JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Polyphenol Extracts from *Hibiscus sabdariffa* Linnaeus Attenuate Nephropathy in Experimental Type 1 Diabetes

WEN-CHIN LEE,[†,‡,§] CHAU-JONG WANG,[§,||] YU-HSIN CHEN,[||] JEN-DONG HSU,[⊥] SU-YA CHENG,[#] HONG-CHEN CHEN,[‡] AND HUEI-JANE LEE*,[||]

Division of Nephrology, Department of Internal Medicine, Show-Chwan Memorial Hospital, Changhua, Taiwan, Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan, Institute of Biochemistry and Biotechnology, Medical College, Chung Shan Medical University, Taichung, Taiwan, and Department of Pathology and Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan

Diabetic nephropathy progressed to end-stage renal disease (ESRD) is found in type 1 or type 2 diabetes. Oxidative stress is one of the precipitation factors in diabetic nephropathy. Previously, *Hibiscus sabdariffa* Linnaeus and its polyphenol extracts were found to possess antioxidative effects. This study is aimed to investigate the effect of *Hibiscus sabdariffa* L. polyphenol extract (HPE) in streptozotocin (STZ) induced diabetic nephropathy. The results show that HPE reduced kidney mass induced by STZ significantly, as well as improving hydropic change of renal proximal convoluted tubules in the rats. HPE also significantly reduced serum triglyceride, total cholesterol and LDL in STZ induced rats. Treatment with HPE significantly increased the activity of catalase and glutathione and reduced lipid peroxidation (thiobarbituric acid-reactive substances, TBARS). The findings of this research show the beneficial effects of HPE on STZ induced diabetic nephropathy including pathology, serum lipid profile and oxidative marker in kidney.

KEYWORDS: ***Hibiscus sabdariffa*** **L.; polyphenols; diabetic nephropathy; oxidative stress**

INTRODUCTION

Diabetic mellitus is a common but serious metabolic disorder associated with many functional and structural complications. In general, about 1 out of 3 patients with type 1 or type 2 diabetes proceed to developing significant diabetic nephropathy (*1*). Initially, diabetes is accompanied with renal hypertrophy−hyperfunction which is followed by detectable glomerular lesions with normal albumin excretion rate. After 7 or more years, microalbuminuria appears gradually. At this stage, glomerular lesions are generally more severe and hypertension develops. During the progression of this disease, overt proteinuria happens and glomerular filtration rate (GFR) reduction is common. After 5−15 years of the development of proteinuria, progression to end-stage renal disease will occur (*2*).

Numerous theoretical premises and experimental or clinical studies point to the participation of oxidative stress in diabetic pathogenesis and its late vascular complications, including diabetic nephropathy (*3*−*5*). Hyperglycemia is one of the most important factors that are responsible for oxidative stress intensification in diabetes. While reactive oxygen species (ROS) are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation, excess generation in oxidative stress has pathological consequences including damage to proteins, lipids and DNA (*6*). ROS can stimulate oxidation of low-density lipoprotein (LDL) to generate ox-LDL, which can be taken up by scavenger receptors in macrophages leading to "foam" cell formation and atherosclerotic plaques (*6, 7*). Ujihara et al. measured plasma levels of ox-LDL in type 2 diabetes mellitus patients (*8*). They found that the ox-LDL level was significantly higher in the macroalbuminuria group than in the microalbuminuria group, normoalbuminuria group or normal control, suggesting that ox-LDL may play an important role in diabetic nephropathy. Experimentally, oxidative level of LDL can be determined by detecting lipid peroxidation or apolipoprotein B100 (apoB 100) fragmentation.

A wide variety of phenolic substances present in dietary and medicinal plants possess striking antioxidative and anti-inflammatory properties, which to some extent, contribute to their cancer chemopreventive potential. The flower of *Hibiscus sabdariffa* Linnaeus calyx (family Malvaceae, local name

* To whom correspondence should be addressed. Tel: 886-4-24730022 ext 11675. Fax: 886-4-23248195. E-mail: lhj@csmu.edu.tw.
[†] Show-Chwan Memorial Hospital.
[‡] National Chung Hsing University.
[§] These authors contributed equally to this work.
[||] Medical College, Chung Shan Medical University.
[⊥] Department of Pathology, Chung Shan Medical University Hospital.
[#] Clinical Laboratory, Chung Shan Medical University Hospital.

