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ORIGINAL CONTRIBUTION

Houttuynia cordata aqueous extract attenuated glycative and oxidative stress in heart and kidney of diabetic mice

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Abstract

Purpose The anti-glycative and anti-oxidative effects from *Houttuynia cordata* leaves aqueous extract (HCAE) in heart and kidney of diabetic mice were examined.

Methods HCAE, at 1 or 2 %, was supplied in drinking water for 8 weeks. Plasma glucose and blood urea nitrogen (BUN) levels and creatine phosphokinase (CPK) activity were measured. The production of oxidative and inflammatory factors was determined. Activity and protein expression of associated enzymes or regulators were analyzed.

Results HCAE intake at both doses lowered plasma glucose and BUN levels, and CPK activity and also restored creatinine clearance rate in diabetic mice. HCAE intake, only at 2 %, retained plasma insulin levels ($P < 0.05$). HCAE reduced reactive oxygen species, protein carbonyl, interleukin-6, tumor necrosis factor-alpha, N^{ε}-(carboxymethyl)-lysine, pentosidine and fructose levels, and reserved glutathione content in heart and kidney of diabetic mice ($P < 0.05$). Diabetes enhanced aldose reductase (AR) activity and protein expression in heart and kidney ($P < 0.05$). HCAE intake at both doses decreased renal AR activity and protein expression, but only at 2 % lowered cardiac AR activity and protein expression ($P < 0.05$). Diabetes increased protein expression of RAGE, p47[phox] and gp91[phox], nuclear factor kappa-B (NF-κB) p50, NF-κB p65 and mitogen-activated protein kinase in heart and kidney ($P < 0.05$). HCAE intake only at 2 % limited RAGE expression, but at 1 and 2 % downregulated p47[phox], NF-κB p65 and p-p38 expression in these organs ($P < 0.05$).

Conclusions These findings suggest that *Houttuynia cordata* leaves aqueous extract could ameliorate cardiac and renal injury under diabetic condition.

Keywords *Houttuynia cordata* · Diabetes · Glycation · Polyol pathway · NADPH oxidase

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Abbreviations

AGEs	Advanced glycation endproducts
AR	Aldose reductase
BUN	Blood urea nitrogen
CCr	Creatinine clearance rate
CML	N^{ε}-(carboxymethyl)-lysine
CPK	Creatine phosphokinase
Cr	Creatinine
DCFH-DA	2′,7′-Dichlorofluorescein diacetate
DNP	Dinitrophenylhydrazine
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSH	Glutathione
HCAE	*Houttuynia cordata* leaves aqueous extract
IL	Interleukin
MAPK	Mitogen-activated protein kinase
NF-κB	Nuclear factor kappa-B
RFU	Relative fluorescence unit
ROS	Reactive oxygen species
TNF-alpha	Tumor necrosis factor-alpha

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Introduction

Hyperglycemia enhances polyol pathway for glucose metabolism, and aldose reductase (AR) is the rate-limiting enzyme in this pathway [1, 2]. AR metabolizes glucose to sorbitol, which is further converted to fructose. Excessive glucose and fructose occurred in diabetic condition promote the generation of advanced glycation endproducts (AGEs). AGEs and their modified derivatives cause cellular damage and organ malfunctions, and even elicit diabetic complications [3, 4]. N^{ε}-(carboxymethyl)-lysine (CML) and pentosidine are predominant AGEs contributing to the occurrence of diabetic complications like diabetic cardiomyopathy and nephropathy in diabetic patients [5, 6]. Therefore, glycative stress could be diminished by inhibiting AR and decreasing AGEs production. In addition, AGEs upregulate their membrane-anchored receptor (RAGE), and the RAGE–AGE interaction evokes intracellular reactive oxygen species (ROS) generation and activates mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B (NF-κB) signaling pathways [7, 8]. The activation of both pathways further accelerates diabetic deterioration via increasing the formation of glycative, oxidative, inflammatory and fibrotic factors. Thus, the suppression on RAGE, NF-κB and/or MAPK pathways may benefit diabetic prevention and/or attenuation.

Houttuynia cordata Thumb is traditionally applied as a medicinal plant in Asian countries including China, Japan and Taiwan [9]. Wang et al. [10] reported that this plant was used to treat several symptoms such as clearing heat, eliminating carbuncle, detoxification and promoting diuresis. Furthermore, those authors indicated that the decoction of this plant protected renal tissue of diabetic rats through reducing protein expression of transforming growth factor-beta1 [10]. The study of Kumar et al. [11] revealed that ethanol extract prepared from whole plant of *H. cordata* exhibited anti-hyperglycemic effect in diabetic mice via raising antioxidant activity and glucose transporters. Those previous studies already suggest that *H. cordata* could provide antidiabetic activity. On the other hand, the fresh leaf part of this plant is consumed as a vegetable in China [12]. Our previous study found that the aqueous extract of *H. cordata* leaves contained many phenolic acids and flavonoids such as kaempferol, quercetin, myricetin, ellagic acid, ferulic acid and gallic acid, and the intake of this extract protected mice against high saturated fat diet-induced obesity, hepatic inflammatory and oxidative injury [13]. So far, the antidiabetic and antiglycative effects as well as the action modes of the aqueous extract prepared from *H. cordata* leaves remain unknown. Furthermore, heart and kidney are two organs more vulnerable to diabetes. Thus, the antidiabetic protection for these organs seems relatively more important.

