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Quercetin and Ferulic Acid Aggravate Renal Carcinoma in Long-Term Diabetic Victims

Chiu-Lan Hsieh,[†] Chiung-chi Peng,[⊥,⊗,⊕] Yu-Ming Cheng,[#] Li-Yun Lin,[#] Yaw-Bee Ker,[#] Chi-Huang Chang,[△] Kuan-Chou Chen,[*,§] and Robert Y. Peng[*,△]

[†]Graduate Institute of Biotechnology, National Changhua University of Education, 1 Jin-De Road, Changhua, Taiwan 500, [⊥]School of Physical Therapy, [⊗]Graduate Institute of Rehabilitation Science, and, [⊕]Department of Nutrition, College of Health Care, China Medical University, 91 Hsueh-Shih Road, Taichung, Taiwan 40202, [#]Research Institute of Food Biotechnology, Hungkuang University, 34 Chung-Chie Road, Shalu County, Taichung Hsien, Taiwan 43302, [§]Department of Urology, Taipei Medical University Shuan-Ho Hospital, 250 Wu-Xin Street, Xin-Yi District, Taipei, Taiwan 110, and [△]Research Institute of Biotechnology, Hungkuang University, 34 Chung-Chie Road, Shalu County, Taichung Hsien, Taiwan 43302.

Many phytoantioxidants have therapeutic drawbacks due to their potent prooxidant bioactivity. It is hypothesized that phytoantioxidants (PAO) are beneficial only to the early-stage diabetes mellitus (DM) and will become ineffective once renopathy occurs. Gallic acid, rutin, EGCG, ferulic acid (FA), and quercetin were tried on the streptozotocin (STZ)-induced DM rat model for a 28 week experimental period. All of these PAO were shown to be ineffective for hypoglycemic action. The incidence of cataract (50%), injured glomerules, and renal cell carcinoma (RCC) was very common, among which the most severely affected involved the quercetin- and the FA-treated groups. The tumorigenicity of ferulic acid is still unclear. However, for quercetin, this can be attributted to (i) the prooxidant effect, (ii) the insulin−secretagogue bioactivity, and (iii) the competitive and noncompetitive inhibition on the *O*-methyltransferase to enhance the estradiol-induced tumorigenesis. Conclusively, quercetin and FA are able to aggravate, if not induce, nephrocarcinoma. It is time to reevaluate the tumorigenic detrimental effect of PAO, especially those exhibiting prooxidant bioactivity.

KEYWORDS: Quercetin; renal carcimoma; streptozotocin; diabetes mellitus

INTRODUCTION

Streptozotocin (STZ), chemically named *N*-(methylnitrosocarbamoyl)-α-D-glucosamine, is a well-known type 1 diabetes inducing agent. STZ cytotoxically and specifically damages the pancreatic β cells and DNA (*1*,*2*). Although the tumorigenicity of STZ has been well cited (*3*,*4*), Horton was the pioneer who found renal mesenchymal carcinoma induced by STZ in rats (*3*). The overall incidence rate reached 45% (36/80) over 7−14 months after administration of a single dose of STZ at 25 mg/kg. Microscopically, the mixed tumors resembled the nephroblastomas seen in man (*3*).

Quercetin and ferulic acid widely occur in a diversity of plants and plant-related sources (*5*,*6*). Onion leaves, Semambu leaves, bird chili, black tea, and guava are the richest, the content of quercetin aglycone usually exceeding 1120 mg/kg (*5*), whereas vegetables and fruits almost exclusively contain quercetin glycosides (*5*). Quercetin has been shown to be beneficial to a variety of pathological events including the hypotensive effect in vivo and the antiinflammatory activity, potent antioxidant activity, and vitamin C sparing action in vitro (*7*). Quercetin reduces renal oxidative injury and facilitates repair (*8*), exhibiting the potential for inhibiting mitosis and apoptosis of glomerular cells both in vitro and in vivo (*9*). In mice, quercetin administered per os increased gene expression of mitochondrial biomarkers and improved exercise endurance (*10*). Quercetin played a preventive role in preneoplasms (*11*), and ferulic acid significantly decreased plasma glucose and increased insulin concentration in normal rats (*6*).

