JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Rutin (Quercetin Rutinoside) Induced Protein-Energy Malnutrition in Chronic Kidney Disease, but Quercetin Acted Beneficially

Chiu-Lan Hsieh,[†,‡] Chiung-Chi Peng,[‡,§] Kuan-Chou Chen,[*,∥,⊥] and Robert Y. Peng[#]

[†]Graduate Institute of Biotechnology, Changhua University of Education, 1 Jin-De Road, Changhua, Taiwan 50007

[§]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, 250 Wu-Hsing Street, Taipei 11031

[∥]Department of Urology, School of Medicine, College of Medicine, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan 11031

[⊥]Department of Urology, Shuang Ho Hospital, Taipei Medical University, 291, Zhongzheng Road, Zhonghe, Taipei, Taiwan 23561

[#]Research Institute of Biotechnology, Hungkuang University, 34 Chung-Chie Road, Shalu County, Taichung Hsien, Taiwan 43302

ABSTRACT: Nutraceutically, much of the literature has indicated that an aglycon and its related glycoside would act similarly. However, controversial reports are accumulating. We hypothesize that rutin (RT) and quercetin (QT) pharmacodynamically could act differently. To confirm this, doxorubicin (DR) (8.5 mg/kg) was used to induce rat chronic kidney disease (CKD) and then treated with QT and RT (each 70 mg/kg body weight per day) for 13 weeks. QT exhibited better body weight gaining effect (420 ± 45) vs RT, 350 ± 57 g/rat ($p < 0.001$). DR raised the ratio kidney-to-body weight (%) to 0.82 ($p < 0.001$) vs RT, 0.62 ($p < 0.01$), and QT, 0.35 ($p < 0.01$). DR reduced the glomerular filtration rate to 25.2 vs RT, 48 ± 11.3; QT, 124.7 ± 12.8 ($p < 0.001$) and the control, 191.5 ± 15.7 mL/h ($p < 0.001$). DRCKD reduced hematocrit to 29 ± 5; RT, to 28 ± 5 ($p < 0.05$); QT, to 36 ± 6 vs the control 37.5 ± 4%, ($p < 0.01$). DRCKD reduced the serum albumin (s-Ab) to 2.1 ± 0.2 ($p < 0.001$); QT, to 2.7 ± 0.2 ($p < 0.05$) vs the normal 4.3 ± 0.5 g/dL, yet RT was totally ineffective. DRCKD raised serum cholesterol level to 340 ± 30; vs RT, 260 ± 12; QT, 220 ± 25; and the normal value, 70 ± 25 mg/dL. DRCKD increased serum triglyceride to 260 ± 15 ($p < 0.001$), RT and QT restored it to 170 ± 25 and 200 ± 15 ($p < 0.05$) vs the normal 26−145 mg/dL. DRCKD elevated blood urea nitrogen to 38 ± 3 vs RT, to 98 ± 6 mg/dL ($p < 0.001$), implicating "protein-energy malnutrition". RT stimulated serum creatinine (sCr) production to reach 6.0 ± 0.9 mg/dL ($p < 0.001$). QT did not alter the sCr level. RT but not QT induced uremia and hypercreatininemia. DR significantly downregulated Bcl-2, but highly upregulated Bax, Bad, and cleaved caspase-3, implicating the intrinsic mitochondrial pathway. DR damaged DNA, but QT completely rescued such an effect and recovered renal amyloidosis and collagen deposition. Conclusively, RT and QT act differently, and RT is inferior to QT with respect to treating CKD.

KEYWORDS: *quercetin, rutin, chronic kidney disease, apoptosis, flavonol, hypercreatininemia*

INTRODUCTION

Rutin, a phytochemical having a quercetin aglycon portion and rutinose, chemically is named quercetin rutinoside (Figure 1). Rutin exhibits a beneficial effect on the kidney of streptozotocin-induced diabetic rats via modulation of metalloproteinase levels in kidneys and reduction of plasma glucose levels.[1,2] Rutin helps inflammation recovery,[1] arthritis,[3] and cancers.[4] In addition, rutin exhibited cardiac protective, cholesterol- and blood pressure-lowering effects.[5] Rutin has benefits of blood pressure-lowering thromboxane A formation and platelet aggregation.[6,7] Tests on chronic toxicity with the enzymatically decomposed rutin during 52 weeks demonstrated sexual variation of responses to rutin hydrolysate (mainly isoquercitrin).[8]

Quercetin is often used for treating atherosclerosis, hypercholesterolemia, vascular cardiac diseases, diabetes, cataracts, inflammation, asthma, and preventing cancer. Quercetin reduces systolic blood pressure and plasma oxidized low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype.[9] The ability of quercetin to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical is suggested to be involved in these possible beneficial health effects.[10] Recently we pointed out that nephrocarcinoma could be induced by long-term administration of quercetin.[11] Quercetin is generally considered safe. Side effects may include headache and upset stomach. Preliminary evidence suggests that a byproduct of quercetin can lead to a loss of protein function. Very high doses of quercetin may damage the kidneys. Taking quercetin with periodic breaks has been suggested.[12] Epidemic report indicated that pregnant and breastfeeding women and people with kidney disease should avoid quercetin. At high doses (greater than 1 g per day), there are some reports of damage to the kidneys,[10] alternatively after a long-term administration.[11] Chemically quercetin aglycon, quercetin glucosides, and quercetin rutinoside are closely related, but pharmacokinetically these four chemicals act quite differently.[13] Richelle et al. studied the absorption of isoflavones in soy foods and indicated that previous hydrolysis of glycosides to aglycons does not

Received: October 30, 2012
Revised: May 1, 2013
Accepted: May 3, 2013
Published: July 22, 2013

ACS Publications © 2013 American Chemical Society

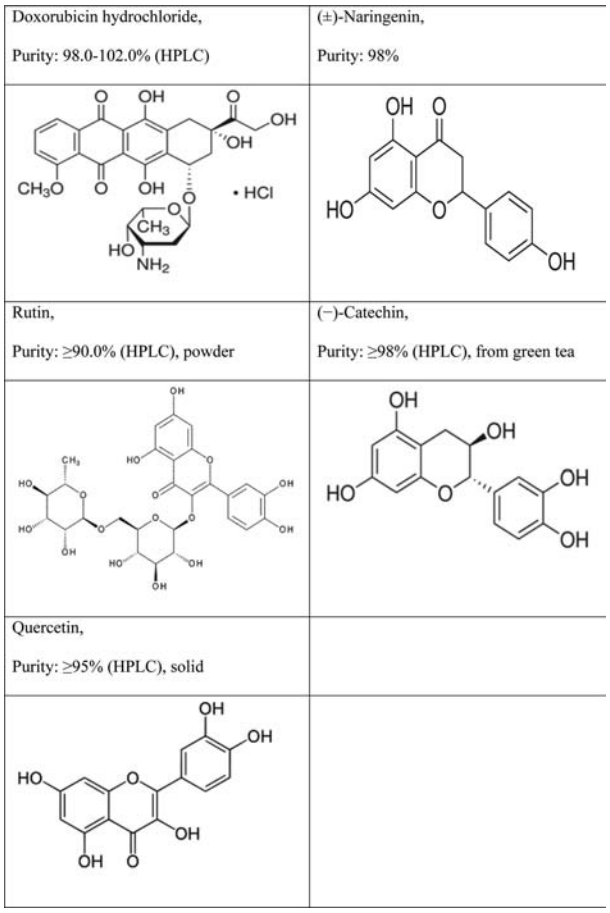

Figure 1. Structures and molecular weight of quercetin and rutin.

enhance the bioavailability of isoflavones in humans.[14] Izumi et al. pointed out that soy isoflavone aglycons are absorbed faster and in higher amounts than their glucosides in humans.[15] The vasodilatation effect of quercetin is more potent than that of rutin.[16] Kidney is the major site for elimination of many cytokines, and the delicate equilibrium of pro-inflammatory cytokines and their inhibitors is clearly dysregulated in CKD patients.[17] The glomerular filtration rate (GFR) indexing allows correlation of severity of kidney function loss and the prevalence of comorbidities associated with kidney disease.[18] High blood pressure can damage the blood vessels in the kidney.[19,20] Interventions such as controlling hypertension, specific pharmacologic options, and lifestyle modification are recommended when appropriate.[21] Previously, we recognized that quercetin and rutin exhibited different body weight gaining effects. Considering that rutin (a quercetin rutinoside) and quercetin pharmacologically may not exert similar benefits to CKD patients, we used the doxorubicin-induced rat chronic kidney disease model to verify this hypothesis. The relevant histological examination, biochemical tests, ELISA, and Western blot of some cytokines and signals were carried out. Their therapeutic effects on CKD were evaluated and compared.