The major purpose of this study was to examine the antiglycative effects of aqueous extract of *H. cordata* leaves in heart and kidney of diabetic mice. The effects of this extract upon the production of AGEs and ROS as well as the activity and/or protein expression of AR, RAGE, NF-κB and MAPK were also evaluated. These results could provide evidence regarding the application of *H. cordata* leaves aqueous extract as a medicinal supplement against diabetes.

Materials and methods

Materials

Fresh *H. cordata* leaves were obtained from Nantou County, Taiwan, in 2012. *H. cordata* leaves aqueous extract (HCAE) was made by mixing 50 g chopped fresh leaves and 150 ml distilled water, homogenizing for 1 min in a Waring blender and cooking for 15 min at 100 °C. After filtrating through a filter paper, the filtrate was then freeze-dried to a powder. Our previous study indicated that the content of total phenolic acid and total flavonoid in HCAE was 2175 ± 210 and 1766 ± 182 mg/100 g dry weight, respectively. In order to standardize HCAE, the content of both total phenolic acid and total flavonoid in HCAE used in the present study was analyzed by the methods of Singleton et al. [14] and Zhishen et al. [15]. HCAE powder at 1 or 2 g was further mixed with 99 or 98 ml double-distilled water to prepare 1 or 2 % HCAE drinking water for experiments.

Animals and diets

Male 3-week-old Balb/cA mice were purchased from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice with 22.7 ± 0.6 g body weight were treated with a single i.v. injection of streptozotocin (50 mg/kg) into the tail vein of mice after 12 h fast to induce diabetes. Blood glucose levels were measured on day 10 from the tail vein by a one-touch blood glucose meter (Lifescan Inc., Milpitas, CA, USA) after 12 h fast. Mice with fasting blood glucose levels ≥200 mg/dl were used. Animal experiments were performed in accordance with protocols approved by the Animal Care Research Committee of China Medical University (102-36-N).

Experimental design

After diabetes has been induced, mice were divided into three groups (10 mice/group): diabetic mice with 0, 1 or 2 % HCAE treatment. In addition, two groups of non-diabetic mice were also used, in which 0 (control) or 2 %

HCAE was supplied. All mice were free to access food and water at all times. Consumed feed and water volume were recorded. Body weight and plasma glucose levels were measured weekly. Urine was collected for 24 h from mice in metabolic cages. After 8 weeks of supplementation, mice were fasted for 12 h and treated with carbon dioxide. Blood, heart and kidney from each mouse were collected. Protein concentration of cardiac or renal homogenate was determined by an assay kit purchased from Pierce Biotechnology Inc. (Rockford, IL, USA).

Blood and urinary analyses

Plasma glucose levels were measured by glucose HK kit bought from Sigma Chemical Co. (St. Louis, MO, USA). Plasma insulin levels were measured by insulin radioimmunoassay kit (Linco Research Inc., St. Charles, MO, USA). Plasma creatine phosphokinase (CPK) activity was assayed by an ELISA kit (Randox, UK). Plasma blood urea nitrogen (BUN), plasma and urinary creatinine concentrations were measured by a Beckman Autoanalyzer (Beckman Coulter, Fullerton, CA, USA). Creatinine clearance rate (CCr) was calculated according to a Cockcroft-Gault formula: [urinary volume (ml) × urinary creatinine (mg/dl)]/[plasma creatinine (mg/dl) × urine collection length (min)]. Results were expressed as ml/min/100 g body weight. Glycated albumin level was analyzed by chromatography on phenylboronate agarose for the separation of nonglycated from glycated albumin by eluting the glycated fraction with 0.35 mol/l sorbitol.

Measurement of CML, pentosidine and fructose levels

Cardiac or renal tissue was homogenized and digested with proteinase K (1 mg/ml) for 3 h at 37 °C and followed by adding 2 mmol/l phenylmethylsulfonyl fluoride to stop reaction. CML was determined by a competitive ELISA kit purchased from Roche Diagnostics (Penzberg, Germany) with 4G9, a CML-specific monoclonal antibody. Absorbance at 405 nm was read in a microtiter Bio-Rad plate reader (Hercules, CA, USA). Pentosidine levels were analyzed by a HPLC method described by Miyata et al. [16]. Sample was lyophilized and hydrolyzed in 500 µl of 6 N HCl at 110 °C for 16 h. NaOH was used for neutralization, and sample was used for HPLC measurement. HPLC (Waters, Tokyo, Japan) equipped with a fluorescence detector and a C18 reverse-phase column was used, and excitation and emission wavelength was set at 335 and 385 nm, respectively. In addition, 50 mg of heart or kidney was homogenized with phosphate buffer containing U-[^{13}C]-sorbitol as an internal standard. After protein precipitation and centrifugation, the supernatant was lyophilized. Fructose content in lyophilized sample was assayed by liquid chromatography according to the method of Guerrant and Moss [17].

Assay of AR activity

The method of Nishinaka and Yabe-Nishimura [18] was used to determine AR activity. D/L-glyceraldehyde was sued as a substrate. The decrease in absorbance at 340 nm was monitored to reflect NADPH oxidation.

