RESEARCH ARTICLE

Anti-glycative and anti-inflammatory effects of caffeic acid and ellagic acid in kidney of diabetic mice

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Protective effects of caffeic acid (CA) and ellagic acid (EA) in kidney of diabetic mice were examined. CA or EA at 2.5 and 5% was mixed in diet and supplied to diabetic mice for 12 wk. Results showed that the intake of CA or EA increased renal content of these compounds, alleviated body weight loss, decreased urine output, increased plasma insulin and decreased blood glucose levels at weeks 6 and 12 ($p<0.05$). The intake of these compounds dose dependently reduced plasma blood urea nitrogen and elevated creatinine clearance ($p<0.05$). CA or EA at 5% significantly decreased the levels of plasma HbA1c, urinary glycated albumin, renal carboxymethyllysine, pentosidine, sorbitol and fructose ($p<0.05$), and significantly diminished renal activity of aldose reductase and sorbitol dehydrogenase, as well as suppressed renal aldose reductase mRNA expression ($p<0.05$). CA or EA dose dependently lowered renal levels of IL-6, IL-1β, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein 1 (MCP-1) ($p<0.05$). Furthermore, CA or EA dose dependently down-regulated tumor necrosis factor-α and monocyte chemoattractant protein-1 mRNA expression in kidney ($p<0.05$). Based on the observed anti-glycative and anti-inflammatory effects, the supplement of CA or EA might be helpful for the prevention or attenuation of diabetic kidney diseases.

Keywords:
Aldose reductase / Diabetes / Glycation / Monocyte chemoattractant protein-1 / Tumor necrosis factor-α

1 Introduction

Diabetic renal injury, or so-called diabetic nephropathy, is one of the diabetic complications, which exacerbates the severity and the mortality of diabetes. Non-enzymatic glycation with the formation of Maillard reaction products, also known as advanced glycation endproducts (AGEs), such as glycated hemoglobin, carboxymethyllysine (CML), pentosidine and glycated albumin, has been implicated in the pathogenesis of diabetic nephropathy and other complications of diabetes [1–3]. On the other hand, disturbed balance between Th1 and Th2 cytokines and overproduced pro-inflammatory cytokines not only enhanced systemic inflammatory stress in diabetic individuals but also promoted the progression of diabetes-associated renal injury [4, 5]. Thus, any agent with anti-glycative and/or anti-inflammatory effect may potentially prevent or delay the development of diabetic nephropathy.

It is well known that hyperglycemia enhances glucose metabolism *via* the polyol pathway [6, 7]. Aldose reductase (AR), the first and rate-limiting enzyme in this polyol pathway, reduces glucose to sorbitol, which could be further metabolized to fructose by sorbitol dehydrogenase (SDH), the second enzyme in the polyol pathway [7, 8]. It is reported that

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Abbreviations: AGE, advanced glycation endproduct; **AR,** aldose reductase; **BUN,** blood urea nitrogen; **CA,** caffeic acid; **CCr,** creatinine clearance; **CML,** carboxymethyllysine; **EA,** ellagic acid; **GAPDH,** glyceraldehyde-3-phosphate dehydrogenase; **MCP-1,** monocyte chemoattractant protein-1; **SDH,** sorbitol dehydrogenase; **TNF-alpha,** tumor necrosis factor-alpha

the flux through SDH and elevated fructose level may increase AGEs formation, which facilitates diabetes-induced microvascular abnormalities [9]. As AR and SDH are involved in diabetic-associated glycation reactions, any agent with the ability to affect the activity and/or mRNA expression of these enzymes might potentially mediate these glycation reactions.

Caffeic acid (CA) and ellagic acid (EA) are phenolic acids naturally occurring in many plant foods such as potato, carrot, tomato, apple, strawberry and blueberry [10, 11]. It has been documented that these phenolic acids possess anti-oxidative activities such as free radical scavenging and iron-chelating activities [12, 13]. The anti-diabetic effect of CA has been examined [14] and these authors observed that this compound could improve glycemic control. On the other hand, it is reported that EA is an AR inhibitor and could reduce sorbitol accumulation in erythrocytes, lens and sciatic nerves of diabetic rats [15]. Those previous studies implied that CA and EA could delay or retard diabetic deterioration *via* reducing blood glucose and mediating polyol pathway. However, it remains unknown that these compounds could protect kidney against diabetes-associated glycative and inflammatory injury.