MATERIALS AND METHODS

Chemicals. Doxorubicin hydrochloride (98.0−102.0%, HPLC) was a product of Pfizer (Milano, Italia); chemiluminescent HRP substrate was the product of Millipore (Billerica, MA, USA). (±)-Naringenin, (−)-catechin, and quercetin were supplied by Sigma Aldrich (St. Louis, MO). The purity was as follows: (±)-naringenin 98%, (−)-catechin ≥98% (HPLC), from green tea; and quercetin ≥95% (HPLC), solid, respectively. Rutin >90% purity was supplied by Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Their chemical structures are shown in Figure 1.

Antibodies and Kits. Pro-PREP lysis buffer was purchased from iNTRON Biotechnology (Seongnam, Korea). Antibodies Bcl-2 (1:1000), Bax (1:1000), Bad (1:1000), cleaved caspase 3 (1:1000), and β-actin (1:1000) were purchased from Cell Signaling Co. (MA, USA). The ELISA was performed using rat IL-6 and TNF-α ELISA kits provided by PeproTech (Rocky Hill, NJ, USA). Kits for SOD and TBARS were manufactured by Cayman Chemical Company (Ann Arbor, MI, USA).

Preparation of the Rat Chows. The basic rat chow was purchased from Fu-So Stock Company. The basic diet was macerated and pulverized by milling machine. The required amounts of nutraceutics were blended thoroughly. To the blended powder was added double distilled water to raise the moisture content to 18% w/w. The moisturized powder was subjected to the granulator. The granules obtained were stored at −20 °C for regular feeding. The average amount of diet consumed by per rat per day approximately was 45−55 g, hence the nutraceutics were compounded to contain 556 mg per kg finished granular chow.

Animal CKD Model. This experimental protocol was approved by the China Medical University Ethic Committee of Experimental Animals (Taichung, Taiwan). The principles of laboratory animal care (NIH publication) were followed. Thirty-six Sprague−Dawley male rats (BioLASCO Taiwan Co., Ltd. Resources), age 4 weeks and weighing 225−250 g, were purchased from BioLASCO. The rats were acclimated during the first week by feeding on ordinary laboratory chow. The rats were housed in the animal room maintained at a relative humidity (RH) of 65−75% within 23 ± 1 °C with a 12 h/12 h light/dark cycle and allowed free access to water and ordinary laboratory chow containing 1.8−2.2% of calcium, 1.1% of phosphorus, and 2650 kcal/kg energy. These rats were randomly assigned to six groups: group 1, the normal; group 2, the doxorubicin control (DR) (DRCKD); group 3, DR + naringenin (DR+N); group 4, DR + rutin (DR+R); group 5, DR + catechin (DR+C); and group 6, DR + quercetin (DR+Q), each having 6 rats. These six groups were separately housed in 12 colony cages, 3 rats in each. These rats were acclimated for the first week (week 0 to week 1); CKD was induced in the beginning of week 1 by a single sc injection of 7.5 mg/kg of doxorubicin (DR).[11] At the same time, the nutraceutics-containing chow granules were applied to administer approximately 70 mg of nutraceutics/kg body weight per day. The treatment was conducted for a course of 12 weeks, i.e., from week 1 to week 11.

Blood, Urine, and Tissue Sample Collection. *Urine Collection.* On finishing the treatment at week 11, rats were moved to the metabolic cage two days before the end of week 11. The urine was collected from 8.00 a.m. to 8.00 a.m. of the next day. The total volume of urine per day for each rat was taken. The urine samples were analyzed fresh for their urinary protein, creatinine, and urinary urea nitrogen (UUN) or immediately stored in the freezer at 0−4 °C when not in use. UUN and creatinine were measured by reagent (Siemens, Bakersfield, CA, USA) and automatic analyzer (Ciba-Corning Express Plus) (Ciba-Corning, USA). The urinary protein concentration was measured using an ELISA reader.

Blood Collection. After urine collection, the blood samples were immediately withdrawn from the abdominal aorta under ether intraperitoneal ketamine and xylazine anesthesia. The sample blood was centrifuged at 3000*g* to separate the serum. The serum obtained were used for measurement of parameters including cell count, serum albumin, cholesterol, triglyceride, BUN, and creatinine using reagent provided by Siemens, Bakersfield (CA, USA) and the automatic analyzer (Ciba-Corning Express Plus) (Ciba-Corning, USA).

Tissue Collection. After the rats were euthanized, the kidneys were excised and immediately frozen with liquid nitrogen and stored in −80 °C for cytokine assays. Part of the tissues was paraffin embedded.

Glomerular Filtration Rate (GFR). The GFR is typically recorded in units of volume per time, e.g., milliliters per minute (mL/min) by the expression[22]

$$\text{GFR} = (\text{urine concentration} \times \text{urine flow})/\text{plasma concentration} \quad (1)$$

GFR was measured by the method of creatinine clearance, C_{Cr}. Briefly 24 h urine was collected to determine the amount of creatinine that was removed from the blood over a 24 h interval.

Creatinine clearance (C_{cr}) is calculated from the creatinine concentration in the collected urine sample (U_{cr}), urine flow rate (V), and the plasma concentration (P_{cr}). Since the product of urine concentration and urine flow rate yields creatinine excretion rate, which is the rate of removal from the blood, creatinine clearance is calculated as removal rate per min ($U_{cr}V$) divided by the plasma creatinine concentration.[23] This is commonly represented mathematically as

$$C_{cr} = (U_{cr}V)/P_{cr} \quad (2)$$

Histochemical Examination. The excised organs were fixed by immersion with 10% formalin in PBS (pH 7.4) at 4 °C for 24 h and processed for paraffin embedding. Paraffin sections were dewaxed in xylene and rehydrated in a series of ethanol washes. The nuclei of these specimens were subjected to Weigert's Hematoxylin−Eosin stain. Otherwise, the collagen content was stained with Sirius Red.

TUNEL Assay. Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) reaction was carried out according to the protocol given by the manufacturer. Paraffin-embedded tissue sections were stained with the *In Situ* Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN, USA). Briefly, the whole procedure was as follows: The paraffin-embedded sections were deparaffinized in 2 changes of xylene for 5 min each, and hydrated with 2 changes of 100% ethanol for 3 min each, and 95% ethanol for 1 min. The sections were rinsed in distilled water. For frozen sections on slides, samples should be pretreated with 0.2% Triton X-100 in PBS−Tween for 30 min before proteinase K digestion treatment. These sections were rinsed in 2 changes of PBS−Tween 20, 2 min each. The rinsed sections were preincubated in TdT reaction buffer for 10 min, and then followed by TdT reaction, i.e., incubated in TdT reaction mixture for 1−2 h at 37−40 °C in a humidified chamber. To stop the reaction, the sections were rinsed in stop wash buffer for 10 min, and then rinsed in PBS−Tween 20 for 6 min. For detection, the sections were incubated in reaction mixture (34 mU/mL terminal transferase, 280 pmol of dATP, 90 pmol of flourescein-11 dUTP, 30 mM Tris-HCl, 140 mM sodium cacodylate, 1 mM $CoCl_2$, pH 7.2) for 1 h at 37 °C in the dark. Cells were subsequently washed with PBS and examined under a fluorescence microscope. Positive controls were carried out by incubating sections with DNase I (3000 U/mL in 50 mM Tris-HCl, pH 7.5, 1 mg/mL BSA) for 10 min at 15−25 °C to induce DNA strand breaks, prior to labeling procedure. Negative controls were conducted by incubating sections with label solution only (without terminal transferase) instead of TUNEL reaction mixture.

ELISA for Tissue IL-6, TNF-α, and Serum SOD, MDA. All ELISA protocols for tissue IL-6, TNF-α, and serum SOD, MDA were performed by following the manufacturer's instruction. The SYSMEX K-1000 Reader used was a product of San-Tong Instrument Co. (Taipei, Taiwan).