Measurement of ROS, glutathione (GSH), protein carbonyl, interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha levels

Cardiac or renal tissue was homogenized with cold phosphate buffer containing 1 mM EDTA and 0.05 % Tween. An oxidation-sensitive dye, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), was used to determine ROS level. Briefly, 500 µl of homogenate was mixed with 500 µl of 2 mg/ml DCFH-DA for 30 min. Fluorescence at 488 nm excitation and 525 nm emission was recorded by a fluorescence plate reader. Results are expressed as relative fluorescence unit (RFU) per mg protein. GSH concentration was quantified by a colorimetric glutathione kit purchased from OxisResearch Co. (Portland, OR, USA). Protein carbonyls were analyzed by the Zentech PC kit bought from BioCell Chem. Co. (Auckland, New Zealand). Briefly, 50 µl of tissue homogenate was blended with a 200 µl dinitrophenylhydrazine (DNP) solution. Then, adsorbed DNP-protein reacted with an anti-DNP-biotin antibody, and the absorbance at 450 nm was measured. IL-6 and TNF-alpha levels in heart or kidney homogenate were measured using ELISA kits purchased from R&D Systems (Minneapolis, MN, USA).

Western blot analyses

Cardiac or renal tissue, 40 mg, was homogenized in buffer containing protease inhibitor cocktail purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and 0.5 % Triton X-100. Homogenate was then mixed with buffer (60 mmol/L Tris–HCl, 2 % β-mercaptoethanol and 2 % SDS, pH 7.2) and followed by boiling for 5 min. Protein sample at 40 µg was electrophoresed on 10 % SDS-polyacrylamide gel and further transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA) for 1 h. After blocking with a protein solution containing 5 % skim milk for 1 h, membranes were treated with monoclonal antibody (Boehringer-Mannheim, Indianapolis, IN, USA) against RAGE (1:500), AR, p47^{phox}, gp91^{phox}, NF-κB p50, NF-κB p65 (1:1000) and MAPK (1:2000) at 4 °C overnight, and followed by incubating with horseradish peroxidase-conjugated antibody at room temperature for 3.5 h.

Table 1 Body weight (g/mouse), water intake (ml/mouse/day), feed intake (g/mouse/day), urine output (ml/mouse/day), heart weight (mg/mouse) and kidney weight (mg/mouse) in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks

	Normal		DM		
	HCAE, 0 %	HCAE, 2 %	HCAE, 0 %	HCAE, 1 %	HCAE, 2 %
Body weight	28.2 ± 1.8^d	29.0 ± 2.1^d	$14.2 \pm 0.6^a, P = 0.001$	$17.1 \pm 1.1^b, P = 0.009$	$19.5 \pm 0.9^c, P = 0.037$
Water intake	2.1 ± 0.4^a	2.0 ± 0.6^a	$5.5 \pm 1.0^c, P = 0.008$	$4.1 \pm 0.5^b, P = 0.025$	$3.7 \pm 0.4^b, P = 0.031$
Feed intake	1.9 ± 0.5^a	1.7 ± 0.7^a	$5.2 \pm 1.2^c, P = 0.01$	$4.0 \pm 0.6^b, P = 0.045$	$3.8 \pm 0.3^b, P = 0.043$
Urine output	0.48 ± 0.06^a	0.51 ± 0.03^a	$5.89 \pm 0.21^d, P = 0.001$	$4.21 \pm 0.13^c, P = 0.013$	$2.95 \pm 0.11^b, P = 0.02$
Heart weight	144 ± 8^a	147 ± 7^a	145 ± 5^a	151 ± 6^a	148 ± 4^a
Kidney weight	440 ± 10^a	443 ± 12^a	435 ± 14^a	449 ± 9^a	450 ± 15^a

Values are mean ± SD, $n = 10$

[a–d] Means in a row without a common letter differ, $P < 0.05$

Table 2 Plasma level of glucose (mmol/l), insulin (nmol/l), CPK (IU/l), BUN (mg/dl) and CCr (ml/min/100 g body weight) in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks

	Normal		DM		
	HCAE, 0 %	HCAE, 2 %	HCAE, 0 %	HCAE, 1 %	HCAE, 2 %
Glucose	9.1 ± 0.6^a	9.7 ± 0.8^a	$26.8 \pm 2.1^d, P = 0.001$	$21.3 \pm 1.5^c, P = 0.01$	$17.2 \pm 1.3^b, P = 0.03$
Insulin	14.0 ± 0.7^c	13.5 ± 1.0^c	$5.5 \pm 0.3^a, P = 0.009$	$6.2 \pm 0.5^a, P = 0.01$	$7.8 \pm 0.6^b, P = 0.029$
CPK	42.5 ± 1.7^a	40.6 ± 2.2^a	$103.8 \pm 5.1^d, P < 0.001$	$88.4 \pm 3.7^c, P = 0.008$	$72.6 \pm 4.0^b, P = 0.015$
BUN	5.1 ± 0.4^a	4.9 ± 0.5^a	$54.0 \pm 2.3^d, P = 0.001$	$43.2 \pm 1.9^c, P = 0.011$	$32.4 \pm 1.3^b, P = 0.037$
CCr	1.71 ± 0.22^d	1.67 ± 0.25^d	$0.45 \pm 0.08^a, P = 0.001$	$0.77 \pm 0.1^b, P = 0.01$	$1.09 \pm 0.12^c, P = 0.042$

Values are mean ± SD, $n = 10$

[a–d] Means in a row without a common letter differ, $P < 0.05$

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control, and the bands were quantified by an ATTO image analyzer (Tokyo, Japan).