The major purpose of this study was to investigate the anti-glycative and anti-inflammatory effects of CA and EA in kidney of diabetic mice. The influence of these compounds on activity and mRNA expression of renal AR and cytokines was also examined.

2 Materials and methods

2.1 Materials

CA (99%), EA (99.5%) and other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). All chemicals used in these measurements were of the highest purity commercially available.

2.2 Animals and diets

Male Balb/cA mice, 3–4 wk old, were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice were housed on a 12-h light:dark schedule; water and rat and mouse standard diet were consumed *ad libitum*. The use of mice was reviewed and approved by China Medical University animal care committee (CMU-97-22-N). To induce diabetes, mice with body weights of 23.0 ± 0.7 g were treated with streptozotocin (40 mg/kg body weight in 0.1 mol/L citrate buffer, pH 4.5) intraperitoneally for five consecutive days. The blood glucose level was monitored on days 2, 5, and 10 from the tail vein using a one-touch blood glucose meter (Lifescan). Mice with fasting blood glucose levels ≥ 14.0 mmol/L were used for this study. After diabetes was induced, mice were divided into several groups (15 mice *per* group).

2.3 Experimental design

CA or EA, 2.5 or 5 g, was mixed with 97.5 or 95 g standard power diet containing (g/100 g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture and 3 salt mixture (PMI Nutrition International LLC, Brentwood, MO, USA). All mice had free access to food and water at all times. Consumed water volume, feed and body weight were recorded weekly. Urine output and plasma levels of glucose and insulin were measured at weeks 1, 6 and 12. After 12-wk supplementation, mice were killed with carbon dioxide. Blood and kidney were collected. Plasma was separated from erythrocytes immediately. Kidney at 0.2 g was homogenized on ice in 2 mL PBS (pH 7.2) in a motor-driven Teflon glass homogenizer (Glas-Col, California, CA, USA), and the filtrate was collected. The protein concentration of plasma or kidney filtrate was determined by the method of Lowry *et al.* [16] using bovine serine albumin as a standard. In all experiments, the sample was diluted to a final concentration of 1 g protein/L using PBS, pH 7.2.

2.4 Content of CA and EA in kidney

The method of Seeram *et al.* [17] was used to analyze the renal content of CA and EA.

2.5 Biochemical analyses

The plasma glucose level (mmol/L) was measured by a glucose HK kit (Sigma Chemical). Plasma insulin level (nmol/L) was measured by using a rat insulin RIA kit (SRI-13K, Linco Research, St. Charles, MO, USA). Urine albumin was measured by a competitive ELISA assay according to the manufacturer's instruction (Exocell, Philadelphia, PA, USA). Urinary glycated albumin was determined by affinity chromatography on phenylboronate agarose to separate non-glycated (unbound) from glycated (bound) albumin *via* eluting the bound fraction with 0.3 mol/L sorbitol. Plasma blood urea nitrogen (BUN), plasma creatinine (Cr) and urinary Cr concentrations were detected by a Beckman Autoanalyzer (Beckman Coulter, Fullerton, California, CA, USA). Creatinine clearance (CCr) was calculated and expressed as mL/min/100 g body weight.

2.6 Determination of renal CML and pentosidine

The method described in Inagi *et al.* [18] was used to detect CML. Kidney cortex at 100 mg was minced, followed by adding excess $NaBH_4$ in 0.2 mol/L borate buffer (pH 9.1) for reduction. Proteins were precipitated by adding 20% trichloroacetic acid and centrifugation at $2000 \times g$ for 10 min. The pellet was washed with 1 mL 10% trichloroacetic acid. After drying, the pellet was acid

hydrolyzed in 500 μL 6 N HCl for 16 h at 110°C in screw-cap tubes purged with nitrogen. Hydrolysates were dried, rehydrated in water and used for measuring CML by reverse-phase HPLC. Pentosidine was analyzed by an HPLC method [19] in which a C18 reverse-phase column was equipped.