Western Blotting. Frozen renal cortex tissue samples (approximately 100 mg) were homogenized with the homogenizer (T10 basic, The IKA Company, Germany) in 1 mL of Pro-PREP lysis buffer (pH 7.2). The homogenate was centrifuged at 12000*g* for 20 min at 4 °C, and the supernatant was collected as tissue sample lysate. The sample protein lysates were heated at 100 °C for 10 min before loading and separated on precast 7.5% SDS−PAGE. The protein content was analyzed before loading according to the manufacturer's instruction. Aliquots of the treated lysates containing 50 μg/μL of protein were electrotransferred onto the PVDF membrane in transfer buffer for 1 h. The nonspecific binding to the membrane was blocked for 1 h at room temperature with 5% nonfat milk in TBS buffer. The membranes were then incubated for 16 h at 4 °C with various primary antibodies. After extensive washing in TBS buffer, the membranes were then incubated with secondary antibody in blocking buffer containing 5% nonfat milk for 1 h at room temperature. Membranes were then washed with TBS buffer, and the signals were visualized using the Luminescent Image Analyzer LAS-4000 (Fujifilm, Tokyo, Japan). Levels of Bcl-2, Bax, Bad, cleaved caspase-3, and β-actin were analyzed respectively by immunoassay according to the manufacturers' instruction (see the section Antibodies and Kits). β-Actin was used as the reference protein.

Statistical Analysis. Data obtained in the same group were analyzed by Student's *t* test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL). ANOVA software statistical system was used with the Tukey's testing to analyze the variances and significances of difference between paired means. Significance of difference was judged by a confidence level of $p < 0.05$.

RESULTS

Body Weight Variation. DR induced malnutrition with subsequent body weight loss. All four nutraceutics were shown ineffective to restore such tendency of body weight loss. Only quercetin exhibited better effect (420 ± 45 g/rat) compared to the normal control (630 ± 50 g/rat) and the other nutraceutics ($p < 0.001$) (Figure 2).

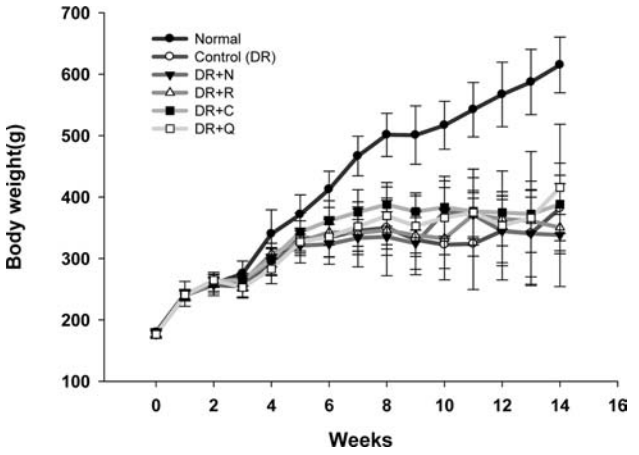

Figure 2. Body weight variation caused by different nutraceutic preparations. The nutraceutic chows were prepared by blending a given amount of nutraceutics, including naringenin, rutin, catechin, and quercetin, with the Fu-So brand basic chow. The feeding amount of nutraceutics was 70 mg/kg body weight per rat per day. Total feeding period was 14 weeks.

Effect on the Percent Ratio Kidney Weight to Body Weight. The percent ratio kidney weight to body weight (% KW/BW) in DR-control was 0.82, an increase of 2.49-fold ($p < 0.001$) due to inflammatory edema (Figure 3A). The rutin-treated victims were seen also severely damaged with a ratio 0.62 ($p < 0.01$). As contrast, quercetin completely ameliorated the kidney swelling (Figure 3A). Thus, such apparently physiological differences between rutin and quercetin (Figure 2, Figure 3A) strongly attracted our attention and inspired us to concentrate our interest only on the differentiated bioactivities of the aglycon quercetin and its rutinoside rutin.

Effect on the Glomerular Filtration Rate. DR greatly reduced the glomerular filtration rate (GFR) to 25.2 mL/h compared to 191.5 ± 15.7 mL/h of the control ($p < 0.001$). Quercetin effectively recovered the GFR to 124.7 ± 12.8 mL/h, approximately 65.1% of the normal ($p < 0.001$) (Figure 3B). DR highly raised the blood pressure to 153 ± 6 mmHg, a status of hypertension ($p < 0.001$). Rutin and quercetin were totally ineffective to suppress such a hypertensive status.

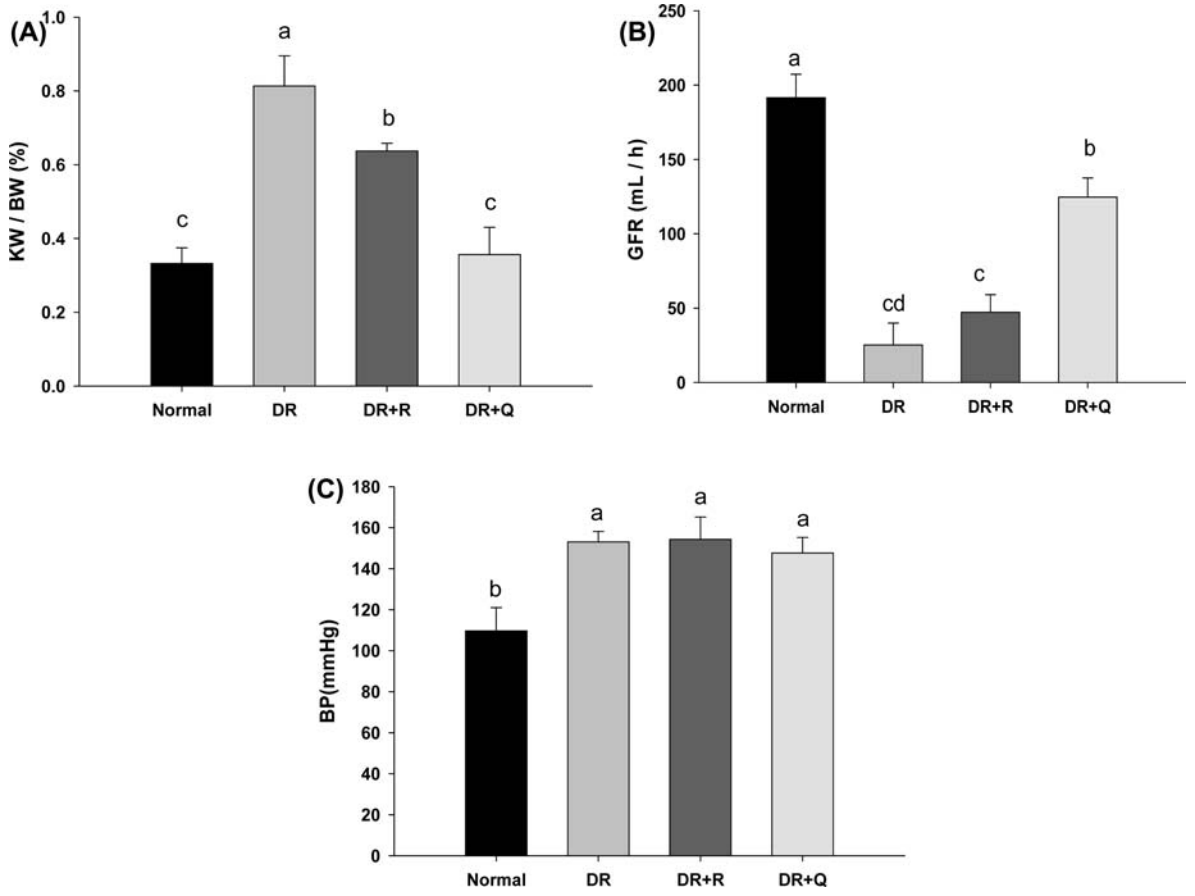

Figure 3. Ratio of kidney to body weight (KW/BW) (A), glomerular filtration rates (B), and blood pressure status (C) affected by nutraceutic rutin and quercetin chows.

Effect on the Hematological and Biochemical Parameters. *Effect on Hematocrit.* The hematocrit (HCT), a parameter indicating anemia, was significantly reduced by DR to 29 ± 5% ($p < 0.05$). Rutin induced further suppression to 28 ± 5% (Figure 4A) ($p < 0.05$; if compared to the control 37.5 ± 4%, $p < 0.01$). Quercetin was found to have completely recovered the level to 36 ± 6% (Figure 4A).

Effect on Serum Albumin Level. The reference serum albumin is 4.3 ± 0.5 g/dL (National Laboratory Animal Center, Taiwan), in contrast with our data 3.6 ± 0.2 g/dL in the normal group. DR highly significantly reduced the serum albumin content to 2.1 ± 0.2 g/dL ($p < 0.001$). Quercetin was shown to be only partially effective in restoring its level to 2.7 ± 0.2 g/dL ($p < 0.05$). Interestingly, rutin did not exhibit any alleviation efficiency regarding the albumin recovery (Figure 4B).