Statistical analysis

Each group had ten mice ($n = 10$). All data were expressed as mean ± standard deviation (SD). Statistical analysis was processed by one-way ANOVA. Post hoc comparisons were carried out by Dunnet's t test. Statistical significance was considered at p value <0.05.

Results

Effect of HCAE on diabetic characteristics

HCAE intake at 2 % did not change body weight, water intake, feed intake, urine output, heart weight and kidney weight in normal mice (Table 1, $P > 0.05$). Diabetes significantly decreased body weight and increased water intake, feed intake and urine output ($P \leq 0.01$); however, diabetic mice with HCAE intake at both doses had significantly higher body weight ($P = 0.009$ and 0.037), lower water intake ($P = 0.025$ and 0.031), lower feed intake ($P = 0.045$ and 0.043) and less urine volume ($P = 0.013$ and 0.02). As shown in Table 2, diabetes raised plasma glucose and BUN levels, and CPK activity, but decreased plasma insulin and CCr levels ($P \leq 0.009$). HCAE intake at both doses reduced plasma levels of glucose ($P = 0.01$ and 0.03) and BUN ($P = 0.011$ and 0.037), and CPK activity ($P = 0.008$ and 0.015) and also restored CCr levels ($P = 0.01$ and 0.042) in diabetic mice. HCAE intake, only at 2 %, retained the insulin level ($P = 0.029$).

Effects of HCAE on oxidative, inflammatory and glycative factors

Diabetes increased ROS, protein carbonyl, IL-6 and TNF-alpha levels, but lowered GSH content in heart and kidney (Table 3, $P \leq 0.002$). HCAE intake decreased cardiac levels of ROS ($P = 0.009$ and 0.039), protein carbonyl ($P = 0.007$ and 0.021), IL-6 ($P = 0.01$ and 0.035) and TNF-alpha ($P = 0.008$ and 0.016), and retained GSH content ($P = 0.01$ and 0.041). HCAE intake at both doses also reduced renal levels of ROS ($P = 0.01$ and 0.043), protein carbonyl ($P = 0.008$ and 0.019), IL-6 ($P = 0.01$ and 0.023) and TNF-alpha ($P = 0.007$ and 0.017), and retained GSH

Table 3 Cardiac or renal level of ROS (RFU/mg protein), GSH (nmol/mg protein), protein carbonyl (pmol/mg protein), IL-6 (pg/mg protein) and TNF-alpha (pg/mg protein) in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks

	Normal		DM		
	HCAE, 0 %	HCAE, 2 %	HCAE, 0 %	HCAE, 1 %	HCAE, 2 %
Heart					
ROS	0.27 ± 0.08^a	0.28 ± 0.05^a	1.13 ± 0.14^d, $P = 0.001$	0.86 ± 0.11^c, $P = 0.009$	0.54 ± 0.09^b, $P = 0.039$
GSH	19.5 ± 1.2^d	19.8 ± 1.0^d	9.9 ± 0.4^a, $P = 0.001$	12.0 ± 0.7^b, $P = 0.01$	14.8 ± 1.1^c, $P = 0.041$
Protein carbonyl	9.6 ± 0.8^a	9.4 ± 0.6^a	79.3 ± 3.1^d, $P = 0.001$	64.1 ± 2.7^c, $P = 0.007$	42.8 ± 2.2^b, $P = 0.021$
IL-6	10.2 ± 0.9^a	9.9 ± 1.1^a	78.5 ± 3.5^d, $P = 0.002$	62.8 ± 3.2^c, $P = 0.01$	48.7 ± 2.4^b, $P = 0.035$
TNF-alpha	12.3 ± 0.7^a	11.8 ± 0.5^a	82.1 ± 2.9^d, $P < 0.001$	70.4 ± 3.1^c, $P = 0.008$	53.6 ± 1.8^b, $P = 0.016$
Kidney					
ROS	0.31 ± 0.06^a	0.29 ± 0.09^a	1.32 ± 0.16^d, $P = 0.001$	0.97 ± 0.13^c, $P = 0.01$	0.62 ± 0.08^b, $P = 0.043$
GSH	18.0 ± 1.3^d	18.4 ± 0.8^d	8.9 ± 1.0^a, $P = 0.001$	10.8 ± 0.7^b, $P = 0.006$	13.1 ± 0.9^c, $P = 0.025$
Protein carbonyl	10.4 ± 0.5^a	10.1 ± 0.8^a	83.8 ± 3.7^d, $P < 0.001$	70.2 ± 3.0^c, $P = 0.008$	52.3 ± 2.2^b, $P = 0.019$
IL-6	11.4 ± 0.7^a	10.5 ± 1.0^a	90.2 ± 3.1^d, $P = 0.001$	73.9 ± 2.8^c, $P = 0.01$	57.2 ± 1.6^b, $P = 0.023$
TNF-alpha	13.5 ± 1.1^a	12.7 ± 0.9^a	94.3 ± 2.5^d, $P < 0.001$	77.5 ± 3.0^c, $P = 0.007$	62.9 ± 2.4^b, $P = 0.017$