However, controversial evidence is accumulating. Quercetin enhanced estradiol-induced tumorigenesis (*12*, *13*), primarily the growth of benign tumors on renal tubular epithelium, and no carcinogenicity at other sites (*14*). Although quercetin is a genotoxic chemical, the neoplastic response observed in the kidney was suggested to be probably caused in part by a combination of nongenotoxic and genotoxic events (*14*). As often cited, high dosages of flavonoids exhibit prooxidative properties. Quercetin decreased glutathione *S*-transferase activity at concentrations ≥200 mM, resulting in DNA damage (*15*).

Normally, nephrocarcinoma is notoriously difficult to treat when metastatic due to its resistance to conventional chemotherapy (*16*). Consequently, complementary alternative medicine (CAM) was suggested to intervene the clinical use. Considering that "the chronic disease can only be examined by a long term follow-up", we hypothesize that the phytoantioxidants (PAO), flavonoids and polyphenolics, could exhibit bioactivity beneficial only to the early stage

*Corresponding authors [(K.-C.C.) e-mail kc.chen416@msa.hinet.net, phone +886-2-27299723; (R.Y.P.) e-mail ypeng@seed.net.tw, mobile +886-953-002-092, phone +886-2-27585767].

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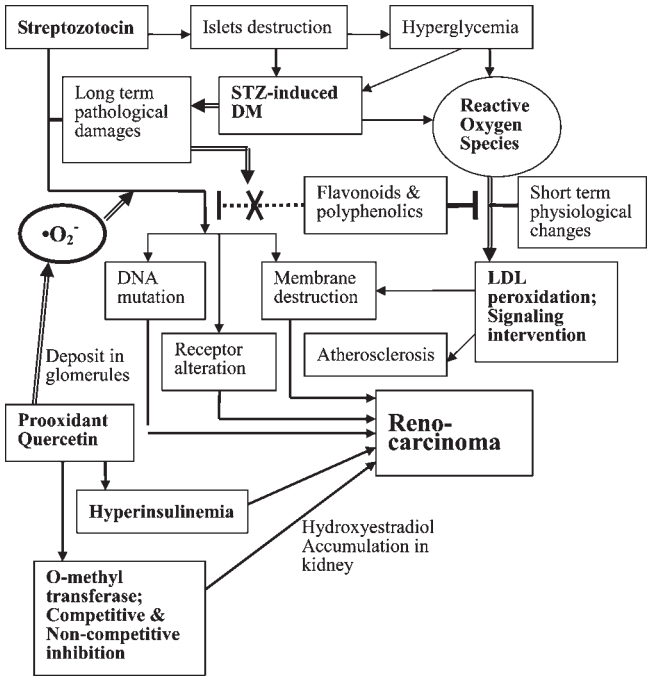

Figure 1. Nephrocarcinogenesis hypothesized to be synergistically elicited by streptozotocin and quercetin in the long-term DM status.

or the short-term physiological changes in DM patients, whereas for the long-term DM victims the PAO may become ineffective after the occurrence of significant pathological changes (**Figure 1**). To prove this, we carried out this experiment.

MATERIALS AND METHODS

Chemicals. All chemicals used in this experiment were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Animals. Forty-eight male Sprague−Dawley rats aged 4 weeks were purchased from the National Laboratory Animal Centre. All studies performed with this rat model were approved by the Hungkuang University Human and Ethic Committee in accordance with Helsinki Declaration of 1975. The animal room was conditioned at 24 ± 1 °C with a relative humidity maintained at 40−60% and light cycle changed every 12 h. Water and meal were taken ad libitum. For the first week, rats were acclimated by supplying only regular diets for experimental rats (Fu-Sow brand; Chinese meaning "Long-Live"). Rats were randomly grouped into eight groups, six in each stainless cage, and fed special ingredient-blended diets. At the beginning of the second week, rats were ip administered 50 mg/kg of body weight STZ and fed special ingredient incorporated diet. Each diet was thoroughly mixed to ensure a homogeneous compositional distribution and stored at 4 °C. The rats were divided into the diet control (group a) and the STZ-DM control (group b), which had a blood sugar level over 220 mg/dL and was given the same regular diet without any treatment. Group c was fed glibenclamide (600 μg/kg) incorporated into the diet to act as the clinical DM therapy control. The PAO used included gallic acid (group d), rutin (group e), EGCG (group f), ferulic acid (group g), and quercetin (group h). The dose of PAO supplement was 70 mg/kg of diet. The experimental period extended for 28 weeks. At the end of experiment, rats were euthanized with CO_2. During the experimental period, body weights were recorded every week. The blood sugar, insulin, and serum malondialdehyde (MDA) levels were taken twice: at the beginning of experiment and before euthanization. After euthanization, the organs or tissues were immerged into 10% formalin for 3 days. The tissues were subsequently dehydrated with 60, 70, 80, and 95% alcohol and finally with xylene. The dewatered tissues were embedded in wax and sectioned with a microtome. The tissue sections were placed in distilled water, and the nuclei were stained with alum hematoxylin. The stained tissues were rinsed under running tap water and then differentiated with 0.3% of acidic ethanol. The tissues slices were rinsed first under running water and then in Scott's water substitute. The tissues were again rinsed with running tap water and then stained with eosin for 2 min. The stained tissues were dehydrated, cleared, and mounted for microscopic examination.