Effect on Plasma Hemoglobin Level. The levels of plasma hemoglobin (Hb, or HGB) among the normal, DR, and quercetin groups were all comparable. DR and quercetin did not alter the hemoglobin content in CKD victims, compared with the normal 14.6 ± 0.7 g/dL (Figure 4C). Astonishingly, the DR + rutin group was shown to exhibit lower plasma Hb 12.2 ± 0.7 g/dL (Figure 4C), although still acceptable by the official standard range of plasma Hb 11−18 g/dL.[24]

Effect on Serum Cholesterol Level. The level of serum cholesterol was raised in CKD to 340 ± 30 mg/dL, compared to the value 70 ± 25 mg/dL for the experimental control and standard range 40−130 mg/dL established by the National Laboratory Animal Centre (Taipei) (NLAC) (Figure 4D). Rutin and quercetin were seen to be partially effective in reducing the serum cholesterol levels to 260 ± 12 mg/dL and 220 ± 25 mg/dL, respectively, and quercetin showed better bioactivity in this regard (Figure 4D).

Effect on Serum Triglyceride Level. The normal serum triglyceride (TG) level of rats ranged within 100 ± 20 mg/dL compared to the reference standard range 26−145 mg/dL of NLAC. Similar to cholesterol, DRCKD was characterized by hypertriglyceridemic (260 ± 15 mg/dL) ($p < 0.001$). Rutin and quercetin all were shown to be only partially active in suppressing this elevation ($p < 0.05$) to 170 ± 25 and 200 ± 15 mg/dL, respectively (Figure 4E). Rutin was more efficient than quercetin with respect to lowering of serum TG (Figure 4E) ($p < 0.05$).

Effect on Serum Blood Urea Nitrogen Level. DRCKD was characteristic of elevated blood urea nitrogen (BUN) level. The reference range given by NLAC is 15−21 mg/dL. The level of BUN was upregulated to 38 ± 3 mg/dL in DRCKD victims, compared to the control value 10 ± 2 mg/dL ($p < 0.05$) (Figure 4F). Amazingly, rutin prominently further increased BUN to 98 ± 6 mg/dL ($p < 0.001$), but quercetin did not improve nor alleviate the serum BUN elevation in DRCKD (Figure 4F).

Effect on Serum Creatinine Level. The experimental normal serum creatinine level was within 0.6 ± 0.2 mg/dL (Figure 4G) in contrast to the reference range 0.2−0.8 mg/dL of NLAC. DR slightly induced the elevation of serum creatinine, yet insignificantly (Figure 4G). Quercetin did not alter any of the situations. Worth noting, DRCKD + rutin strongly stimulated

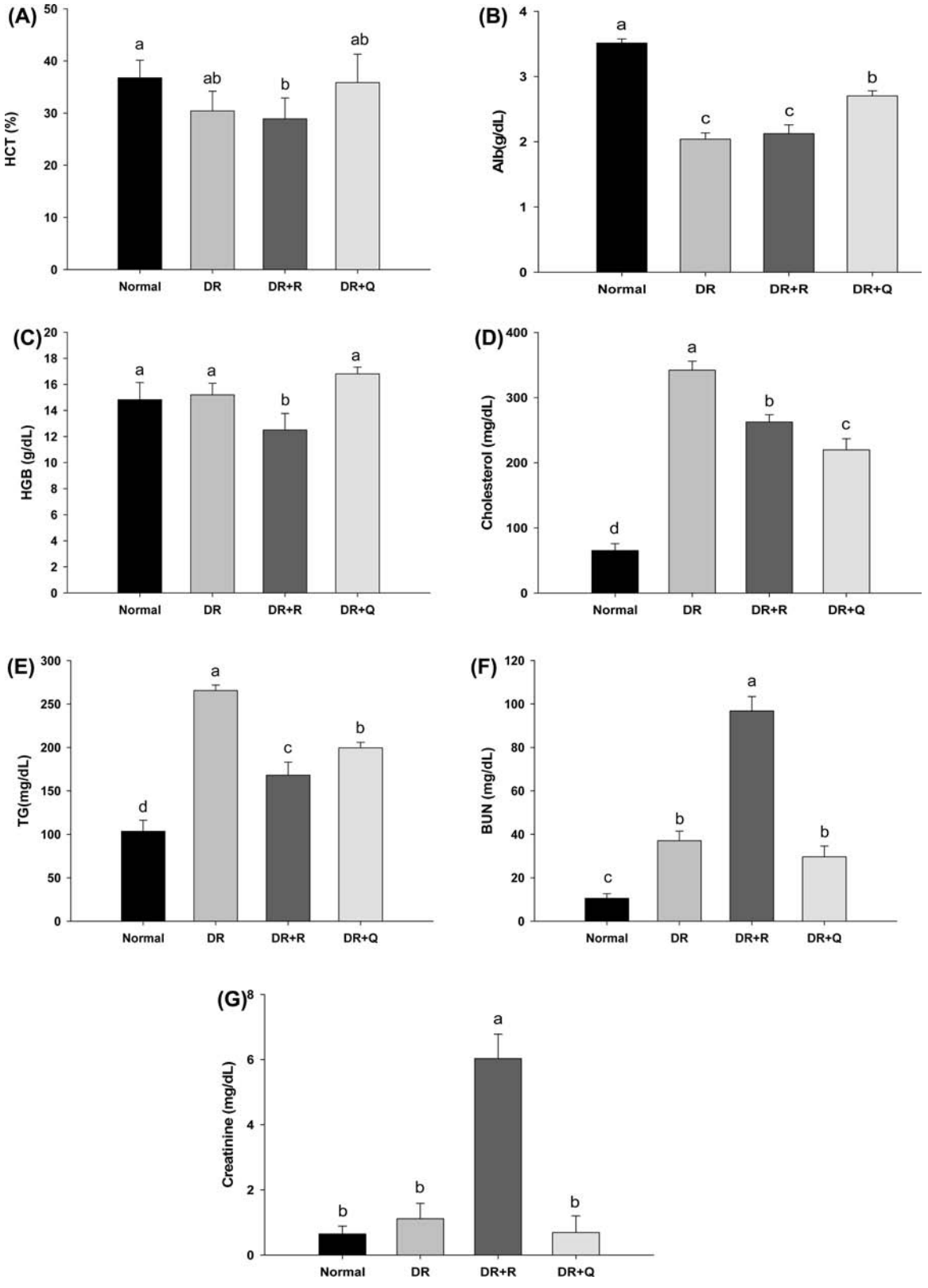

Figure 4. Hematocrit status (A), and levels of serum albumin (B), plasma hemoglobin (C), cholesterol (D), triglyceride (E), BUN (F), and creatinine (G) affected by nutraceutic rutin and quercetin chows.

the serum creatinine production to reach 6.0 ± 0.9 mg/dL ($p < 0.001$), a 10-fold-increase as the control (Figure 4G).

Effect on Urinary Urea Nitrogen (UUN) and Creatinine Levels. The normal UUN and creatinine levels were 2200 ± 62 mg/dL and 152 ± 62 mg/dL, respectively (Figure 5A,B). In DRCKD the levels were severely reduced to 900 ± 168 mg/dL and 75 ± 20 mg/dL. Rutin and quercetin were revealed to be only slightly too moderately effective in restoring BUN (Figure

dx.doi.org/10.1021/jf304595p | J. Agric. Food Chem. 2013, 61, 7258−7267

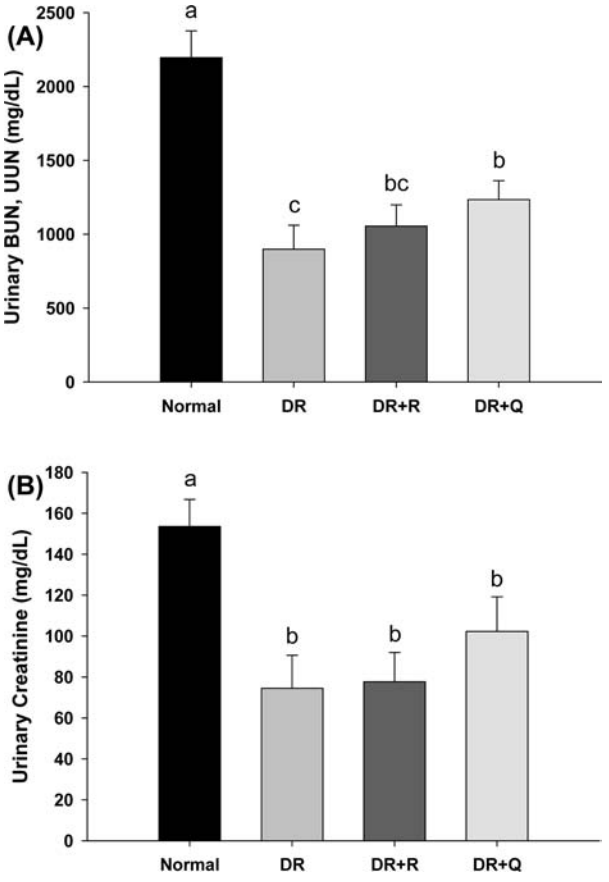

Figure 5. Urinary levels of urea nitrogen (UUN) (A) and creatinine (B) affected by nutraceutic rutin and quercetin chows.