10.1021/jf802993s CCC: $40.75 © 2009 American Chemical Society
Published on Web 02/16/2009

Karkaday) is commonly used in cold and hot beverages and as a supplement due to its perceived potential of health benefits. The flower extract has been reported to decrease blood pressure, and have antitumor characteristics as well as immune-modulating and antileukemic effects (*9, 10*). *Hibiscus sabdariffa* L. extract contains polyphenolic acids, flavonoids, protocatechuic acid (PCA) and anthocyanins. In our previous studies, *Hibiscus sabdariffa* L. extract has been found to contain various polyphenols and was shown to have antioxidative potential to inhibit the development of atherosclerosis in cholesterol-fed rabbits, LDL oxidation and ox-LDL-mediated macrophage apoptosis (*11, 12*).

This study is aimed to assess the beneficial effects of the polyphenol extracts from *Hibiscus sabdariffa* L. (HPE) in streptozotocin (STZ)-induced diabetic nephropathy, including the serum biochemistry, the morphologic change, and the oxidative parameters in the kidneys.

MATERIALS AND METHODS

Preparation and Assay of HPE. *Hibiscus sabdariffa* L. obtained from Taitung Hsien Farmers' Association, Taiwan, was identified by Associate Professor Yi-Ching Li, Chung Shan Medical University. A voucher specimen has been kept for future reference at the Department of Pharmacology, Chung Shan Medical University. The extraction of HPE was carried out as previously described (*13*). Briefly, dried flowers were extracted with methanol; and then the samples were filtered and concentrated with a vacuum rotary evaporator. The residue was treated with hexane to remove pigments and further extracted with ethyl acetate. The extracted portion was redissolved in water and lyophilized to obtain HPE. The components of HPE, including protocatechuic acid (8.83%), catechin (9.97%), EGC (10.23%), EGCG (20.20%), and caffeic acid (18.10%), were determined by HPLC analysis.

Animals and Experimental Design. All animal experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of the Chung Shan Medical University (IACUC, CSMU), Taichung, Taiwan. Male Sprague−Dawley (SD) rats (180 ± 10 g) used in the studies was purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The animals were housed in laboratory conditions (18−23 °C, humidity 55−60%, 12 h light/dark cycle) for at least 1 week before each study. Rats were made diabetic by intraperitoneal injection (IP) of STZ (dissolved in 0.05 M of citrate buffer, pH4.5) one time, 65 mg/kg body weight. Seventy-two hours after STZ administration, fasting blood was collected from the tail vein of all the animals taking part in the experiment to determine the glucose concentration in the blood. Animals which were administered STZ and in which glucose concentration was <250 mg/dL were excluded from the study. Blood glucose and body weight were checked every week. The rats were provided with standardized food (Purina Laboratory Chow, obtained from Purina Mills, Inc., U.S.A.) and water ad libitum and divided into five groups (five rats per group). Group 1, control group: fed with standardized diet, without any treatment. Group 2, buffer control group: ip injection with 0.05 M citrate buffer, pH4.5, fed with standardized diet. Group 3, STZ group: diabetic rats fed with standardized diet. Group 4, HPE 100 mg/kg group: diabetic rats tube-fed with HPE 100 mg/kg per day. Group 5, HPE 200 mg/kg group: diabetic rats tube-fed with HPE 200 mg/kg per day.

The rats were sacrificed after 8 weeks of treatment. Blood and kidney tissues were collected for analysis. The relative organ weight was calculated as the ratio of organs and body weight to represent the level of weight change.

Determination of Glucose, Creatinine, Albumin and Blood Urea Nitrogen (BUN). Plasma glucose, creatinine, albumin and BUN were measured by enzymatic colorimetric methods using automatic analyzer (Olympus AU2700, Olympus Co., Tokyo, Japan).