2.7 Determination of renal sorbitol and fructose content

One hundred milligrams kidney were homogenized with PBS (pH 7.4) containing U-[^{13}C]-sorbitol as an internal standard. After precipitating protein by ethanol, the supernatant was lyophilized. The content of sorbitol and fructose in each lyophilized sample was determined by LC with tandem mass spectrometry, according to the method of Guerrant and Moss [20].

2.8 Activity of AR and SDH

The method of Nishinaka and Yabe-Nishimura [21] was used to measure AR activity. Renal cortices were separated from the medulla, and glomeruli were isolated by differential sieving with stainless steel meshes under sterile condition. After sonication, sample was centrifuged and the supernatant was used for analysis. AR activity was measured in glomeruli by monitoring the decrease in absorbance at 340 nm due to NADPH oxidation. SDH activity was assayed according to the method of Bergmeyer [22] by mixing 100 μL kidney homogenate, 200 μL NADH (12 mM) and 1.6 mL triethanolamine buffer (0.2 M, pH 7.4), and monitoring the absorbance change at 365 nm.

2.9 Renal cytokines determination

Perfused renal tissues were homogenized in 10 mM Tris-HCl-buffered solution (pH 7.4) containing 2 M NaCl, 1 mM ethylenediaminetetraacetic acid, 0.01% Tween 80, 1 mM phenylmethylsulfonyl fluoride and centrifuged at 9000 × *g* for 30 min at 4°C. The resultant supernatant was used for cytokine determination. The levels of inflammatory cytokines, IL-1β, IL-6, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) were measured by ELISA using cytoscreen immunoassay kits (BioSource International, Camarillo, California, CA, USA). The samples were assayed in duplicates according to the manufacturer's instructions.

2.10 Quantitative RT-PCR for mRNA expression

Quantitative RT-PCR was used to examine the mRNA expression of TNF-α, MCP-1 and AR in kidney. Renal cells were lysed, and RNA was extracted using TRIizol reagent and further digested with DNase. Total RNA was isolated using the SV Total RNA Isolation kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Two micrograms of total RNA were used to generate cDNA. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The following primers were used. AR: forward, 5′-CCC AGG TGT ACC AGA ATG AGA-3′, reverse, 5′-TGG CTG CAA TTG CTT TGA TCC-3′; TNF-α: forward, 5′-GCA TGA TCC GCG ACG TGG AA-3′, reverse, 5′-AGA TCC ATG CCG TTG GCC AG-3′; MCP-1: forward, 5′-ATG CAG GTC CCT GTC ATG-3′, reverse, 5′-GCT TGA GGT GGT TGT GGA-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5′-TGA TGA CAT CAA GAA GGT GGT GAA G-3′, reverse, 5′-CCT TGG AGG CCA TGT AGG CCA T-3′. The target concentration was expressed relative to the concentration of a reference housekeeping gene, GAPDH. The cDNA was amplified under the following reaction conditions: 94°C for 1 min, 57°C for 1 min and 72°C for 1 min. In total, 28 cycles were performed for GAPDH, and 35 cycles for AR, TNF-α and MCP-1. The corresponding PCR products were analyzed by 1% w/v agarose gel electrophoresis and revealed with ethidium bromide. Finally, quantitative analysis was performed with a BAS 2000 BIO-image analyzer (Fuji Photo Film, Tokyo, Japan). In this study, mRNA level was calculated as percentage value of the non-diabetic group (without CA or EA treatment).

2.11 Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance, and *post hoc* comparisons were carried out using Dunnett's *t*-test. p-Values < 0.05 were considered as significant.