5A), and both were totally ineffective to restore urinary creatinine secretion (Figure 5B).

Cytokines IL-6 and TNF-α and Superoxide Dismutase (SOD) and Malondialdehyde (MDA) Levels. DRCKD victims characteristically exhibited highly lowered tissue IL-6 and TNF-α levels (Figure 6A,B). Rutin not only was unable to alleviate but also further aggravated such trends. Interestingly, quercetin was shown to be partially effective for amelioration of IL-6 level, and completely for the restoration of TNF-α levels (Figure 6A,B).

The antioxidative defensive weapon SOD was severely downregulated in DRCKD victims (31 ± 4 U/mL) contrasted with the control (54 ± 2 U/mL) ($p < 0.05$) (Figure 6C), while both rutin and quercetin were shown only partially effective in alleviation of such reducing effect exerted by DR (Figure 6D). Similar results were seen in MDA. DR stimulated the MDA production to a level of MDA 25 ± 3 μM, which was seen only partially alleviated by rutin, but entirely unaffected by quercetin (Figure 6D).

Histochemical Examinations. Histochemical examination revealed severe amyloidosis in renal tissue, especially apparent in the DRCKD and DR + rutin victims. As contrast, quercetin did not show any damage in this regard (Figure 7A). Sirius Red staining indicated huge amount of collagen deposition in the DRCKD and the DR + rutin victims, while DR + quercetin did not show any degree of collagen deposition (Figure 7B).

TUNEL Assay. TUNEL assay revealed severe DNA damage caused by DR. Rutin was found only partially, but quercetin completely, to have ameliorated this damage (Figure 8).

Western Blot Analysis. Western blot indicated that DR significantly downregulated Bcl-2, but highly upregulated Bax, Bad, and cleaved caspase-3 (Figure 9), implicating that the intrinsic mitochondrial pathway had been triggered toward apoptosis. Rutin was only partially and quercetin was almost completely effective in rescuing such an effect.

DISCUSSION

Body Weight Loss Caused by Rutin Could Be Due to Protein-Energy Malnutrition. Rutin highly stimulated the production of BUN, indicating the protein degradation was actively taking place under the influence of rutin (Figure 4F). Rutin, a bioflavonoid, has several possible modes of action, including protein removal from lymphatic vessels and increased proteolysis and lymph removal from tissues.[25] As contrast, quercetin neither improved nor enhanced the BUN level (Figure 4F). Quercetin but not rutin effectively restored the antiapoptotic cytokine Bcl-2 and suppressed the upregulation of the apoptotic cytokines Bax and Bad that were otherwise significantly upregulated by DRCKD (Figure 9). Similarly, rutin was also less effective than quercetin in suppressing the cleaved caspase-3 (Figure 9). The overall effect revealed DRCKD + rutin with apparent apoptosis which was totally unseen in DRCKD + quercetion (Figure 8). Potential mechanisms of muscle wasting in renal failure can occur through the insulin and IGF-1 receptor-mediated signaling via the insulin receptor substrate (IRS)/phosphoinositide-3 kinase (PI3K)/Akt pathway, which drives anabolic and antiapoptotic processes, potentially leading to a catabolic state with body weight loss.[26,27] A reduction in circulating amino acid levels, as is often seen in renal failure patients, would reduce the anabolic stimulus functioning via the IRS/PI3K/Akt pathway as well.[26,27] Thus, severe body weight loss was seen in the DRCKD victims and DRCKD + rutin (Figure 2). DRCKD + rutin group exhibited the lowest HCT %, HGB, and serum albumin and, additionally, the highest serum BUN level (Figure 4A−C,F), a status similar to the "protein-energy malnutrition" described by Fock et al. (2007)[28] and Peng et al. (2012).[29]

Rutin (350 ± 57 g/rat) was revealed to be far less effective than its corresponding aglycon quercetin in ameliorating body weight loss (Figure 2). Different aglycons and sugar moieties may influence the absorption, metabolism, and phase II action of polyphenolic compounds.[30] The O-methylation metabolism may not be affected, but the glucuronyl conjugation in both liver and small intestine can be influenced.[31] Thus all these effects are considered to have more or less effect on growth and metabolism.

Rutin and Quercetin Failed To Ameliorate Hyperlipidemia Status in DRCKD, Hence Hypertension Was Sustained. The typical symptoms of CKD are renal swelling due to inflammation and edema and protein urea.[29] In CKD victims, rutin was inferior to quercetin regarding the hypocholesterolemic (Figure 4D) and anti-inflammatory action (Figure 6A,B; Figure 7A), consequently rutin could not show any better effect than quercetin in sustaining the normal kidney to body weight ratio (Figure 3A), and the DRCKD + rutin group exhibited severely reduced GFR compared to the quercetin-treated CKD rats (Figure 3B). Thus for treatment of CKD, in many aspects quercetin relatively was revealed to have a better effect than rutin. Otherwise, rutin showed a slightly better effect than quercetin to suppress the serum triglyceride level in CKD rats (Figure 4E). However, such a hypertriglyceridemic effect apparently could not compensate its

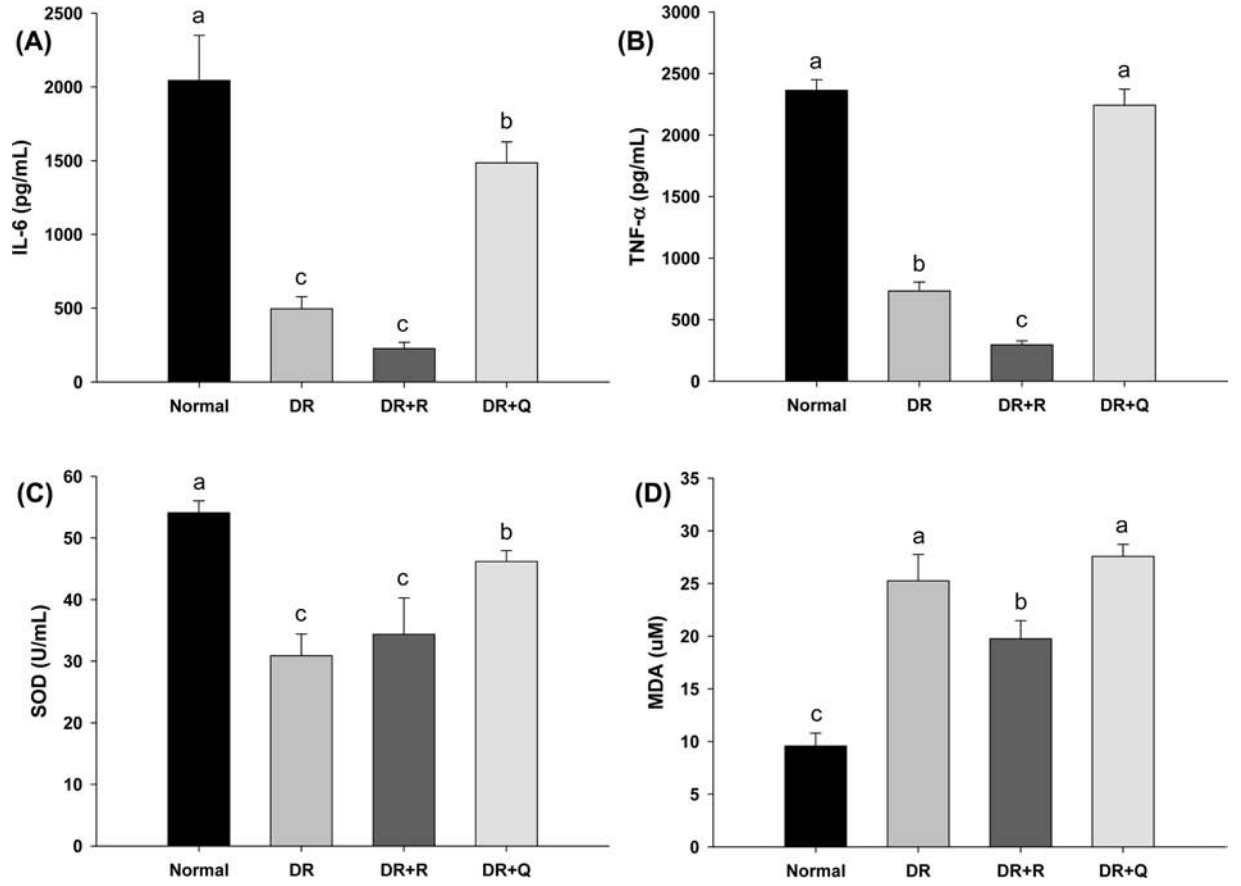

Figure 6. Levels of tissue IL-6 (A) and TNF-α (B) and serum superoxide dismutase (SOD) (C) and malondialdehyde (MDA) (D) affected by nutraceutic rutin and quercetin chows.

overall adverse effect. In addition, both quercetin and rutin also revealed no apparent promising hypocholesterolemic effect in CKD victims (Figure 4D). As a result, although quercetin had alleviated renal inflammation and edema (Figure 3A), due to hyperlipidemia, both rutin and quercetin failed to completely alleviate the highly reduced GFR, resulting in obstructive renal type hypertension (Figure 3C).