Values are mean $\pm$ SD, $n = 10$

[a–d] Means in a row without a common letter differ, $P < 0.05$

Table 4 Cardiac or renal content of CML (pmol/mg protein), pentosidine (pmol/mg protein) and fructose (nmol/mg protein), and urine glycated albumin (μg/ml) in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks

	Normal		DM		
	HCAE, 0 %	HCAE, 2 %	HCAE, 0 %	HCAE, 1 %	HCAE, 2 %
Heart					
CML	9.4 ± 1.2^a	9.1 ± 0.8^a	74.8 ± 4.1^d, $P < 0.001$	65.1 ± 3.5^c, $P = 0.008$	50.4 ± 2.6^b, $P = 0.029$
Pentosidine	0.27 ± 0.08^a	0.25 ± 0.05^a	1.37 ± 0.19^d, $P = 0.001$	1.16 ± 0.14^c, $P = 0.01$	0.86 ± 0.11^b, $P = 0.036$
Fructose	16.9 ± 1.1^a	16.3 ± 0.8^a	83.4 ± 3.2^d, $P < 0.001$	69.8 ± 2.7^c, $P = 0.009$	51.5 ± 2.2^b, $P = 0.018$
Kidney					
CML	12.1 ± 0.9^a	12.5 ± 1.1^a	83.0 ± 3.9^d, $P < 0.001$	71.4 ± 2.7^c, $P = 0.01$	57.2 ± 2.1^b, $P = 0.021$
Pentosidine	0.34 ± 0.11^a	0.31 ± 0.07^a	1.68 ± 0.42^d, $P = 0.001$	1.35 ± 0.38^c, $P = 0.011$	1.04 ± 0.25^b, $P = 0.04$
Fructose	15.8 ± 0.7^a	15.2 ± 1.0^a	79.6 ± 3.5^d, $P = 0.001$	70.4 ± 1.9^c, $P = 0.009$	58.9 ± 2.1^b, $P = 0.023$
Urine					
Glycated albumin	133 ± 18^a	135 ± 22^a	1086 ± 98^d, $P < 0.001$	818 ± 46^c, $P = 0.01$	608 ± 55^b, $P = 0.042$

Values are mean $\pm$ SD, $n = 10$

[a–d] Means in a row without a common letter differ, $P < 0.05$

content ($P = 0.006$ and 0.025). Diabetes increased CML, pentosidine and fructose levels in heart and kidney and also raised urinary glycative albumin level (Table 4, $P \leq 0.001$). HCAE intake lowered the generation of CML ($P = 0.008$ and 0.029), pentosidine ($P = 0.01$ and 0.036) and fructose ($P = 0.009$ and 0.018) in heart. HCAE intake at 1 or 2 % also decreased renal level of CML ($P = 0.01$ and 0.021), pentosidine ($P = 0.011$ and 0.04), fructose ($P = 0.009$ and 0.023) and urinary glycative albumin level ($P = 0.01$ and 0.042). As shown in Fig. 1, diabetes enhanced AR activity and protein expression in heart and kidney ($P < 0.05$).

HCAE intake at both doses decreased renal AR activity and protein expression and only at 2 % lowered cardiac AR activity and expression ($P < 0.05$).

Effects of HCAE on expression of RAGE, NADPH oxidase, NF-κB and MAPK

Diabetes promoted protein expression of RAGE, p47[phox] and gp91[phox] in heart and kidney (Fig. 2, $P < 0.05$). HCAE intake only at 2 % limited RAGE expression in both organs, but at 1 and 2 % lowered p47[phox] expression in these organs