3 Results

Feed intake, water intake, body weight and urine output at weeks 1, 6 and 12 are summarized in Table 1. Compared with diabetic control group, mice with CA or EA intake had significantly lower water intake, lower feed intake, higher body weight and lower urine output at weeks 6 and 12 ($p < 0.05$), in which dose-dependent effect was presented in increasing body weight and decreasing urine output ($p < 0.05$). Renal content of CA or EA is summarized in Table 2. The intake of CA or EA dose dependently increased the content of these compounds in kidney ($p < 0.05$). Plasma levels of glucose and insulin at weeks 1, 6 and 12 are shown in Fig. 1. When compared with diabetic control group, CA or EA treatments caused significantly lower plasma glucose and higher plasma insulin at weeks 6 and 12 ($p < 0.05$). CA

Table 1. Water intake (mL/mouse/d), Feed intake (g/mouse/d), body weight (g/mouse) and urine output (mL/mouse/d) of non-diabetic mice (non-DM), diabetic mice (DM) and DM consumed 2.5 or 5% CA or EA at weeks 1, 6 and 12

	Non-DM	DM	DM+ CA, 2.5%	DM+ CA, 5%	DM+ EA, 2.5%	DM+ EA, 5%
WI						
1	$2.2\pm0.7^{a)}$	$3.7\pm0.8^{b)}$	$3.3\pm0.6^{b)}$	$3.6\pm0.9^{b)}$	$3.2\pm0.5^{b)}$	$3.5\pm0.7^{b)}$
6	$2.6\pm1.0^{a)}$	$6.0\pm1.0^{c)}$	$5.1\pm1.2^{b)}$	$4.5\pm0.8^{b)}$	$4.9\pm0.7^{b)}$	$4.3\pm0.9^{b)}$
12	$2.5\pm0.8^{a)}$	$8.2\pm1.7^{d)}$	$6.9\pm1.4^{c)}$	$5.5\pm1.1^{b)}$	$6.7\pm1.6^{c)}$	$5.3\pm1.2^{b)}$
FI						
1	$2.0\pm0.6^{a)}$	$2.3\pm0.4^{a)}$	2.4 ± 0.6^{a}	$2.1\pm0.5^{a)}$	$2.5\pm0.7^{a)}$	$2.3\pm0.4^{a)}$
6	$2.6\pm0.8^{a)}$	$5.6\pm0.9^{c)}$	$4.5\pm0.8^{b)}$	$4.2\pm0.9^{b)}$	$4.6\pm1.0^{b)}$	$4.4\pm0.6^{b)}$
12	$3.1\pm1.2^{a)}$	$7.3\pm1.3^{d)}$	$6.3\pm1.0^{c)}$	$5.3\pm0.7^{b)}$	$6.2\pm1.1^{c)}$	$5.0\pm0.8^{b)}$
BW						
1	$22.3\pm1.1^{b)}$	$20.8\pm1.2^{a)}$	$21.0\pm1.5^{a)}$	$20.7\pm1.1^{a)}$	$21.1\pm1.0^{a)}$	$21.3\pm1.4^{a)}$
6	$26.9\pm2.0^{d)}$	$15.6\pm1.8^{a)}$	$16.8\pm1.7^{b)}$	$18.0\pm1.2^{c)}$	$17.0\pm1.3^{b)}$	$18.4\pm1.6^{c)}$
12	$29.3\pm2.2^{d)}$	$11.4\pm1.7^{a)}$	$13.4\pm1.3^{b)}$	$15.5\pm1.5^{c)}$	$14.1\pm1.0^{b)}$	$16.0\pm1.2^{c)}$
UO						
1	$0.53\pm0.12^{a)}$	0.66 ± 0.09^{a}	$0.72\pm0.12^{a)}$	$0.70\pm0.05^{a)}$	$0.69\pm0.08^{a)}$	$0.67\pm0.05^{a)}$
6	$0.56\pm0.10^{a)}$	$3.68\pm0.23^{d)}$	$2.71\pm0.19^{c)}$	$1.94\pm0.21^{b)}$	$2.90\pm0.16^{c)}$	$2.03\pm0.18^{b)}$
12	$0.52\pm0.09^{a)}$	$6.32\pm0.35^{d)}$	$5.57\pm0.38^{c)}$	$4.72\pm0.20^{b)}$	$5.36\pm0.24^{c)}$	$4.65\pm0.27^{b)}$

Data are mean $\pm$ SD, $n = 15$.
a–d) Means in a row without a common letter differ, $p < 0.05$.