Alternatively, literature elsewhere indicated that rutin decreases the levels of fasting blood sugar, systolic and diastolic blood pressure, high density lipoprotein, serum urea, and creatinine significantly ($p < 0.05$), whereas significant increases ($p < 0.05$) in TG, low density lipoprotein, and very low density lipoprotein were seen in patients with diabetes mellitus,[32] suggesting such an in vivo complication highly depending on different medicines and disease.

Quercetin Showed More Efficient Antioxidative Defensive Capability than Rutin, Hence More Effectively Alleviated the DRCKD Status. The DR-induced CKD is a well-known model. Much of the literature demonstrated that the plasma levels of procytokines (such as TNF-α, IL-1β), creatinine, and BUN are significantly upregulated after DR-treatment.[33−35] Controversially, we showed that the tissue IL-6, TNF-α, and serum creatinine levels were all downregulated (Figure 4G, Figure 6A,B). As we determined the IL-6 and TNF-α in tissues not in serum, such a difference could cause different results between the cited[33] and our findings. These cytokine levels in tissues are closely associated with malnutrition.[28] Mohammed and Alsaif indicated that treatment with rutin and vitamin C in streptozotocin-induced diabetic rats lowered MDA and increased the antioxidant levels to near control values,[36] verifying the presence of oxidative stress in diabetes and suggesting beneficial effects of rutin and vitamin C combinations in combating the oxidative stress in this disease.[36] Similar action mechanism had been found for other rutinosides. Dietary lemon flavonoids eriocitrin (eriodictyol 7-*O*-β-rutinoside) and hesperidin (hesperetin 7-*O*-β-rutinoside) played a role as antioxidant in serum, liver, and kidney of diabetes rats.[37] Quercetin restored the oxidative defensive SOD more effectively than rutin (Figure 6C), however, was totally inefficient in suppressing the MDA level, much less effective than rutin (Figure 6D). Suggestively, the highly sustained level of MDA by quercetin could be attributed to its potent prooxidant bioactivity.[38] Thus although quercetin exhibited better anti-inflammatory cytokine expression (Figure 6A,B), quercetin seemed moderately better than rutin in alleviating the inflammatory status of kidney tissue (Figure 7A), implicating that the ROS attack would have more severely elicited renal inflammation,[29] and glomerular amyloidosis in DRCKD and DRCKD + quercetin than DRCKD + rutin. Increased ROS production leads to decreased cell proliferation and increased glomerular collagen IV accumulation that is reversed by antioxidants both in vivo and in vitro.[39]

The compromised pathological changes showed more severely cytoplasmic inflammation of DRCKD + rutin and less collagen deposition in DRCKD + quercetin (Figure 7A,B).

Highly Raised Serum BUN, Creatinine Levels, and Reduced Urinary Creatinine Implicated More Severe Renal Damages Caused by Rutin than Quercetin on

dx.doi.org/10.1021/jf304595p | J. Agric. Food Chem. 2013, 61, 7258−7267

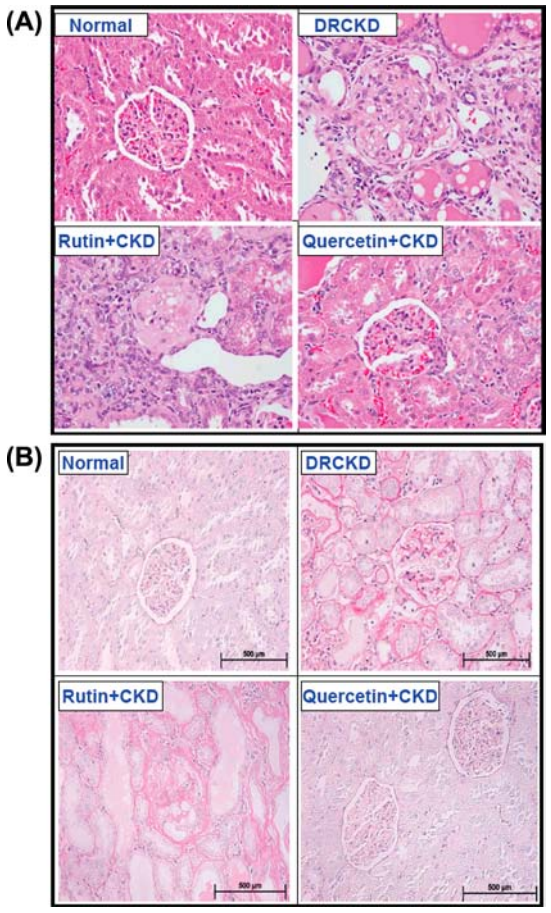

Figure 7. Hematoxylin–Eosin (A) and Sirius Red (B) stainings of renal glomerular tissues. HE staining shows amyloidosis of renal tissues. Sirius Red staining revealed severe collagen deposition in DRCKD and rutin CKD victims.

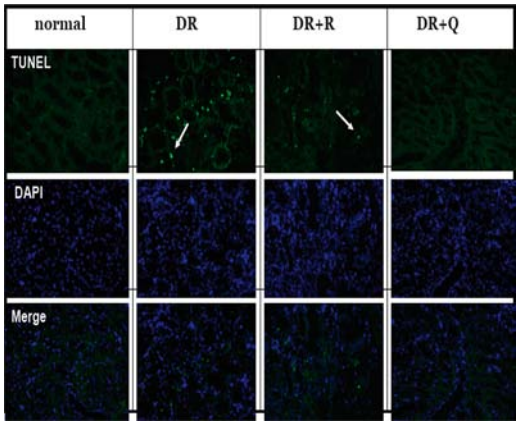

Figure 8. TUNEL assay. Normal: normal control. DR: Doxorubicin-induced CKD (DRCKD). DR+R: DRCKD + rutin. DR+Q: DRCKD + quercetin.

CKD. The serum creatinine level is an indicator of how well the kidneys are working. The amount of creatinine the body produces each day normally depends on the person's muscle mass, or pathologically on the muscle protein degradation.[40] Amidinotransferase (transamidinase, L-arginine: glycine amidinotransferase, EC 2.1.4.1) is an enzyme that catalyzes the first step in creatine synthesis primarily in the kidney and pancreas.[41] Creatinine is usually produced at approximately

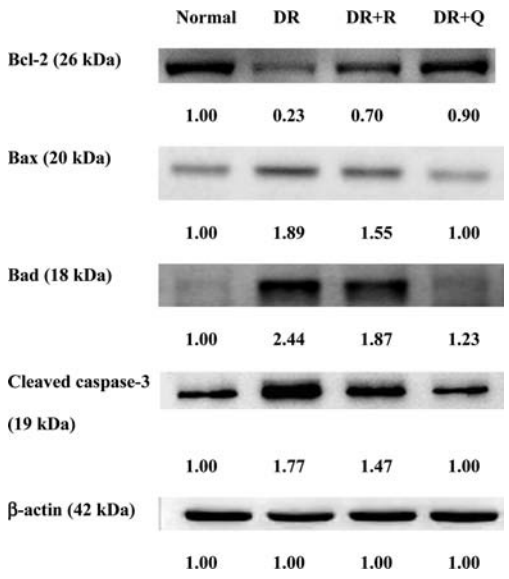

Figure 9. Western blot for BcL2, Bax, Bad, and cleaved caspase-3. The protein loading was 50 $\mu g/\mu L$ per well. β-Actin was internal control.

the same rate every day in each healthy person. It ends up as a waste product in the blood that is transported to the kidney, where it is filtered out of the blood and removed from the body in the urine as the so-called "urinary creatinine". Thus, creatinine clearance reflects the rate at which the kidneys filter blood, as often called "the glomerular filtration rate (GFR)". This is clinically a routine measure of kidney function.[22]

Mercury intoxication was shown to have decreased renal transamidinase activity ($p < 0.001$).[41] Supposedly, rutin might have stimulated the pancreatic transamidinase (Figure 4G) and simultaneously actively stimulated protein catabolism to elicit uremic syndrome (elevation of serum BUN) (Figure 4F). Moreover, rutin was seen ineffective to restore the urinary creatinine excretion due to suppressed GFR (Figure 3B), pointing to the astonishingly damaging effect of rutin in DRCKD. Normally, serum creatinine assay is more sensitive than the BUN test for kidney function.