Preparation of Blood. Blood was immediately centrifuged at 3200 rpm for 15 min using a Kubota-3740 centrifuge to separate the serum and stored at −20 °C for determination of insulin level.

RESULTS

Pathological Incidence. The overall pathological changes in each group within the 28 week experiment are listed in **Table 1**. Briefly, the aorta was totally unaffected. Degeneration in eyes all reached 100% (6/6), except the gallic acid-treated group (83%, 5/6). Focal necrosis of the heart was found only in the DM control rats (33.3%, 2/6) (**Table 1**). The main manifestations found in renopathy involved inflammation, multiple hydropic degeneration of proximal tubule, and renal cell tumor. Renal inflammation occurred in the DM control, the gallic acid-treated, and the ferulic acid-treated groups (all 33.3%, 2/6) (**Table 1**). The incidence of multiple hydropic degeneration reached 100% (6/6) in groups gallic acid, rutin, EGCG, and quercetin. In contrast, the DM control and ferulic acid groups were only moderately affected (66.6%, 4/6). Renal cell tumor occurred in all rats except the gallic acid treated group (**Table 1**); the incidence rates, respectively, reached were 33.3% (2/6) for the DM control, 83.3% (5/6) for the glibenclamide-treated group, 66.6% (4/6) for the rutin-treated group, 33.3% (2/6) for the EGCG-treated group, (50% (3/6) for the ferulic acid-treated group, and 100% (6/6) for the quercetin-treated group; however, a similar event was not found in the group gallic acid-treated group (**Table 1**). Pathologically, the severity of lesion was very comparable in groups DM, glibenclamide, gallic acid, rutin, and EGCG (**Table 1**). The degree of severity was moderate to severe (51−75% severity) for ferulic acid and severe to high (76−100% severity) for queretin (**Table 1**). In livers, the common feature was infiltration and diffusion of glycogen in groups glibenclamide (83.3%, 5/6), rutin (33.3%, 2/6), and EGCG (50%, 3/6) (**Table 1**). Muscle rhabdomyosarcoma was found in the DM control group. Pancreas was found severely destroyed in groups STZ-DM, glibenclamide, gallic acid, rutin, and quercetin (all 100%, 6/6), whereas only 66.6% (4/6) occurred in group ferulic acid (**Table 1**). Of note, β cell adenoma was found only in groups EGCG (33.3%, 2/6) and quercetin (33.3%, 2/6) (**Table 1**). Otherwise, the normal control rats revealed 50% (3/6) of cardiac necrosis and 16.7% (1/6) of focal cardiac fibrosis (**Table 1**). Also of note, the occurrene of cardiac necrosis (50%, 3/6) and focal cardiac fibrosis (16.7%, 1/6) in the normal control was also perceived (**Table 1**).

Change in Body Weight. The body weight was significantly reduced in all DM groups (**Figure 2**). Compared to the control (620 ± 60 g), change from 250 ± 30 g to an overall range of 292−384 g was perceived during a total of 35 weeks (**Figure 2**).