Fig. 1 Cardiac or renal activity (nmol/min/mg protein) and protein expression of AR in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks. Values are mean ± SD, $n = 10$. [a–d]Means among *bars without a common letter differ*, $P < 0.05$
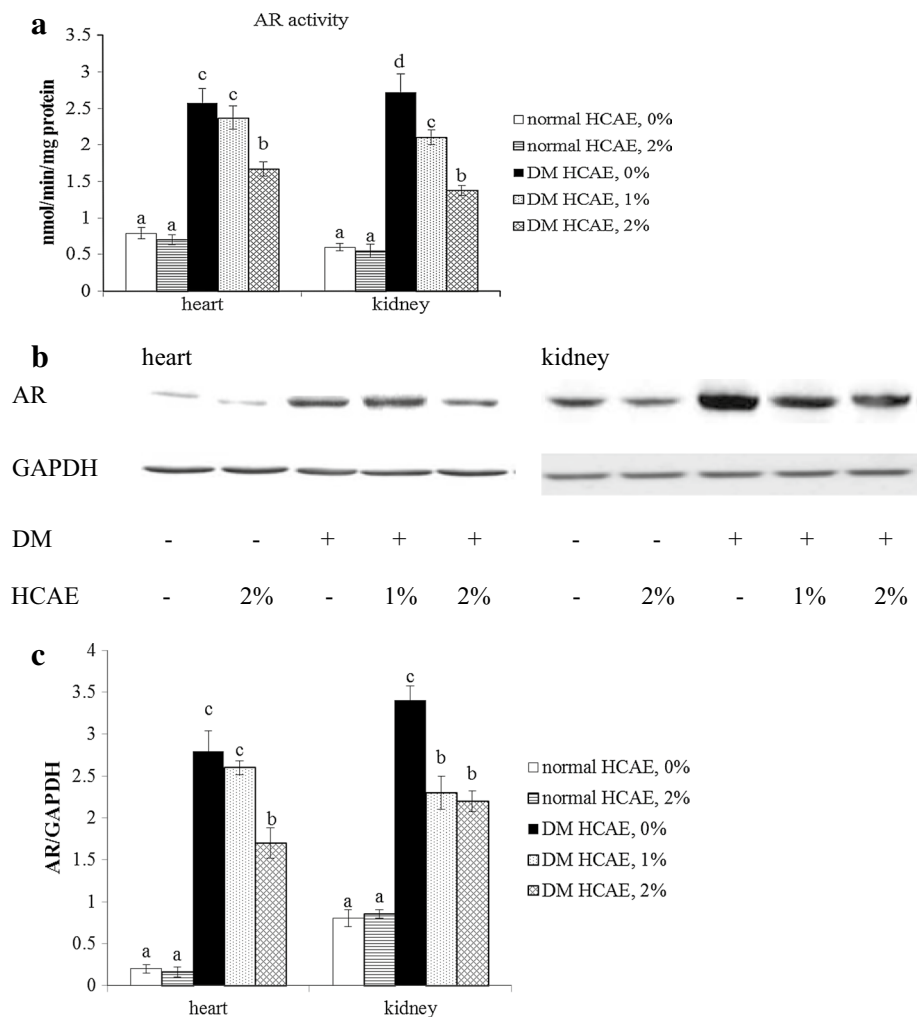
($P < 0.05$). HCAE intake did not affect gp91[phox] expression ($P > 0.05$). Diabetes also upregulated cardiac and renal expression of NF-κB p50, NF-κB p65 and MAPK (Fig. 3, $P < 0.05$). HCAE intake at both doses downregulated NF-κB p65 and p-p38 expression in two organs ($P < 0.05$).

Discussion

The effects of *H. cordata* upon glycemic control and renal protection in diabetic animals have been reported [11, 12]. However, decoction of crude *H. cordata* was used by Wang et al. [11], while Kumar et al. [12] used ethanol extract prepared from whole plant of *H. cordata*. In our present study, the aqueous extract of *H. cordata* leaves was used, and our data revealed that the intake of this extract decreased plasma glucose, BUN and CPK levels or activity, retained plasma insulin level and raised CCr in diabetic mice. These novel findings suggest that the aqueous extract of *H. cordata* leaves could exhibit antidiabetic activity. Furthermore, we found that this extract repressed polyol, NF-κB

and MAPK pathways in heart and kidney of diabetic mice, which consequently diminished the formation of glycative, inflammatory and oxidative factors including AGEs, ROS and inflammatory cytokines in these two organs. These data support that *H. cordata* leaves aqueous extract could provide multiple protective actions against diabetic deterioration.

Hyperglycemia enhances oxidative and inflammatory stress, which contribute to the development of diabetic cardiomyopathy and/or nephropathy [19, 20]. In our present study, diabetes caused overproduction of ROS, protein carbonyl, IL-6 and TNF-alpha in heart and kidney. These findings agreed those previous studies and indicated that these two organs were vulnerable to diabetes. NADPH oxidase complex plays an important role in diabetes-induced ROS generation [21, 22]. We examined the influence of aqueous extract of *H. cordata* leaves upon the expression of p47[phox] and gp91[phox], NADPH oxidase cytosolic and membrane components, and our data revealed that this extract effectively downregulated cardiac and renal protein expression of p47[phox], which in turn led to lower ROS