Plasma Lipid Measurements. Concentration of total cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were measured by enzymatic colorimetric methods using commercial kits (Boeheringer Mannheim, Germany). The atherosclerotic index (AI) was determined for different groups. It is defined as the ratio of LDL-C and HDL-C.

Pathological Histology of Kidney. After removal from the animals, kidneys were immediately fixed in 10% buffered formaldehyde, and processed for histological examination by conventional methods with hematoxylin and eosin (H&E) stain. The kidney lesions were observed according to morphology changes, such as hydropic change. The severity of kidney damage was evaluated by examining the section under five randomly selected high power fields (×200). Image Pro Plus 4.0 was used to quantify the percentages of lesion areas in kidney specimen.

Thiobarbituric Acid-Reacting Substances (TBARS). The lipid peroxidation was determined by measuring the TBARS. 0.5 g of kidney specimen was homogenized with 5 mL of 50 mM phosphate buffer, pH 7.4) and centrifuged (1000g) for 30 min to obtain supernatant homogenate. Protein content of the supernatant was determined with Bio-Rad protein assay reagent using bovine serum albumin as a standard. The calibration curve range was from 0 to 400 μg/mL (r^2 = 0.9913). The 0.3 mL of homogenate was added with 0.3 mL of TBA (1% thiobarbituric acid in 0.3% of NaOH) to react for 40 min at 95 °C in the dark. After the reaction, samples were analyzed in a Hitachi F2000 spectrophotofluorimeter with excitation at 532 nm and emission at 600 nm. The calibration curve was prepared with malondialdehyde (MDA) standards of 0−50 nmol (r^2 = 0.9927). The concentrations of TBARs were expressed as equivalents of MDA represented as μM/mg protein.

Catalase Assay. Catalase activity in kidney homogenates was assayed according to a previous method (*14*). Briefly, the concentration of protein was determined with Bio-Rad protein assay reagent and adjusted to 50 mg/mL, and then 20 μL of homogenate was added to 980 μL of H_2O_2 solution (containing 30 μL of ddH_2O, 50 μL of 1 M Tris-HCl and 5 mM EDTA, pH8.0 and 900 μL of 10 mM H_2O_2). After 10 s at room temperature, the optical density of H_2O_2 was recorded at 240 nm for 1 min. The linear portion was used to calculate the catalase activity (average r^2 = 0.9908). A unit of catalase activity was defined in H_2O_2 consumed units/mg protein.

Determination of GSH Content. We determined the kidney GSH content according to the method of Hissin and Hilf (*15*). The stock solution of the fluorescent probe o-phthalaldehyde (OPT) was freshly prepared in methanol (1 mg/mL). 10 μL of homogenate was mixed with 100 μL of OPT to incubate for 15 min in the dark. We then monitored the fluorescence intensity with excitation at 350 nm and emission at 420 nm. A GSH calibration curve range from 0 to 20 ng/mL (r^2 = 0.9836) was established to normalize the GSH concentration. Result was expressed as ng GSH/ mg protein.

Statistical Analysis. The results were reported as means ± SD from individual magnitudes. Statistical differences were analyzed with Student's *t*-test; and the differences were considered to be significant when $P < 0.05$.

RESULTS

Effect of HPE on Glucose, Renal Function and Plasma Lipid of STZ Induced Diabetic Rats. A significant elevation of blood sugar is found in STZ treated rats, but is not influenced by HPE. There is no significant change of serum creatinine level in rats with STZ or HPE treatment. The increase of serum BUN induced by STZ is significant, whereas 100 or 200 mg/kg of HPE treatment reduced the level of BUN. A significant decrease of serum albumin is found in STZ treated rats ($P < 0.01$) and significant improvement in the HPE treated groups ($P < 0.05$) (**Table 1**). The serum levels of lipid profile, such as cholesterol, triglyceride, and LDL, are significantly elevated in STZ treated group ($P < 0.005$) and significantly improved in HPE 100 mg/kg/day and 200 mg/kg/day treated groups ($P < 0.05$, $P < 0.005$) (**Table 2**).