Phytoantioxidants Alone Failed To Suppress Hyperglycemia in Rats with Long-Term DM. All of the blood sugar levels in each group were raised to over 348 mg/dL at 28 weeks after STZ induction. In reality, administration of PAO-incorporated diets failed to suppress such events. The blood sugar levels in all DM rats maintained at 324−498 mg/dL except the quercetin-treated group (218 mg/dL) (**Table 2**), which was very close to the marginal DM level (≥220 mg/dL) defined by Zhang et al. (*17*).

Quercetin Acted as an Insulin Secretagogue. Quercetin was shown to be a potent insulin secretagogoue; it effectively elevated the serum insulin level to 2.2 μg/L compared to 1.25, 1.20, and 0.9 μg/L of the normal, EGCG-, and glibenclamide-treated groups, respectively (**Figure 3**). Interestingly, the serum insulin of ferulic acid-, rutin-, and gallic acid-treated rats remained at levels only comparable to the DM control (0.40 μg/L) (**Figure 3**).

Oxidative Stress Could Not Be Retrieved by Phytoantioxidants. The oxidative stress caused by STZ induction in all STZ-DM

Table 1. Summary of Pathological Changes in the STZ-Induced Diabetic SD Rats[a]

		group[b]							
organ/tissue	histopathology	N	D	G	GA	R	E	FA	Q
aorta		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
eye	degeneration, lens	0/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6
heart	necrosis, focal	3/6	0/6	0/6	0/6				
	necrosis, focal, with bacteria	0/6	2/6	0/6	0/6				
	fibrosis, focal	1/6							
kidney	inflammation, focal, with bact	0/6	2/6		2/6			2/6	
	hydropic degeneration, proximal tubule, multiple	0/6	4/6		6/6	6/6	6/6	4/6	6/6
	renal cell tumor	0/6	2/6	5/6	0/6	4/6	2/6	3/6	6/6
	1. adenoma, clear cell, focal[c]	0	2	2	1	2	2	0	3
	2. carcinoma, papillary, focal[c]	0	2	3	1	0	1	1	2
	3. carcinoma, papillary, extensive[c]	0	2	3	1	1	0	4	4
	4. carcinoma, chromophobe, extensive[c]	0	2	1	1	1	0	4	5
liver	infiltration, glycogen, diffuse	0/6	0/6	5/6	0/6	2/6	3/6		
muscle	rhabdomyosarcoma	0/6	6/6					N[d]	N
pancreas	decrease of β-cell and islets	0/6	6/6	6/6	6/6	6/6	6/6	4/6	6/6
	β-cell adenoma						2/6		2/6

[a] Each group had six rats. N, control group; D, STZ-DM control group; G, glibenclamide control group; GA, gallic acid group; R, rutin group; E, EGCG group; FA, ferulic acid group; Q, quercetin group. Each phytoantioxidant was given at 70 mg/kg diet. The dose of glibenclamide was 600 μg/kg. [b] Incidence: expressed in number of affected rats/total number of rats in the same group; 0/6 means no abnormities observed. [c] Severity of lesions was graded according to the methods described by Shackelford et al. (*Toxicol. Pathol.* **2002**, *30*, 93−96). Degree of lesions was graded from 1 to 5 depending on severity: 1 = minimal (<1%); 2 = slight (1-25%); 3 = moderate (26-50%); 4 = moderate/severe (51−75%); 5 = severe/high (76−100%). Score of lesions = mean score of lesions in rats examined. [d] N, no tissue submitted.

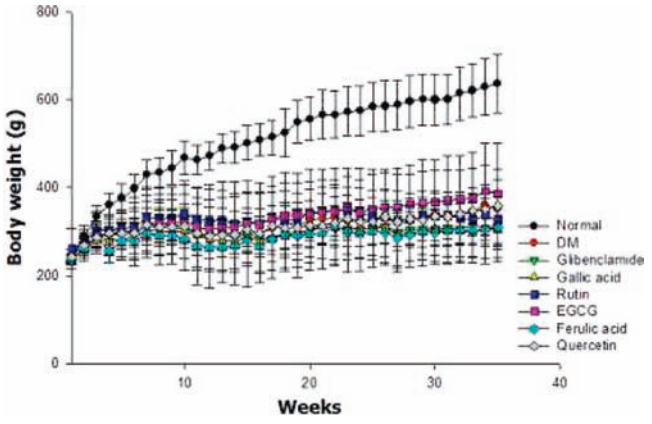

Figure 2. Change of body weight of rats during a 28 week experimental period. The body weight gain was significantly retarded in the DM and DM + nutraceutics groups. The change was roughly from the initial 250 ± 25 g to a final 620 ± 60 g in the control group and from the initial 250 ± 30 g to an overall range of 292−384 g for the other DM and DM + phytoantioxidant groups.

