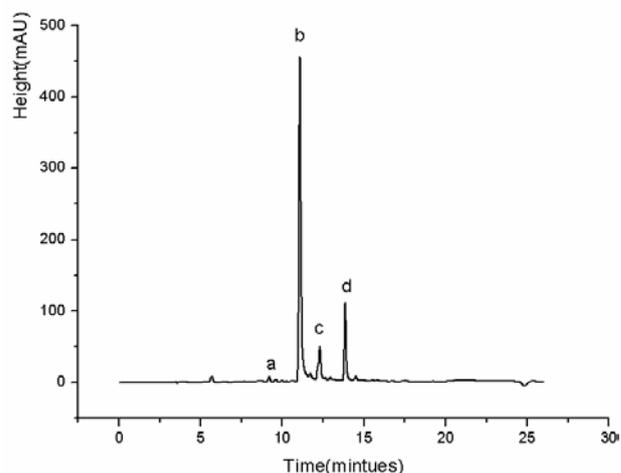


Figure 1. HPLC separation of tea polyphenols. (a) EGC; (b) EGCG; (c) EC; (d) ECG

3.2. Glycation Products in Amino Acid/Glucose Systems

The Maillard reaction between reducing sugars such as glucose and free amine residues of proteins can cause non-enzymatic glycation of proteins. Free amino groups react initially with reducing sugar to form Schiff's bases, which then covalently bond to form Amadori rearrangement products. The reductone formation over furfural production from the Amadori products leads to colour development. These Amadori products undergo a rearrangement reaction to form a heterogeneous group of protein-bound moieties with specific fluorescence and optical absorption called AGEs, such as 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI), pentosidine and crossline [25]. The changes of optic absorption and fluorescence of the reaction mixture during this process [7,26] were employed to monitor the process of glycation. Absorbance at 280 nm was used to determine the intermediate compounds of the Maillard reaction [22]. The excitation and emission wavelength used to measure AGEs' concentration were 300-420 nm and 350-600 nm, while 370nm/440nm were most widely used. Therefore, to evaluate the inhibitory effect of TP on the formation of AGEs *in vitro*, we measured the fluorescence intensity of mixtures at 370nm/440nm. From the results depicted in Fig. 2(A), the absorbance at 280 nm of mixtures increased along the reaction, suggesting the formation of an uncolored compound, which could be the precursor of the AGEs [22]. TP inhibited the optic absorbance at different concentrations, and a significant decrease of the absorbance intensities with increase of TP concentrations was observed. The average inhibition rates by different concentrations of TP on A280 were 19.3%, 24.6% and 35.2% respectively compared with the control. As to the

influence of TP on fluorescence intensity, similar phenomenon was observed [Figure 2(B)]. The fluorescence intensity was quenched by nearly 20.8%,

36.2%, 68.6% at 0.05, 0.5 5.0 mmol/L TP respectively compared with the control, a little higher than that on absorbance at 280 nm.