Fig. 2 Cardiac and renal expression of RAGE, p47^{phox} and gp91^{phox} in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks. Values are mean ± SD, $n = 10$. $^{a-c}$Means among *bars without a common letter* differ, $P < 0.05$
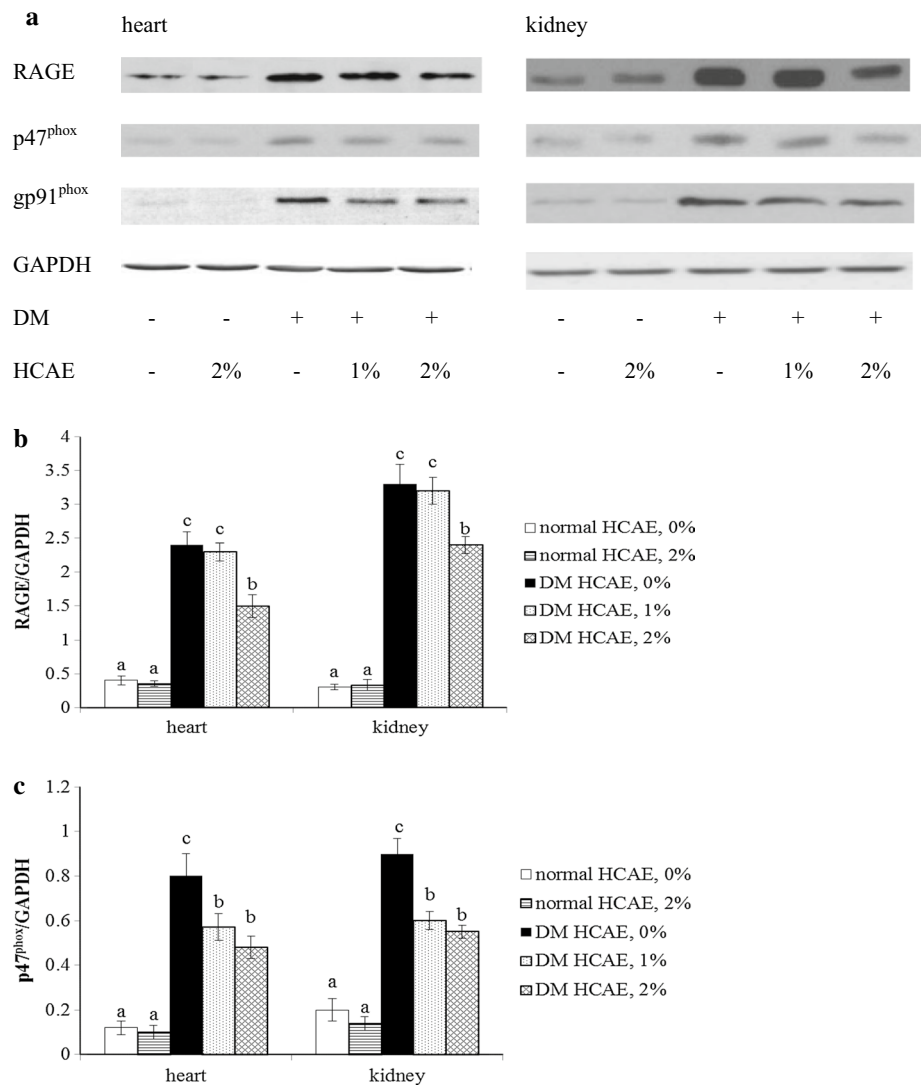

production and spared GSH content in both organs. These findings suggest that aqueous extract of *H. cordata* leaves could mitigate diabetes elicited cardiac and renal oxidative injury via regulating NADPH oxidase. ROS is a stimulator for the formation of inflammatory cytokines like IL-6 and TNF-alpha [23]. Thus, the less ROS level in heart and kidney of mice treated with aqueous extract of *H. cordata* leaves subsequently ameliorated inflammatory stress. Consequently, the lower IL-6 and TNF-alpha levels in heart and kidney were observed. In addition, hyperglycemia and ROS are crucial activators for signal transduction cascades of NF-κB and MAPK [20, 24], and the activation of both pathways facilitates the production of inflammatory cytokines and oxidants. Our results revealed that the intake of aqueous extract from *H. cordata* leaves markedly limited protein expression of NF-κB p65 and p-p38 in heart and kidney of diabetic mice, which in turn decreased inflammatory cytokines in these organs. These results suggest that

aqueous extract of *H. cordata* leaves was able to mediate NF-κB and MAPK signaling pathways under diabetic condition.

CML, pentosidine and urinary glycated albumin are indicators of diabetes associated with glycative stress, especially in patients with elevated risk of diabetic cardiomyopathy and/or nephropathy because the accumulation of these AGEs in organs impairs their functions [25, 26]. The activated polyol pathway, mainly due to enhanced activity and expression of aldose reductase, promotes AGEs formation [27, 28]. In this study, *H. cordata* leaves aqueous extract substantially reduced both activity and protein expression of this enzyme in heart and kidney, which subsequently decreased fructose flux and finally reduced CML, pentosidine and urinary glycated albumin production. These findings indicated that this extract attenuated cardiac and renal glycative stress by repressing polyol pathway. In addition, we found this extract at 2 % downregulated