Effect of HPE on Weight Change of Organs of STZ Induced Diabetic Rats. The relative organ weight increased in liver and kidney in STZ treated rats ($P < 0.05$) and

Table 1. The Effect of HPE on Renal Function in Diabetic Rats[a]

treatment			parameters in plasma			
HPE (mg/kg/day)	STZ	buffer	glucose (mg/dL)	creatinine (mg/dL)	albumin (g/dL)	BUN (mg/dL)
−	−	−	145.3 ± 17.2	0.47 ± 0.06	3.30 ± 0.10	18.2 ± 1.6
−	−	+	141.3 ± 11.1	0.50 ± 0.10	3.33 ± 0.31	15.2 ± 1.3
−	+	+	630.0 ± 77.2[b]	0.52 ± 0.04	2.84 ± 0.30[b]	34.1 ± 5.1[b]
100	+	+	641.0 ± 46.0[b]	0.72 ± 0.04	2.72 ± 0.13	25.3 ± 2.4[c]
200	+	+	651.3 ± 43.0[b]	0.70 ± 0.00	3.10 ± 0.44[c]	23.4 ± 0.2[c]

[a] Data are mean ± SD, $n = 5$. Statistical significance analyzed by Student's *t*-distribution. [b] $p < 0.005$, compared with the normal group. [c] $p < 0.05$, compared with the STZ-treated group.

Table 2. The Effect of HPE on Plasma Lipids in Diabetic Rats[a]

treatment			plasma lipids				
HPE (mg/kg/day)	STZ	buffer	total cholesterol (mg/dL)	triglyceride (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	HDL-C/LDL-C
−	−	−	59.0 ± 5.0	139.7 ± 65.7	8.67 ± 1.53	24.00 ± 1.73	2.81 ± 0.32
−	−	+	50.0 ± 3.6	88.0 ± 35.6	9.07 ± 0.12	22.00 ± 2.65	2.43 ± 0.27
−	+	+	106.7 ± 9.9[b]	858.7 ± 196.1[b]	17.60 ± 3.05[b]	31.00 ± 5.24	1.81 ± 0.48[b]
100	+	+	74.2 ± 10.8[c]	362.7 ± 121.6[c]	11.60 ± 2.97[c]	29.40 ± 2.19	2.67 ± 0.69
200	+	+	50.0 ± 14.4[d]	48.3 ± 22.0[d]	2.00 ± 0.00[d]	26.67 ± 7.57	13.33 ± 3.79[d]

[a] Data are mean ± SD, $n = 5$. Statistical significance analyzed by Student's *t*-distribution. [b] $p < 0.005$ compared with the normal group. [c] $p < 0.05$ compared with the STZ-treated group. [d] $p < 0.005$ compared with the STZ-treated group.

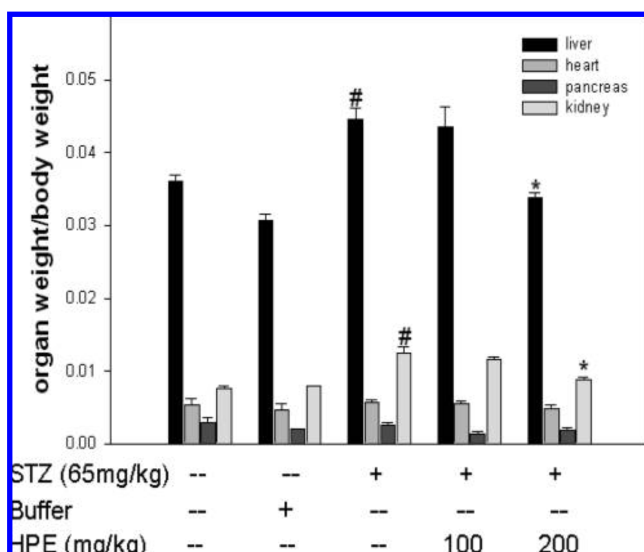

Figure 1. Effects of HPE on weight change of organs in diabetic rats. Results are shown as relative weight (organ weight/body weight) and expressed as mean ± SE. #, $P < 0.05$, significant differences compared with the control; *, $P < 0.05$, compared with the STZ group.