Table 2. Content (mg/100 g tissue) of CA and EA in kidney from non-DM, DM and DM consumed 2.5 or 5% CA or EA at week 12

	non-DM	DM	DM+CA, 2.5%	DM+CA, 5%	DM+EA, 2.5%	DM+EA, 5%
CA	–[a)]	–	31.6 ± 4.3	55.4 ± 6.0	–	–
EA	–	–	–	–	26.9 ± 2.8	46.3 ± 5.1

Data are mean $\pm$ SD, $n = 15$.
a) Means too low to be detected.

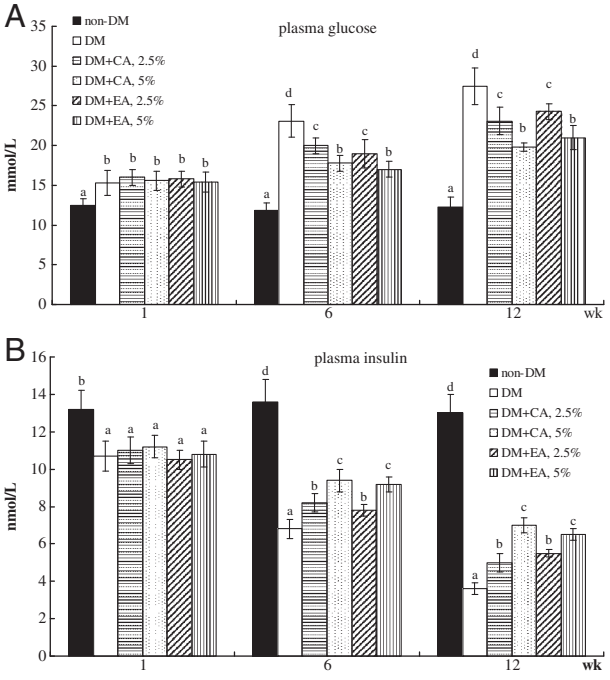

Figure 1. Plasma level of glucose (mmol/L) and insulin (nmol/L) of non-diabetic mice (non-DM), DM and DM consumed 2.5 or 5% CA or EA at weeks 1, 6 and 12. Data are mean $\pm$ SD, $n = 15$. a–d) Means among bars without a common letter differ, $p < 0.05$.

or EA treatments also dose dependently decreased BUN and elevated CCr levels ($p < 0.05$, Fig. 2).

As summarized in Table 3, CA or EA treatments at 5% significantly decreased the levels of plasma HbA1c, urinary glycated albumin, renal CML, pentosidine, sorbitol and fructose ($p < 0.05$). CA or EA intake at 5% also significantly reduced renal activity of AR and SDH ($p < 0.05$, Fig. 3). Renal levels of cytokines are summarized in Table 4. CA or EA treatments dose dependently lowered renal IL-1β, IL-6, TNF-α and MCP-1 levels ($p < 0.05$). At equal concentration, EA was greater than CA in decreasing TNF-α and MCP-1 levels ($p < 0.05$). The effects of CA or EA on mRNA expression of renal AR, TNF-α and MCP-1 are shown in Fig. 4. CA or EA treatments at 5% significantly down-regulated AR mRNA expression; but these two compounds dose dependently down-regulated mRNA expression of TNF-α and MCP-1 ($p < 0.05$).

4 Discussion

The results of our study revealed that the intake of CA or EA effectively improved glycemic control, decreased the

formation of glycative products in kidney, diminished renal AR activity and suppressed renal inflammatory cytokines release, which consequently attenuated renal glycative and inflammatory stress in diabetic mice. Furthermore, we found that the intake of these two phenolic acids effectively decreased urine output and BUN level, as well as elevated CCr in diabetic mice. These findings support that CA or EA could improve renal functions and protect kidney against the development of diabetic nephropathy *via* their anti-glycative and anti-inflammatory effects.