Fig. 3 Cardiac and renal expression of NF-κB and MAPK in normal or diabetic mice treated with HCAE at 0, 1 or 2 % for 8 weeks. Values are mean ± SD, $n = 10$. [a–d]Means among *bars without a common letter differ*, $P < 0.05$
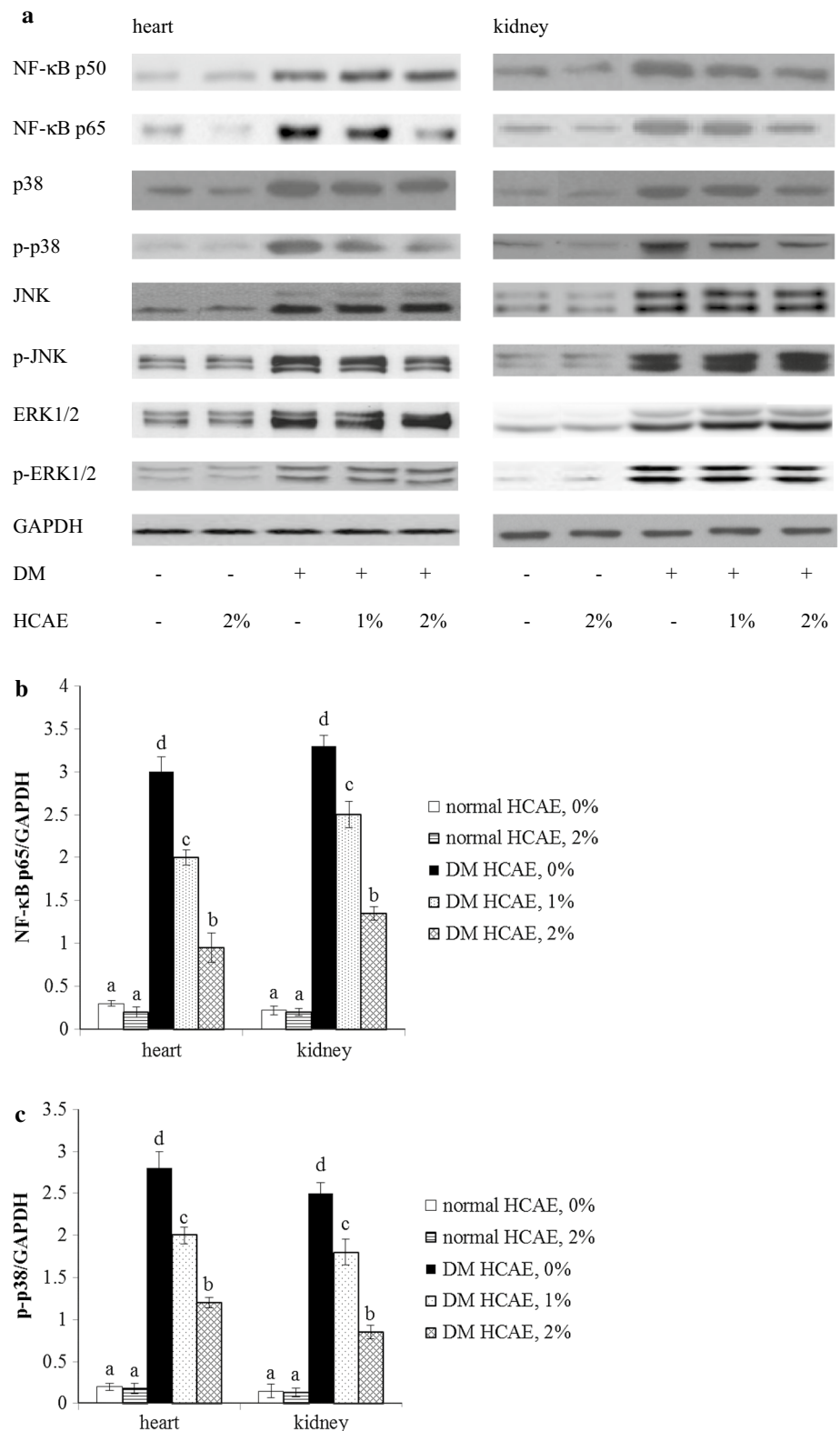
RAGE expression in two organs. The suppression on RAGE expression definitely contributed to mitigate glycative stress in these tissues by inhibiting the de novo synthesis of AGEs. On the other hand, the engagement of AGEs and RAGE

benefits NF-κB and MAPK pathways activation and consequently accelerates the formation of glycative, oxidant, inflammatory and coagulant factors [29, 30]. Because the available RAGE has been reduced by *H. cordata* leaves

aqueous extract, the interaction between AGEs and RAGE could be substantially suppressed. Thus, the less AGEs and ROS levels, as well as the limited NF-κB p65 and p-p38 expression in heart and kidney of diabetic mice, could be partially ascribed to this extract and downregulate RAGE in these organs. Since oxidative, inflammatory and glycative injuries in these organs of diabetic mice have been alleviated, the observed improvement in cardiac and renal functions, evidenced by the changes in CPK activity, BUN and CCr levels in those diabetic mice, could be explained.

Our previous study reported that aqueous extract of *H. cordata* leaves was rich in phytochemicals, and the content of kaempferol, myricetin, quercetin, ferulic acid, ellagic acid or gallic acid was in the range of 104–245 mg/100 g dry weight [13]. So far, the antidiabetic and even antiglycative effects of kaempferol, ferulic acid and/or gallic acid have been reported [31–34]. Therefore, it seems highly possible that these compounds presented in this extract of *H. cordata* leaves executed the observed antidiabetic protection. Furthermore, our previous study found that this extract contained other compounds such as naringenin, rutin and rosmarinic acid, although at relatively lower amount, in the range of 62–94 mg/100 g dry weight. Certainly, the contribution from these compounds for antidiabetic actions of this extract should not be ignored. *H. cordata* is commonly used as a vegetable or for medicinal purpose in Asia. Our previous and present studies supported that the aqueous extract from the leaf part of this plant benefited liver, heart and kidney protection. Thus, *H. cordata* leaves aqueous extract could be developed as a supplement for health promotion. However, further human study is necessary to verify the safety and effects of this extract.

In conclusion, the intake of *H. cordata* leaves aqueous extract attenuated glycative, oxidative and inflammatory stress in heart and kidney of diabetic mice through mediating NADPH oxidase, aldose reductase, RAGE, NF-κB and MAPK. These findings suggest that *H. cordata* leaves aqueous extract may benefit diabetic prevention and/or alleviation.

Compliance with ethical standards

Conflict of interest There was no conflict of interest regarding this manuscript.

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