When DRCKD is treated with flavonoids, rutin upregulated the tissue protein contents of liver and kidney but downregulated that in the heart.[42] DRCKD victims exhibited tissue protein contents of 282.33, 201.33, and 125.00 mg/dL in liver, heart, and kidney, respectively. On treatment with rutin, the values changed to 249.00, 108.33, and 178.33 mg/dL. Conversely, quercetin significantly downregulated the corresponding tissue proteins to 96.40, 46.60, and 51.98 mg/dL, respectively.[42]

Previously we indicated quercetin tends to induce renal carcinoma under the condition of prolonged administration.[11] However for short-term medication, as the above-mentioned, much of the data were favoring quercetin that acted as the most effective protective nutraceutics against the DRCKD. CKD is a renopathy accompanied with renal inflammation,[29] glomerular amyloidosis (Figure 7A), and collagen deposition (Figure 7B), hence progressive fibrosis and necrosis are inevitably accompanying apoptosis.[29] Thus, to ameliorate these symptoms by simple nutraceutic therapy obviously would be impossible.

Western Blot Analysis Implicated That Rutin but Not Quercetin Elicited Apoptosis in CKD through the Intrinsic Mitochondrial Pathway. DR and rutin significantly downregulated Bcl-2, but highly upregulated Bax, Bad, and

dx.doi.org/10.1021/jf304595p | J. Agric. Food Chem. 2013, 61, 7258–7267

cleaved caspase-3 (Figure 9), implicating that the intrinsic mitochondrial pathway had been triggered toward apoptosis in DRCKD. Quercetin alleviated these apoptotic signals as evidenced by the disappearance of renal amyloidosis (Figure 7A) and collagen deposition (Figure 7B). Rutin was shown partially effective, but quercetin completely, in rescuing the apoptosis. In intrinsic apoptotic pathway, mitochondria usually are damaged to release cytochrome *c* (not shown).[43] As a consequence, energy−ATP metabolism would be impaired in the DRCKD + rutin group,[43] with extensive in vivo protein degradation triggered (Figure 4F) to result in the so-called "protein-energy malnutrition" and severe body weight loss (Figure 2).

In addition, pharmacokinetic and pharmacodynamic literature has pointed out that aglycon and its related glycoside may act quite differently.[13] Pharmacokinetically, humans absorb appreciable amounts of quercetin and that absorption is enhanced by conjugation with glucose (52 ± 15%),[13] contrasted with 17 ± 15% for quercetin rutinoside, and 24 ± 9% for quercetin aglycon.[13]

Similar results were reported by Erlund et al. (2000).[44] The overall kinetic behavior of quercetin differed remarkably after ingestion of quercetin aglycon or rutin.[44] The mean area under the plasma concentration−time curve from 0 to 32 h [AUC(0−32)] and maximum plasma concentration (C_{max}) values of the two treatments were similar. However, the time to reach C_{max} (t_{max}) was significantly shorter for quercetin aglycon than rutin (1.9, 2.7, and 4.8 h versus 6.5, 7.4, and 7.5 h, for doses 1, 2, and 3, respectively).[44] Quercetin and rutin were found in plasma as glucuronides and/or sulfates of quercetin and as unconjugated quercetin aglycon, but no free rutin was detected.[44] In pharmacodynamical evaluation of the antiasthma effect, quercetin caused vasorelaxation of the preconstricted aorta ring with or without endothelium intact; while rutin also caused vasorelaxation in preconstricted endothelium-intact rings, however not in aorta rings without endothelium. The vasodilatation effect of quercetin was shown more potent than that of rutin.[16]

Taken together, these data have strongly supported the fact that rutin and quercetin, although chemically belonging to the same chemical category flavonol, in reality acted differently in view of pharmacology. Rutin induced apoptosis by taking the intrinsic mitochondrial pathway and DNA damage, accelerating in vivo protein degradation and causing the so-called "protein-energy malnutrition", uremia, and hypercreatininemia in CKD. In contrast, quercetin in all aspects has shown much more beneficial effects to CKD therapeutics.

In conclusion, pharmacokinetically and phamacodynamically rutin and quercetin act differently in DRCKD. Rutin aggravates, while quercetin is much more beneficial to CKD treatment.

AUTHOR INFORMATION

Corresponding Author

*E-mail: kc.chen416@msa.hinet.net. Tel: +886-2-22490088 ext 8112. Fax: +886-2-27585767.

Author Contributions

‡C.-L.H. and C.-C.P. contributed equally.

Notes

The authors declare no competing financial interest.

This work was supported by the financial support from the National Science Council NSC-99-2313-B-018-002-MY3, NSC-99-2320-B-038-011-MY3, NSC 101-2320-B-038-030 and from Taipei Medical University-Shuang Ho Hospital 102 TMU-SHH-11.

ABBREVIATIONS USED

BUN, blood urea nitrogen; CAM, complementary adjuvant medicines; C_{cr}, creatinine clearance; CKD, chronic kidney disease; DR, doxorubicin; DR+C, DR + catechin; DR+N, DR + naringenin; DR+Q, DR + quercetin; DR+R, DR + rutin; DRCKD, doxorubicin-induced chronic kidney disease; GFR, glomerular filtration rate; Hb or HGB, hemoglobin; HCT, hematocrit; HDL, high density lipoprotein; IL 6, interleukin 6; IRS, insulin receptor substrate; MDA, malondialdehyde; NOAEL, no observed adverse effect level; P_{cr}, plasma concentration; PI3K, phosphoinositide-3 kinase; SOD, superoxide dismutase; TG, triglyceride; TNF-α, tumor necrosis factor α; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling; U_{cr}, urinary creatinine clearance; UUN, urinary urea nitrogen

REFERENCES

(1) Kamalakkannan, N.; Prince, P. S. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats. *Basic Clin. Pharmacol. Toxicol.* **2006**, *98*, 97−103.

(2) Kamalakkannan, N.; Stanely Mainzen Prince, P. The influence of rutin on the extracellular matrix in streptozotocin-induced diabetic rat kidney. *J. Pharm. Pharmacol.* **2006**, *58*, 1091−1098.

(3) Guardia, T.; Rotelli, A. E.; Juarez, A. O.; Pelzer, L. E. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco* **2001**, *56*, 683−687.

(4) Kwon, K. H.; Murakami, A.; Tanaka, T.; Ohigashi, H. Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. *Biochem. Pharmacol.* **2005**, *69*, 395−406.

(5) Karthick, M.; Stanely Mainzen Prince, P. Preventive effect of rutin, a bioflavonoid, on lipid peroxides and antioxidants in isoproterenol-induced myocardial infarction in rats. *J. Pharm. Pharmacol.* **2006**, *58*, 701−707.

(6) Chen, W. M.; Jin, M.; Wu, W. Experimental study on inhibitory effect of rutin against platelet activation induced by platelet activating factor in rabbits. *Zhongguo Zhongxiyi Jiehe Zazhi* **2002**, *22*, 283−285.

(7) Sheu, J. R.; Hsiao, G.; Chou, P. H.; Shen, M. Y.; Chou, D. S. Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonol quercetin, in human platelets. *J. Agric. Food Chem.* **2004**, *52*, 4414−4418.

(8) Tamura, T.; Mitsumori, K.; Muto, S.; Kasahara, H.; Kobayashi, S.; Okuhara, Y.; et al. Fifty-two week chronic toxicity of enzymatically decomposed rutin in Wistar rats. *Food Chem. Toxicol.* **2010**, *48*, 2312−2318.

(9) Egert, S.; Bosy-Westphal, A.; Seiberl, J.; Kürbitz, C.; Settler, U.; Plachta-Danielzik, S.; et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *Br. J. Nutr.* **2009**, *102*, 1065−1074.

(10) Boots, A. W.; Li, H.; Schins, R. P.; Duffin, R.; Heemskerk, J. W.; Bast, A.; et al. The quercetin paradox. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 89−96.

(11) Hsieh, C. L.; Peng, C. C.; Cheng, Y. M.; Lin, L. Y.; Ker, Y. B.; Chang, C. H. Quercetin and Ferulic Acid Aggravate Renal Carcinoma in Long-Term Diabetic Victims. *J. Agric. Food Chem.* **2010**, *58*, 9273−9280.