Plasma HbA1c, renal CML and pentosidine, and urinary glycated albumin are biomarkers for evaluating glycative injury in diabetic progression. It has been documented that the elevation or accumulation of these AGEs in plasma or organs means diabetic deterioration, and favors the occurrence of diabetic complications [1, 3]. Our present animal study found that CA and EA effectively decreased the formation of these glycative products in kidney, which not only attenuated renal glycative injury but also alleviated systemic glycative stress. AR and SDH are two enzymes involved in polyol pathway, in which AR is the first and rate-limiting enzyme in this pathway. It is reported that the increased activity and expression of these enzymes facilitate the generation of sorbitol and fructose; then, fructose and its metabolites promote non-enzymatic glycation of cellular proteins and lipids in kidney and other organs, which exacerbates glycative injury [23, 24]. Thus, these enzymes have been considered as therapeutic targets for improving diabetic nephropathy. The *in vitro* inhibitory effect of EA on AR activity has been observed [25]. Our present study further found that dietary supplement of CA and EA diminished renal activity of AR and SDH, which subsequently led to lower production of sorbitol and fructose in kidney. This finding indicated that these two phenolic acids could attenuate diabetic renal glycative injury *via* suppressing polyol pathway and decreasing AGEs formation. Furthermore, we found that the intake of these compounds

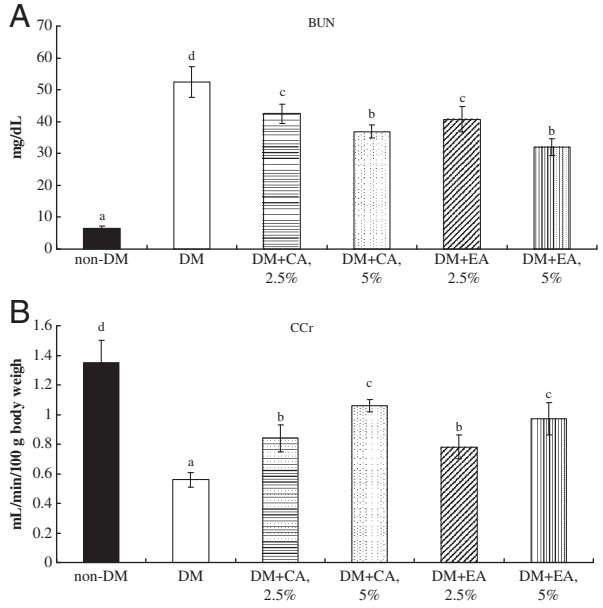

Figure 2. Plasma level of BUN (mg/dL) and CCr (mL/min/100 g body weight) in non-DM, DM, DM consumed 2.5 or 5% CA or EA at week 12. Data are mean ± SD, $n = 15$.
a–d) Means among bars without a common letter differ, $p < 0.05$.

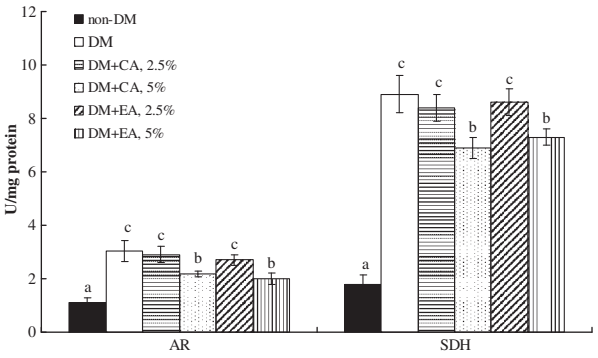

Figure 3. Renal activity (U/mg protein) of AR and SDH in non-DM, DM, DM consumed 2.5 or 5% CA or EA at week 12. Data are mean ± SD, $n = 15$.
a–c) Means among bars without a common letter differ, $p < 0.05$.