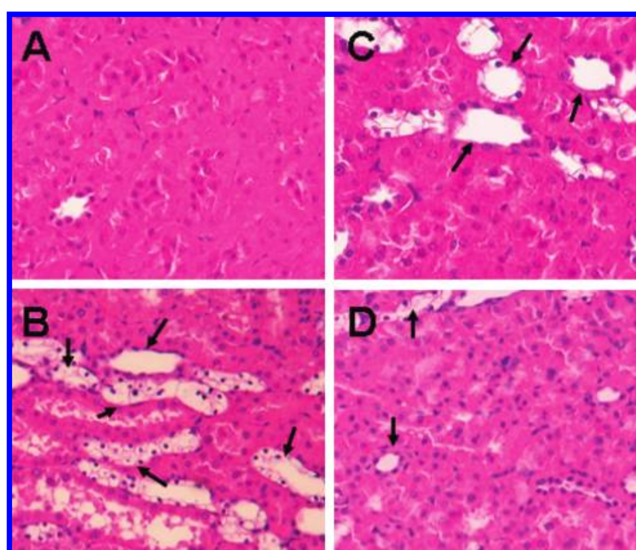

Figure 2. Effects of HPE on diabetic nephropathy in rats. Histological examination (200×) in rat kidney of (**A**) normal group, (**B**) STZ-induced diabetic group, (**C**) STZ + 100 mg/kg of HPE, and (**D**) STZ + 200 mg/kg of HPE. Arrows represent the hydropic changes shown as pale and swollen change of the proximal convoluted tubules.

significantly regressed in those rats treated with 200 mg/kg of HPE ($P < 0.05$). The relative weight changes for the heart and pancreas between the groups is not significant (**Figure 1**).

Effect of HPE on Kidney Lesions of STZ Induced Diabetic Rats. The pathological change of HPE on STZ induced diabetic kidneys was evaluated. We found a significant hydropic change in proximal convoluted tubules in STZ treated rats ($P < 0.05$), and a significant decrease in 100 mg and 200 mg of HPE treated groups ($P < 0.05$; $P < 0.005$) (**Figures 2**, **3**).

Effect of HPE on Oxidative Stress of STZ Induced Diabetic Rats. The levels of catalase, glutathione and TBARS in rat kidney were examined. The activity of catalase is decreased in STZ treated group ($P < 0.05$), and significantly increased in 100 mg/kg and 200 mg/kg of HPE groups ($P < 0.05$; $P < 0.005$). The level of GSH is decreased in STZ treated group ($P < 0.05$), and significantly increased in 200 mg/kg of HPE treated group ($P < 0.05$). To prove the level of lipid peroxidation, TBARS assay is carried out to show that a significantly increased level exists in the STZ treated group as represented by the substrate MDA ($P < 0.05$). Treatment of 100 mg or 200 mg of HPE decreased the level of lipid peroxidation significantly ($P < 0.05$; $P < 0.005$) (**Table 3**).

DISCUSSION

Diabetes mellitus is a syndrome with disordered metabolism and inappropriate hyperglycemia due either to a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate (*16*). Several studies suggest that patients with diabetes mellitus have increased rates of lipoprotein oxidation (*8, 17, 18*). Elevated plasma levels of lipid peroxides are found in patients with poorly controlled diabetes and macroangiopathy (*19*). Hyperlipidemia as a risk factor for diabetic nephropathy has been reported in the

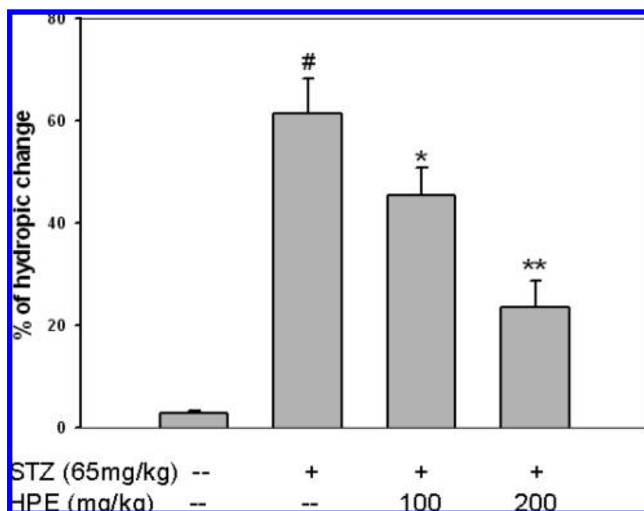

Figure 3. Quantitative determination of the percentage of hydropic change. #, $P < 0.05$, significant differences compared with the control; *, $P < 0.05$, **, $P < 0.005$, compared with the STZ group.