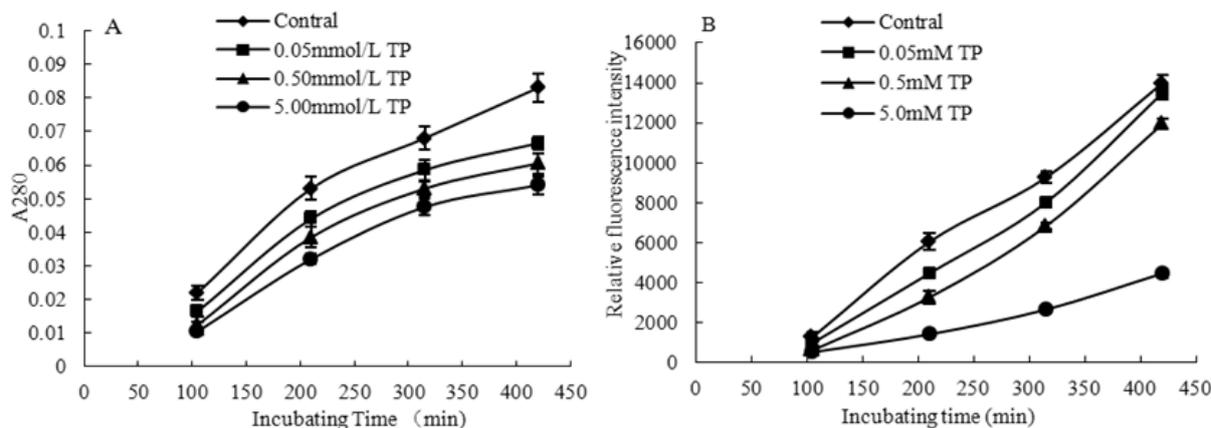


Figure 2. Fluorescence and UV absorption intensity of glucose-glycine system. A: Relative fluorescence intensity at 370nm/440nm; B: Absorption intensity at 280nm

3.3. Glycation products in BSA /Glucose Systems

It is well known that the glycation of albumin occurs in biological tissues. BSA usually undergoes marked reversible changes in conformation under non-physiological conditions. Therefore, in the method adopted in the present study, BSA was chosen as the model protein for the formation of AGEs through glycation reaction in an *in vitro* glucose-BSA system. The products were characterized by their fluorescence after incubation.

As shown in Figure 3, the result suggested that the fluorescence intensity of AGEs produced was highly increased through incubation of BSA with glucose at 37°C for 4 weeks and was weakened by different concentrations of TP at the rate of 17.7%, 36.3%, 76.4% in a dose-dependent manner. However, after incubation for 2 weeks, low dose of TP (0.005mmol/L and 0.05mmol/L) had little influence on the fluorescence intensity while high dose (0.5mmol/L) worked at the inhibition rate of 45.8%. This result indicated that the inhibition activity of TP on glycation may be weak at the initial reaction, and became more and more efficient as the reaction went on.

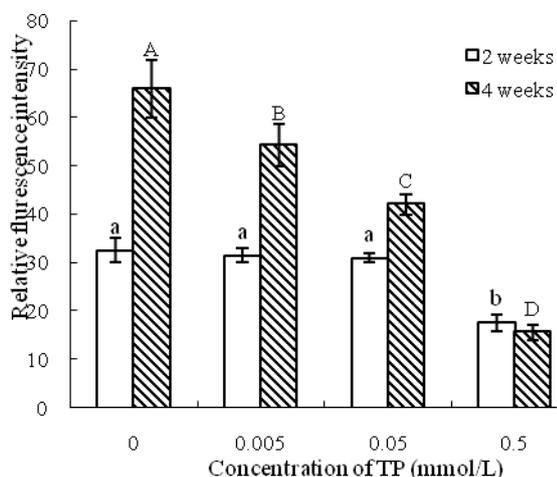


Figure 3. Fluorescence intensity of glucose-BSA system after incubation for 2 and 4 weeks. Means not sharing a common superscript letter are significantly different ($p < 0.05$) between groups (one-way analysis of variance with turkey's post hoc test used for all between-group analysis)

Interestingly, it can be seen from Figure 4 that the fluorescence emission wavelengths of AGEs produced after 4 weeks incubation have a red shifts in the glucose-BSA system at the presence of TP. When the concentration of TP is 0.5mmol/L, the fluorescence emission wavelength of AGEs has been shifted about 15 nm. Similar shift has been observed in the study on protein glycation inhibitory activity of feruloyl oligosaccharides [19] which indicated that some amino acid residues in proteins have been brought to a more hydrophilic environment without reacting with reducing sugar. This spectral contribution is attributed to the fact that glycated BSA turns into a less polar molecule owing to exposing its hydrophobic sites in respect to the native molecules [19,27]. Treatment with TP resulted in a profound prevention of such structural changes, keeping the protein molecule closer to its native polar conformation. The behavior of TP in this respect resembles that of molecular chaperones which block the exposed hydrophobic surfaces of their substrate proteins.