(12) Harwood, M.; Danielewska-Nikiel, B.; Borzelleca, J. F.; Flamm, G. W.; Williams, G. M.; Lines, T. C. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo

toxicity, including lack of genotoxic/carcinogenic properties. *Food Chem. Toxicol.* **2007**, *45*, 2179−2205.
(13) Hollman, P. C.; de Vries, J. H.; van Leeuwen, S. D.; Mengelers, M. J.; Katan, M. B. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr.* **1995**, *62*, 1276−1282.
(14) Richelle, M.; Pridmore-Merten, S.; Bodenstab, S.; Enslen, M.; Offord, E. A. Previous hydrolysis of glycosides to aglycones does not enhance the bioavailability of isoflavones in humans. *J. Nutr.* **2002**, *132*, 2587−2592.
(15) Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000**, *130*, 1695−1699.
(16) Zhou, X. M.; Yao, H.; Xia, M. L.; Cao, C. M.; Jiang, H. D.; Xia, Q. Comparison of vasodilatation effect between quercetin and rutin in the isolated rat thoracic aorta. *Zhejiang Daxue Xuebao, Yixueban* **2006**, *35*, 29−33.
(17) Carrero, J. J.; Yilmaz, M. I.; Lindholm, B.; Stenvinkel, P. Cytokine dysregulation in chronic kidney disease: How can we treat it? *Blood Purif.* **2008**, *26*, 291−299.
(18) Curtis, B. M.; Levin, A.; Parfrey, P. S. Multiple risk factor intervention in chronic kidney disease: management of cardiac disease in chronic kidney disease patients. *Med. Clin. North Am.* **2005**, *89*, 511−523.
(19) United States Renal Data System. *USRDS 2007 Annual Data Report*; Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U.S. Department of Health and Human Services: 2007.
(20) Tremblay L. Heart Attack and Chronic Kidney Disease. *ABOUT.COM, Heart Health Center*; updated November 4, 2008; http://heartdisease.about.com/lw/Health-Medicine/Conditions-and-diseases/Heart-Attack-and-Chronic-Kidney-Disease.htm, **2008**.
(21) Curtis, B. M.; Parfrey, P. S. Congestive heart failure in chronic kidney disease: disease-specific mechanisms of systolic and diastolic heart failure and management. *Cardiol. Clin.* **2005**, *23*, 275−284.
(22) Stevens, L. A.; Coresh, J.; Greene, T.; Levey, A. S. Assessing kidney function—measured and estimated glomerular filtration rate. *N. Engl. J. Med.* **2006**, *354*, 2473−2483.
(23) National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am. J. Kidney Dis.* **2002**, *39*, S1−S266.
(24) Sevinc, A.; Davutoglu, V.; Barutcu, I.; Kocoglu, M. E. Unusual course of infective endocarditis: acute renal failure progressing to chronic renal failure. *J. Natl. Med. Assoc.* **2006**, *98*, 651−654.
(25) Thompson, M. S.; Cohan, L. A.; Jordan, R. C. Use of rutin for medical management of idiopathic in four cats. *J. Am. Vet. Med. Assoc.* **1999**, *215*, 346−348.
(26) Adams, G. R.; Vaziri, N. D. Skeletal muscle dysfunction in chronic renal failure: effects of exercise. *Am. J. Physiol.* **2006**, *290*, F753−F761.
(27) Silveira, E. M. S.; Rodrigues, M. F.; Krause, M. S.; Vianna, D. R.; Almeida, B. S.; Rossato, J. S.; et al. Acute exercise stimulates macrophage function: possible role of NF-kB pathways. *Cell Biochem. Funct.* **2007**, *25*, 63−73.
(28) Fock, R. A.; Vinolo, M. A.; de Moura Sá Rocha, V.; de Sá Rocha, L. C.; Borelli, P. Protein-energy malnutrition decreases the expression of TLR-4/MD-2 and CD14 receptors in peritoneal macrophages and reduces the synthesis of TNF-alpha in response to lipopolysaccharide (LPS) in mice. *Cytokine* **2007**, *40*, 105−114.
(29) Peng, C. C.; Chen, K. C.; Lu, H. Y.; Peng, R. Y. Treadmill exercise improved adriamycin-induced nephropathy. *J. Biol. Regul. Homeostatic Agents* **2012**, *26*, 15−28.
(30) Wu, X.; Pittman, H. E., 3rd; Prior, R. L. Pelargonidin is absorbed and metabolized differently than cyanidin after marionberry consumption in pigs. *J. Nutr.* **2004**, *134*, 2603−2610.
(31) Ichiyanagi, T.; Shida, Y.; Rahman, M. M.; Sekiya, M.; Hatano, Y.; Matsumoto, H.; Hirayama, M.; Konishi, T.; Ikeshiro, Y. Effect on both aglycone and sugar moiety towards Phase II metabolism of anthocyanins. *Food Chem.* **2008**, *110*, 493−500.
(32) Sattanathan, K.; Dhanapal, C. K.; Umarani, R.; Manavalan, R. Beneficial health effects of rutin supplementation in patients with diabetes mellitus. *J. Appl. Pharm. Sci.* **2011**, *1*, 227−231.
(33) Abo-Salem, O. M. The protective effect of aminoguanidine on doxorubicin-induced nephropathy in rats. *J. Biochem. Mol. Toxicol.* **2012**, *26*, 1−9.
(34) Zhu, C.; Huang, S.; Ding, G.; Yuan, Y.; Chen, Q.; Pan, X.; Chen, R.; Zhang, A. Protective effects of Huang Qi Huai granules on adriamycin nephrosis in rats. *Pediatr. Nephrol.* **2011**, *26*, 905−913.
(35) Nazmi, A. S.; Ahmad, S. J.; Rashikh, A.; Akhtar, M.; Pillai, K. K.; Najmi, A. K. Protective effects of 'Khamira Abresham Hakim Arshad Wala', a unani formulation against doxorubicin-induced cardiotoxicity and nephrotoxicity. *Toxicol. Mech. Methods* **2011**, *21*, 41−47.
(36) Alsaif, M. A. Combined Treatment of Rutin and Vitamin C Improves the Antioxidant Status in Streptozotocin-Induced Diabetic Rats. *J. Med. Sci.* **2009**, *9*, 1−9.
(37) Miyake, Y.; Yamamoto, K.; Tsujihara, N.; Osawa, T. Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids* **1998**, *33*, 689−695.
(38) Shin, J. K.; Kim, G. N.; Jang, H. D. Antioxidant and pro-oxidant effects of green tea extracts in oxygen radical absorbance capacity assay. *J. Med. Food* **2007**, *10*, 32−40.
(39) Chen, X.; Moeckel, G.; Morrow, J. D.; Cosgrove, D.; Harris, R. C.; Fogo, A. B.; Zent, R.; Pozzi, A. Lack of integrin $\alpha1\beta1$ leads to severe glomerulosclerosis after glomerular injury. *Am. J. Pathol.* **2004**, *165*, 617−630.
(40) Patel, S. S.; Molnar, M. Z.; Tayek, J. A.; Ix, J. H.; Noori, N.; Benner, D.; Heymsfield, S.; Kopple, J. D.; Kovesdy, C. P.; Kalantar-Zadeh, K. Serum creatinine as a marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature. *J. Cachexia, Sarcopenia Muscle* **2013**, *4* (1), 19−29.
(41) Nikolic, J.; Sokolovic, D. Lespeflan, a bioflavonoid, and amidinotransferase interaction in mercury chloride intoxication. *Renal Failure* **2004**, *26*, 607−611.
(42) Parabathina, R. K.; Swamy, P. L.; Harikrishna, V. V. S. N.; Rao, G. S.; Rao, K. S. Vitamin E, morin, rutin, quercetin prevents tissue biochemical changes induced by doxorubicin in oxidative stress conditions: Effect on heart, liver and kidney homogenates. *J. Chem. Pharm. Res.* **2010**, *2*, 826−834.
(43) Li, H.; Kolluri, S. K.; Gu, J.; Dawson, M. I.; Cao, X.; Hobbs, P. D. Cytochrome *c* Release and Apoptosis Induced by Mitochondrial Targeting of Nuclear Orphan Receptor TR3. *Sciences* **2000**, *289*, 1159−1164.
(44) Erlund, I.; Kosonen, T.; Alfthan, G.; Mäenpää, J.; Perttunen, K.; Kenraali, J.; et al. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur. J. Clin. Pharmacol.* **2000**, *56*, 545−553.