Table 3. The Effect of HPE on Antioxidative Parameter of Kidney in Diabetic Rats[a]

treatment			antioxidative parameter of kidney tissue		
HPE (mg/kg/day)	STZ	buffer	catalase (units/mg protein)	GSH (ng/mg protein)	MDA (μM/mg protein)
−	−	−	11.21 ± 4.78	9.28 ± 0.40	1.59 ± 0.06
−	−	+	11.25 ± 5.22	9.63 ± 0.33	1.86 ± 0.13
−	+	+	6.11 ± 3.03[b]	6.80 ± 0.29[b]	5.34 ± 0.64[b]
100	+	+	24.97 ± 6.93[c]	8.27 ± 0.36	3.26 ± 1.03[c]
200	+	+	26.56 ± 8.81[d]	8.46 ± 0.03[c]	2.59 ± 0.98[d]

[a] Data are mean ± SD, $n = 5$. Statistical significance analyzed by Student's *t*-distribution. [b] $p < 0.05$ compared with the normal group. [c] $p < 0.05$ compared with the group treated by STZ. [d] $p < 0.005$ compared with the group treated by STZ.

past (*20*−*22*). In our study, we found a significant elevation of serum cholesterol, triglyceride and LDL in STZ induced diabetic rats. After HPE treatment, the serum level of cholesterol, triglyceride and LDL decreased. It means that HPE has the effects to lower lipid protein and improve the serum level of lipid, and possibly improve the cardiovascular injury in diabetic nephropathy. We also found that although the serum creatinine is not changed in these groups, the level of albumin is elevated and BUN is decreased significantly. It may be because the disease process is not long enough to cause obvious nephropathy, but HPE still possessed the ability to improve renal function. In fact, it has been found that vitamin C and vitamin E decreased lipid peroxidation, augmented activities of antioxidant enzymes in the kidneys, and decreased kidney weights in streptozotocin induced diabetic rats (*3*).

Diabetic nephropathy encompasses discrete structural alterations, including renal hypertrophy, thickening of basement membranes, and progressive glomerular accumulation of extracellular matrix components (*23, 24*). We found a significant organomegaly in liver and kidney of STZ induced diabetic rats, and it is regressed at HPE concentration of 200 mg/kg/day. It has been noted that the diabetic patients may cause fatty liver, and hyperfiltration of the kidney, and HPE seems improve the organomegaly.

Advanced diabetic glomerulopathy is commonly characterized by diffuse glomerulosclerosis and may sometimes exhibit a distinctive morphological appearance, namely, the nodular form of glomerulosclerosis, as first described by Kimmelstiel and Wilson in 1936 (*25, 26*). Clinical studies have demonstrated that high blood glucose is the main determinant of initiation and progression of diabetic vascular complications including nephropathy (*27*−*29*). In addition, high glucose may cause osmotic diuresis and result in pale and swollen change of the proximal convoluted tubules, called "hydropic change" (*30*), which may be the initial glomerular injury by poor control of blood sugar. In our study, we did not find the specific pathological change of Kimmelstiel−Wilson nodule in STZ treated diabetic kidneys, but we found a significant hydropic change in the proximal convoluted tubules in STZ treated rats. It may be due to the fact that the study period was not long enough to induce the obvious diabetic nephropathy. HPE 100 mg/kg/day or 200 mg/kg/day treatment made the hydropic change subside. It suggested that HPE could reverse the high glucose induced diabetic nephropathy in early stage.