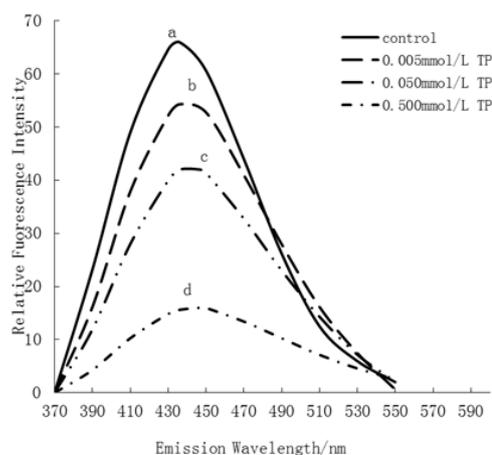


Figure 4. Fluorescence-quenched spectrum of AGEs by TP with different concentrations

3.4. Glycation Products in Diabetic Mice

After DM animal model was induced by intraperitoneal injection of STZ, TP was given to mice at a dose of

200mg/kg continuously once per day for 8 weeks. During the intervention period, fast blood-glucose level (FBG) of all mice measured weekly were shown in Figure 5. STZ can destroy pancreatic β -cells and cause severe hypoinsulinaemia that is responsible for the hyperglycemia and dyslipidemia seen in DM animals [28]. As shown in Figure 5, the result suggested that mice in DM and intervention groups have significant hyperglycemia compared to those in normal group and the hyperglycemia symptom was slightly adjusted by TP without statistical significance.

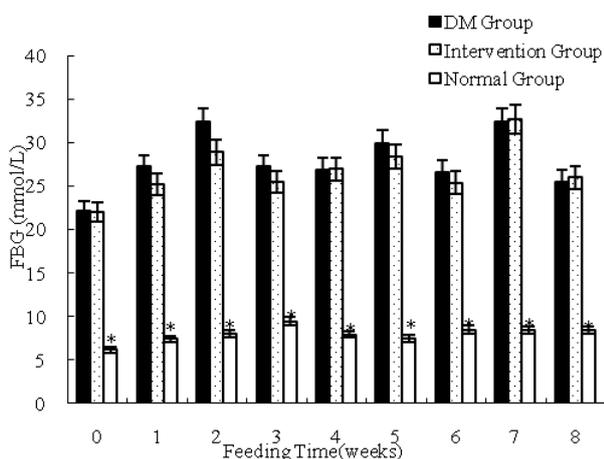


Figure 5. Fast blood glucose level of mice in different groups in diet intervention period. Values are expressed as mean \pm SD, n=6. Means with * are significantly different ($P < 0.05$)

In this study, at the end of TP intervention, all mice were sacrificed and serums were obtained from blood samples for the detection of GHbA1c level and relative fluorescence intensity of AGEs. The relative fluorescence intensity and GHbA1c level of serum were shown in Figure 6. It is clear that fluorescence intensity of DM group increased significantly compared with normal group ($p < 0.05$) while that of intervention group decreased and no statistic difference of fluorescence intensity between normal group and diet intervention group was observed. Similar phenomenon was found in the differences of concentration of GHbA1c, glycated protein which is used as a golden standard in the diagnosis and monitoring of diabetes mellitus [29]. These results showed that the inhibition of TP on glycation occurred in diabetic mice.

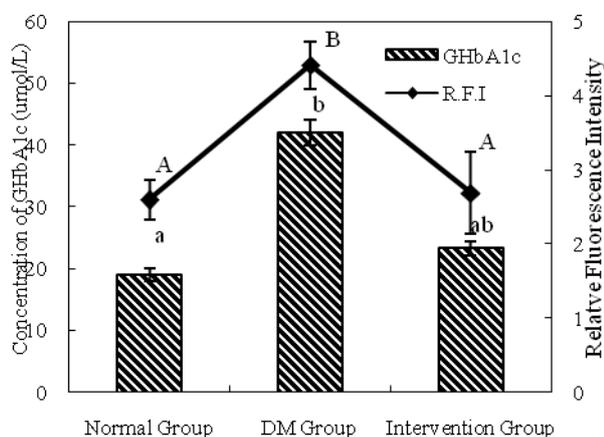


Figure 6. Concentration of GHbA1c and relative fluorescence intensity (R.F.I) of AGEs in serum

3.5. Kidney Injury in diabetic mice

As depicted in Figure 7, after TP intervention for 8 weeks, the concentration of Cr and UN in intervention group decreased significantly and almost restored to the level of normal group. These results indicate that TP may relieve the symptoms of diabetic nephropathy (DN), an important indicator of diabetic complications in diabetic mice.