Table 3. Level of plasma HbA1c (%), urinary glycated albumin (μg/mL), renal CML (pmol/mg), pentosidine (pmol/mg), sorbitol (nmol/mg protein) and fructose (nmol/mg protein) in non-DM, DM and DM consumed 2.5 or 5% CA or EA at week 12

	non-DM	DM	DM+CA, 2.5%	DM+CA, 5%	DM+EA, 2.5%	DM+EA, 5%
Plasma						
HbA1c	$3.1 \pm 0.4^{a)}$	$10.3 \pm 0.6^{c)}$	$9.8 \pm 0.5^{c)}$	$7.5 \pm 0.7^{b)}$	$9.6 \pm 0.4^{c)}$	$7.3 \pm 0.6^{b)}$
Urinary						
Glycated albumin	$41 \pm 7^{a)}$	$1206 \pm 178^{c)}$	$1135 \pm 180^{c)}$	$875 \pm 96^{b)}$	$1112 \pm 167^{c)}$	$824 \pm 86^{b)}$
Renal						
CML	$9 \pm 3^{a)}$	$89 \pm 6^{c)}$	$81 \pm 5^{c)}$	$61 \pm 4^{b)}$	$79 \pm 5^{c)}$	$56 \pm 3^{b)}$
Pentosidine	$0.23 \pm 0.05^{a)}$	$1.97 \pm 0.11^{c)}$	$1.86 \pm 0.14^{c)}$	$1.31 \pm 0.09^{b)}$	$1.82 \pm 0.13^{c)}$	$1.45 \pm 0.12^{b)}$
Sorbitol	$2.5 \pm 0.3^{a)}$	$27.3 \pm 1.9^{c)}$	$26.0 \pm 2.1^{c)}$	$21.5 \pm 1.3^{b)}$	$26.6 \pm 1.0^{c)}$	$22.1 \pm 1.5^{b)}$
Fructose	$10.2 \pm 1.4^{a)}$	$90.7 \pm 4.5^{c)}$	$86.4 \pm 3.9^{c)}$	$67.3 \pm 2.3^{b)}$	$87.4 \pm 4.1^{c)}$	$61.8 \pm 3.6^{b)}$

Data are mean ± SD, $n = 15$.
a–c) Means in a row without a common letter differ, $p < 0.05$.

Table 4. Renal level (pg/mg) of IL-1β, IL-6, TNF-α and MCP-1 in non-DM, DM and DM consumed 2.5 or 5% CA or EA at week 12

	non-DM	DM	DM+CA, 2.5%	DM+CA, 5%	DM+EA, 2.5%	DM+EA, 5%
IL-1β	$15\pm3^{a)}$	$247\pm23^{e)}$	$205\pm19^{d)}$	$153\pm14^{c)}$	$197\pm17^{d)}$	$132\pm16^{b)}$
IL-6	$20\pm4^{a)}$	$226\pm18^{d)}$	$188\pm14^{c)}$	$136\pm10^{b)}$	$171\pm15^{c)}$	$124\pm13^{b)}$
TNF-α	$17\pm4^{a)}$	$307\pm27^{f)}$	$263\pm20^{e)}$	$171\pm17^{c)}$	$219\pm15^{d)}$	$140\pm12^{b)}$
MCP-1	$15\pm5^{a)}$	$281\pm24^{e)}$	$242\pm20^{d)}$	$190\pm18^{c)}$	$201\pm13^{c)}$	$153\pm10^{b)}$

Data are mean ± SD, $n = 15$.
a–f) Means in a row without a common letter differ, $p < 0.05$.