Studies *in vivo* have been performed utilizing antioxidants in experimental diabetic models (*3*). The effects of antioxidants are measured through certain observable biomarkers including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), as well as lipid peroxidation. Our results showed a significant decrease of catalase activity and GSH level in the kidney of STZ treated diabetic rats. After HPE treatment, the levels of catalase elevated significantly in HPE 100 mg/kg/day and 200 mg/kg/day, respectively. On the other hand, the levels of GSH showed significant elevation only in HPE 200 mg/kg/day. In view of MDA level in kidneys, a significant increase in STZ treated group is found. After treatment of HPE, the MDA level is decreased significantly. These results suggest that oxidative stress increased in diabetic nephropathy, and HPE has the effect to suppress the oxidative stress in the kidneys.

In summary, we have demonstrated that HPE has beneficial effects on STZ induced diabetic rats, including serum lipid profile, renal pathologic change, and oxidative marker in kidney. Although the limitation of this study is the relative sample size of animals, the results would provide an alternative viewpoint to clinical usage of natural bioactive products on diabetic nephropathy.

ACKNOWLEDGMENT

We thank Dr. Wen-Huei Chang for her kind release of Image Pro Plus 4.0.

LITERATURE CITED

(1) Zipp, T.; Schelling, J. R. Diabetic nephropathy. In *Nephrology secrets*, 2nd ed.; Hricik, D. E., Miller, R. T., Sedor, J. R., Eds.; Hanley & Belfus, Inc: Philadelphia, 2003; pp 105−108.

(2) Caramori, M. L.; Mauer, M. Diabetic nephropathy. In *Primer on kidney diseases*, 3rd ed.; Greenberg, A., Cheung, A. K., Coffman, T. M., Falk, R. J., Jennette, J. C., Eds.; National Kidney Foundation: FL, 2001; pp 212−218.

(3) Kedziora-Kornatowska, K.; Szram, S.; Kornatowski, T.; Szadujkis-Szadurski, L.; Kedziora, J.; Bartosz, G. Effect of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. *Nephron Exp. Nephrol.* **2003**, *95*, e134−143.

(4) Hunt, J. V.; Wolff, S. P. Oxidative glycation and free radical production: A causal mechanism of diabetic complications. *Free Radical Res. Commun.* **1991**, *1*, 115–123.

(5) Ha, H.; Kim, K. H. Role of oxidative stress in the development of diabetic nephropathy. *Kidney Int.* **1995**, *48* (Suppl. 51), 18–21.

(6) Johansen, J. S.; Harris, A. K.; Rychly, D.; Ergul, A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc. Diabetol.* **2005**, *4*, 5.

(7) Boullier, A.; Bird, D. A.; Chang, M. K.; Dennis, E. A.; Friedman, P.; Gillotre-Taylor, K.; Horkko, S.; Palinski, W.; Quehenberger, O.; Shaw, P. Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann. N.Y. Acad. Sci.* **2001**, *947*, 214–222.

(8) Ujihara, N.; Sakka, Y.; Takeda, M.; Hirayama, M.; Ishii, A.; Tomonaga, O.; Babazono, T.; Takahashi, C.; Yamashita, K.; Iwamoto, Y. Association between plasma oxidized low-density lipoprotein and diabetic nephropathy. *Diabetes Res. Clin. Pract.* **2002**, *58*, 109–114.

(9) Haji Faraji, M.; Haji Tarkhani, A. The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. *J. Ethnopharmacol.* **1999**, *65*, 231–236.

(10) Tseng, T. H.; Kao, T. W.; Chu, C. Y.; Chou, F. P.; Lin, W. L.; Wang, C. J. Induction of apoptosis *Hibiscus* protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. *Biochem. Pharmacol.* **2000**, *60*, 307–315.

(11) Chen, C. C.; Hsu, J. D.; Wang, S. F.; Chiang, H. C.; Yang, M. Y.; Kao, E. S.; Ho, Y. C.; Wang, C. J. *Hibiscus sabdariffa* Extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *J. Agric. Food Chem.* **2003**, *51*, 5472–5477.

(12) Chang, Y. C.; Huang, K. X.; Huang, A. C.; Ho, Y. C.; Wang, C. J. *Hibiscus* anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food Chem. Toxicol.* **2006**, *44*, 1015–1023.