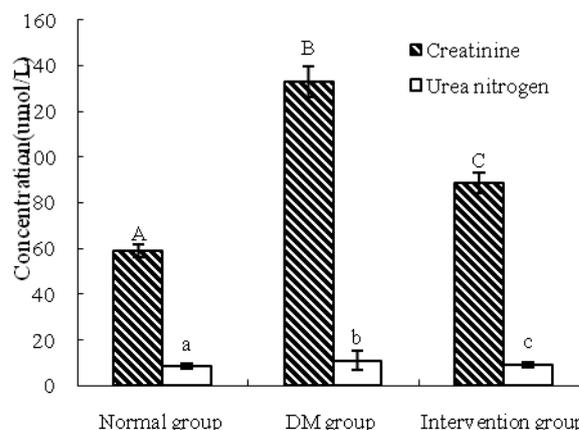


Figure 7. Concentration of serum creatinine and urea nitrogen. Values are expressed as mean \pm SE (n=6). Means not sharing a common superscript letter are significantly different ($p < 0.05$) between groups

Table 1. Correlation among concentration of AGEs and renal damage index

Serum index (umol/L)	Fluorescence Intensity	Creatinine	Urea Nitrogen
Correlation	0.939	0.995*	0.993*
P-Value	0.111	0.031	0.038

Correlation is pearson correlation, and * is significant in the 0.05 level (one-tailed).

The renal histology is shown in Figure 8. Compared to the normal renal architecture of the normal mice (Figure 8A), DM mice appeared to have severe pathological damages, such as mesangial expansion, glomerular hypertrophy, infiltration of inflammatory cell into the renal tubule-interstitium, narrow lumen, and sclerosis in part of glomeruli (Figure 8B). AGEs and metabolic disorder are main injuring factors of DM and DN [30,31,32]. The correlation analysis showed the contents of diabetic kidney damage index, Cr and UN, have significant positive correlation with the concentration of GHbA1c and fluorescence intensity, which are positively correlated with each other (Table 1). These correlations are consistent with previously studies. Many of the pathogenic changes that occur in diabetic nephropathy may be induced by AGEs which are able to directly stimulate the production of extracellular matrix and inhibit degradation. AGEs modification of matrix proteins is also able to disrupt matrix-matrix and matrix-cell interactions, contributing to their profibrotic action and finally leading to glomerular lesion, and renal tubular sclerosis. The TP treatment changed morphology and led to a decreased extent of the expansion in glomerular and mesangial matrix (Figure 8C). Further, there were no clearly histopathological abnormalities found in renal histology in the TP treated mice as compared to normal mice. Studies have suggested that angiotensin-converting enzyme inhibitors are able to reduce the accumulation of AGEs in

diabetes, possibly via the inhibition of oxidative stress [24]. This interaction may be a particularly important

pathway for the development of AGEs-induced damage, as it also can be attenuated by antioxidant therapy.