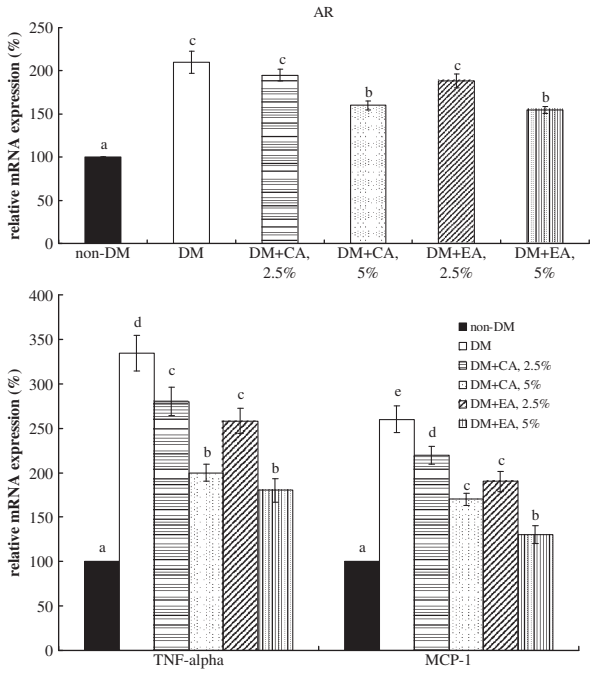

Figure 4. mRNA expression of renal AR, TNF-α and MCP-1 in non-DM, DM, DM consumed 2.5 or 5% CA or EA at week 12. Data are mean ± SD, $n = 15$.
a–e) Means among bars without a common letter differ, $p < 0.05$.

down-regulated the mRNA expression of AR in kidney. This result suggests that the effects of these agents might occur at the level of transcription. It is well known that AGEs formation and glycative stress may be involved in the progression of other diseases such as Alzheimer's disease [23, 24]. Thus, the intake of these phenolic acids might also provide anti-glycative protection for patients with other glycation-associated diseases.

IL-1β, IL-6 and TNF-α, pro-inflammatory cytokines, were central mediators for the regulation of several biomarkers such as C-reactive protein and von Willebrand factor, which consequently enhanced the progression of inflammation, endothelial dysfunction and coagulation in diabetes [26, 27]. It has been indicated that overexpression of these cytokines exacerbates the severity of diabetes [4, 26]. Thus, the suppression on these inflammatory cytokines could retard or alleviate inflammation and endothelial dysfunction. Our present study found that the intake of CA and EA substantially decreased renal IL-1β, IL-6 and TNF-α levels in diabetic mice. These results revealed that the anti-inflammatory effects of these compounds were partially due to their suppression on the release of pro-inflammatory cytokines. MCP-1 is a chemotactic factor for activating monocytes and macrophages and could recruit monocytes to the sites of injury [28, 29]. In this study, the increased renal MCP-1 level indicated that the kidneys of these diabetic mice were injured, and these mice were at risk for further diabetes-associated renal diseases. However, we also found that the intake of these two phenolic acids markedly lowered renal MCP-1 protein level in diabetic mice. These results implied that these two compounds could protect kidney against inflammation *via* diminishing the activation of monocytes/macrophages and lowering the recruitment of monocytes. Furthermore, we found that these two compounds dose dependently down-regulated renal mRNA expression of TNF-α and MCP-1, which also supported that these compounds could mediate these factors through molecular pathway.

It is reported that AGEs could promote mRNA expression and secretion of TNF-α in human umbilical vein cords endothelial cells [30]. Dronavalli *et al.* [31] also indicated that there were interactions between AGEs and cytokines in the pathogenic mechanism of diabetic nephropathy. Obviously, any therapeutic strategy for diabetic nephropathy should consider the interaction of glycation or inflammation. Thus, agents with anti-glycative and anti-inflammatory activities such as CA and EA may provide greater renal protection because both glycation and inflammation reactions could be simultaneously retarded or delayed. It is notified that CA or EA dose dependently decreased renal release of inflammatory cytokines, but these two compounds lowered AGEs formation and AR activity only at high dose (5%). Apparently, these two agents could provide more efficient anti-inflammatory protection. Both CA and EAs are phenolic acids naturally occurred in many vegetables and fruits [10, 11], and our present study indicated that the intake of these two phenolic acids increased their deposit in mice kidney. Thus, it might be possible that the consumption of foods rich in these compounds in considerable amount and

with high frequency for human could cause the accumulation of these compounds in our organs, which allows them to exhibit protective effects such as anti-glycation and anti-inflammation.

In summary, the intake of CA and EA protected kidney of diabetic mice against glycative and inflammatory progression *via* decreasing the formation of glycative biomarkers such as CML and pentosidine, suppressing AR activity, and lowering the release of inflammatory cytokines such as IL-1β and IL-6. The impact of these compounds on mRNA expression of renal TNF-α, MCP-1 and AR revealed that the effects of these compounds might directly occur at the level of transcription. Therefore, the supplement of these agents might be helpful for the prevention or treatment of diabetes-associated kidney diseases.

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The authors have declared no conflict of interest.

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