(13) Lin, H. H.; Huang, H. P.; Huang, C. C.; Chen, J. H.; Wang, C. J. *Hibiscus* polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. *Mol. Carcinog.* **2005**, *43*, 86–99.

(14) Aebi, H. Catalase. In *Methods of enzymatic analysis*; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; pp 673−684.

(15) Hissin, P. J.; Hilf, R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal. Biochem.* **1976**, *74*, 214–226.

(16) Masharani, U. Diabetes mellitus and hypoglycemia. In *2008 Current medical diagnosis and treatment*, 47th ed.; McPhee, S. J., Paradakis, M. A., Tierney, L. M., Eds.; McGraw Hill Companies, Inc: New York, 2008; pp 1032−1073.

(17) Bowie, A.; Owens, D.; Collins, P.; Johnson, A.; Tomkin, G. Glycosylated low density lipoprotein is more sensitive to oxidation: implication for the diabetic patients. *Atherosclerosis* **1993**, *102*, 63–67.

(18) Lyons, T. J. Oxidized low density lipoprotein: a role in the pathogenesis of atherosclerosis in diabetes. *Diabetes Med.* **1991**, *8*, 411–419.

(19) Baynes, J. W. Role of oxidative stress in the development of complications in diabetes. *Diabetes* **1991**, *40*, 405–412.

(20) Mulec, H.; Johnson, S. A.; Wiklund, O.; Bjorck, S. Cholesterol: a renal risk factor in diabetic nephropathy. *Am. J. Kidney Dis.* **1993**, *22*, 196–201.

(21) Krämer-Guth, A.; Quaschning, T.; Pavenstädt, H.; Galle, J.; Nauck, M.; Baumstark, M. W.; Schollmeyer, P.; März, W.; Wanner, C. Uptake and metabolism of lipoproteins from patients with diabetes mellitus type II by glomerular epithelial cells. *Nephrol. Dial. Transplant.* **1997**, *12*, 1336–1343.

(22) Pai, R.; Kirschenbaum, M. A.; Kamanna, V. S. Low-density lipoprotein stimulates the expression of macrophage colony-stimulating factor in glomerular mesangial cells. *Kidney Int.* **1995**, *48*, 1254–1262.

(23) Wolf, G.; Ziyadeh, F. N. Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int.* **1999**, *56*, 393–405.

(24) Ziyadeh, F. N. The extracellular matrix in diabetic nephropathy. *Am. J. Kidney Dis.* **1993**, *22*, 736–744.

(25) Kimmelstiel, P.; Wilson, C. Intercapillary lesions in glomeruli of the kidney. *Am. J. Pathol.* **1936**, *12*, 83–97.

(26) The kidney in metabolic disorders. In *Atlas of renal pathology*; Kern, W. F., Laszik, Z. G., Nadasdy, T., Silva, F. G., Bane, B. L., Pitha, J. V., Eds.; W.B. Saunders Company: Philadelphia, 1999; pp 97−112.

(27) Lee, H. B.; Yu, M.-R.; Yang, Y.; Jiang, Z.; Ha, H. Reactive oxygen species regulated signaling pathways in diabetic nephropathy. *J. Am. Soc. Nephrol.* **2003**, *14* (Suppl. 3), S 241−245.

(28) The diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **1993**, *329*, 977−986.

(29) United Kingdom Prospective Diabetes Study 33. Intensive blood glucose control with sulphonylurea or insulin compared with conventional treatment and the risk of complication in patients with type 2 diabetes. *Lancet* **1998**, *352*, 837−853.

(30) Curran, R. C.; Crocker, J. In *Curran's atlas of histopathology*, 4th revised ed.; Harvey Miller Publisher: London, 2000; pp 174.

Received for review September 25, 2008. Revised manuscript received November 19, 2008. Accepted January 22, 2009. This work was supported by the Show-Chwan Memorial Hospital Grant (RD 96002), partially from National Science Council Grant (NSC93-2320-B-040-052) and Chung Shan Medical University Grant (CSMU94OM-A-025).

JF802993S