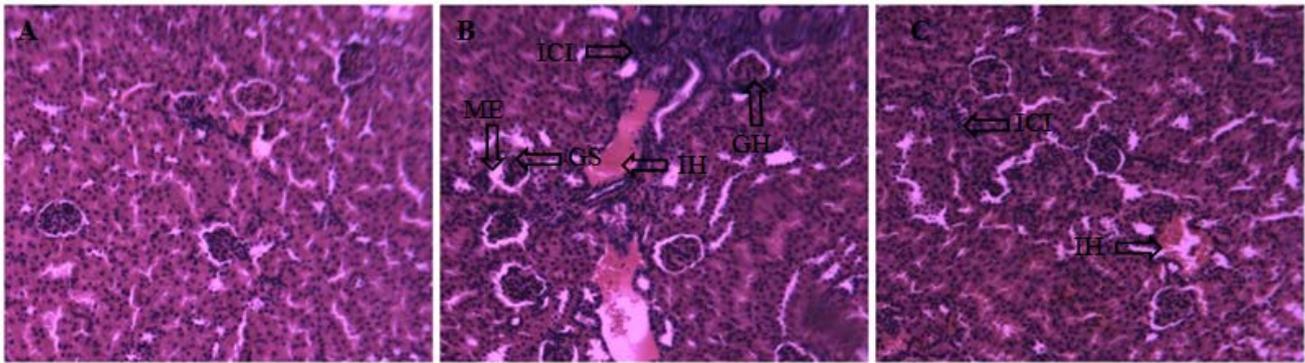


Figure 8. Effects of TP treatment on kidney damage in STZ-induced diabetic mice (H&E stain 400 \times). A: control group (normal mice as control); B:DM group (STZ-induced diabetic mice); C: intervention group (diabetic mice received TPby oral administration). ME mesangial expansion; GH glomerular hypertrophy; ICI inflammatory cell infiltration; GS glomeruli sclerosis; IH interstitium was hyperemia

3.6. Enhanced Antioxidant Capacity of DM Mice

As shown in Table 2, MDA level in serum and kidney was significantly higher ($p < 0.05$) in STZ-induced diabetic mice than control mice whereas total antioxidant capacity and activity of antioxidant enzymes in serum and kidney was significantly lower ($p < 0.05$). The TP treatment reduced MDA level ($p < 0.05$) and increased activity of GSH-Px, SOD, and CAT ($p < 0.05$) in liver and kidney as compared to the STZ treatment alone. Especially, total antioxidant capacity, activity of GSH-Px and SOD in the intervention mice almost returned to their normal level.

Table 2. Effect of TP on MDA and activities of antioxidant enzymes in serum and kidney of STZ-induced diabetic mice

Group	Control group	Diabetic group	Intervention group
<i>Serum</i>			
T-AOC(U/mL)	9.6 \pm 0.8 ^b	5.7 \pm 1.0 ^a	10.8 \pm 0.8 ^c
GSH-Px(U/mL)	880.7 \pm 14.3 ^c	469.2 \pm 50.4 ^a	813.0 \pm 47.7 ^b
SOD (U/mL)	52.0 \pm 2.5 ^b	26.1 \pm 1.9 ^a	55.1 \pm 3.7 ^b
CAT (U/mL)	6.0 \pm 0.5 ^c	1.4 \pm 0.3 ^a	3.7 \pm 0.7 ^b
MDA(nmol/mL)	4.3 \pm 0.8 ^a	10.0 \pm 1.1 ^c	6.5 \pm 1.2 ^b
<i>Kidney</i>			
T-AOC(U/mg.pro.)	3.2 \pm 0.1 ^b	1.9 \pm 0.1 ^a	3.6 \pm 0.3 ^c
GSH-Px(U/mg.pro.)	449.2 \pm 22.8 ^b	263.3 \pm 29.8 ^a	458.9 \pm 14.2 ^c
SOD (U/mg.pro.)	160.2 \pm 14.2 ^b	83.6 \pm 11.0 ^a	149.3 \pm 12.6 ^b
CAT (U/mg.pro.)	82.1 \pm 13.1 ^c	35.4 \pm 6.4 ^a	61.6 \pm 10.2 ^b
MDA(nmol/mg.pro.)	3.0 \pm 0.4 ^a	10.1 \pm 1.4 ^b	4.7 \pm 0.8 ^c

Value is expressed as mean \pm SE (n=6). Means with different letters within the same row are significantly different ($p < 0.05$).

In addition to their immediate effects on protein structure and function, AGEs also induce oxidative stress, leading to inflammation and propagation of tissue damage and play a central role in the development and progression of DM and DN [33]. At the molecular level, this is owing to the contribution of reactive oxygen species as well as reactive nitrogen species to AGE induced damage. At the cellular level, numerous studies support the view that interaction of AGEs with cell surface receptors such as RAGE elicits ROS generation and vascular inflammation [34]. Glycated proteins activate membrane receptors through AGEs, and induce an intracellular oxidative stress and a pro-inflammatory status. Thus, formation of AGEs,

glycation of protein and resultant oxidative stress, which accelerate glycation reactions [36], can initiate an autocatalytic cycle of deleterious reactions in tissues. Inhibition the accumulation of AGEs should improve the prognosis for a broad range of age-related diseases. Since glycation and oxidative stress are closely linked, and oxidation plays a role in the formation of AGEs. A supplement of antioxidants in response to the inhibition of AGEs formation should be a dietary strategy for preventing diabetic complications [37] and has been supported by the clinical results.

In our research, TP increase the activity of antioxidant enzymes, reduce oxidative stress, suggesting a possible mechanism by which TP inhibit AGEs formation and relieve symptoms of diabetic complications. However, it can be found that the inhibition effect of TP on fluorescence intensity and other AGEs related index *in vivo* is not as effective as that *in vitro*. This is reasonable considering the low bioaccessibility and bioavailability of polyphenols *in vivo* [36]. Several chemical and biochemical factors are believed to affect bioaccessibility and bioavailability of polyphenols including: (1) sensitivity to digestive conditions and poor intestinal transport; (2) deconjugation and re-conjugation reactions in metabolism; and (3) enzymes and gut microflora involved in polyphenol metabolism and clearance[37, 38]. Researches show that the majority of digestive loss occurs in the small intestine where elevated pH and presence of reactive oxygen species provide favorable conditions for catechin auto-oxidative reactions. Following the significant digestive loss or low bioaccessibility due to sensitivity to digestive conditions, transport of polyphenols in the intestine is limited by their affinity to efflux transport systems including Multidrug Resistance Proteins (MRP) and P-glycoprotein (PgP) known to limit uptake of many xenobiotics including catechins (Jodoin, Demeule, & Beliveau, 2002; Vaidyanathan & Walle, 2003; Zhang et al., 2004). Polyphenols are extensively metabolised either in tissues once they are absorbed, or by the colonic microflora. All polyphenols are conjugated to form O-glucuronides, sulphate esters and O-methyl ether [39,40]. The formation of derivatives by conjugation with glucuronides and sulphate groups facilitates their urinary and biliary excretion and explains their rapid elimination. The extent of polyphenol methylation should also affect

the biological properties of polyphenols. Therefore, it is essential to take into account the bioavailability of TP to explain the difference of their effects on formation of AGEs between *in vitro* and *in vivo*.

4. Conclusions

In this study, the optic absorption and relative fluorescence intensity of AGEs produced in the *in vitro* evaluation systems was decreased by TP in a dose-dependent manner. As to diabetic animals, the relative fluorescence intensity and GHbA1c level of blood samples were reduced significantly, and the symptoms of diabetic renal damage were relieved after TP intervention compared with the DM mice. Since a correlation between tissue AGEs concentrations and the severity of some chronic diseases and the significance of oxidative stress to protein modification has been demonstrated, our results suggest that a supplement of TP as antioxidants with the benefits against AGEs formation is considered to be one promising approach for the prevention and treatment of diabetes mellitus. However, because of low bioavailability, the potential effect of TP *in vivo* is not as effective as that *in vitro*. In order to translate the *in vitro* results to *in vivo*, one of the challenging areas of work would be to increase the bioavailability of TP to enhance the effect in prevention and treatment of diabetes mellitus using different methods, not only by increasing the polyphenol content of diets. Available literature indicate that disruption of food matrix, addition of fat, cooking, treatment with enzymes, addition of ascorbic acid and sucrose can increase the bioavailability of dietary polyphenols. We believe a good understanding of tea polyphenols and an increase of their bioavailability will go a long way to take full advantage of their bioactivities. In conclusion, the present study provides scientific evidence of the promising therapeutic potential of TP, for glycation related disease. Further investigation to increase the bioavailability of polyphenols and mechanistic studies involving *in vivo* models should be followed.

Acknowledgments

The current investigation was supported by National Natural Science Foundation of China (Project No: 21076095).

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