groups at week 28 was not suppressed by PAO. As seen, the serum MDA levels remained moderately high within 53−63 μM, compared to 30 μM in the control (**Figure 4**). EGCG was revealed to be the most effective, persisting at 40 μM. Of note, even glibenclamide, a medicine commonly prescribed as DM therapy, also failed to exhibit any better protective bioactivity (**Figure 4**).

Cataract Incidence Could Not Be Ameliorated by Phytoantioxidants. The incidence of cataract was common as often seen in many long-term human DM (**Table 3**). The lenses of STZ-DM groups all became opaque (figure not shown), exemplifying the ineffectiveness of PAO for prevention of cataract. The incidence rate of cataract all reached 50% except the quercetin-treated group (16.7%, 1/6) (**Table 3**).

Table 2. Blood Sugar Level of Each Rat Group[a]

	blood sugar (mg/dL)	
group	initial	28 weeks later
normal	136 ± 5	144 ± 10
STZ-DM	366 ± 130	324 ± 32
STZ-DM + glibenclamide	349 ± 64	498 ± 78
STZ-DM + gallic acid	354 ± 61	480 ± 39
STZ-DM + rutin	364 ± 46	442 ± 25
STZ-DM + EGCG	353 ± 13	373 ± 31
STZ-DM + ferulic acid	366 ± 35	396 ± 61
STZ-DM + quercetin	348 ± 33	218 ± 71

[a] Each group had six rats. Each phytoantioxidant was given at 70 mg/kg of diet. The dose of glibenclamide was 600 μg/kg.

Deposition of Microaneurysms in Lenses Was Common in DM Rats. Owing to the deposition of microaneurysm in lenses caused by the long-term DM, all of the lenses of the STZ-DM groups revealed a high degree of opacity (**Figure 5**; **Table 1**). Nonetheless, the deposition pattern of microaneurysms depended on the PAO used; for example, the STZ-DM rats showed tremendous clusters of microaneurysms (**Figure 5b**), the glibenclamide-treated rats revealed very huge porous structures with large precipitates (**Figure 5c**), and the STZ-DM and the EGCG-treated rats showed clusters of large aggregates (**Figure 5b**,**f**). In contrast, quercetin seemed to be partially preventive (**Figure 5h**), and degree of injury was rather comparable among the other groups (**Figure 5d**,**e**,**g**).

Injured Islets Were Incurable by Phytoantioxidant Therapy. The islets in all STZ-DM rats were heavily injured (**Figure 6b−h**). Comparatively, the pathological examination revealed that the islet structure was partially protected in the glibenclamide-, gallic

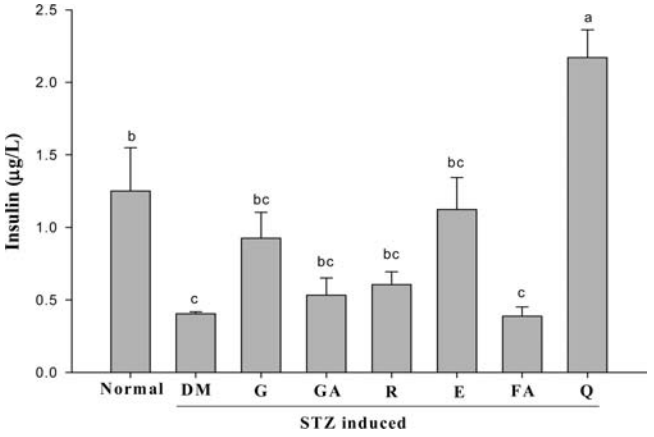

Figure 3. Serum insulin level of rats after treatment for 28 weeks. Each value represents mean ± SD ($n = 6$). Different letters indicate significant difference ($p < 0.05$). Normal, control group; DM, diabetic; G, glibenclamide (600 μg/kg); GA, gallic acid; R, rutin; E, EGCG; FA, ferulic acid; Q, quercetin. All phytoantioxidants were incorporated at 70 mg/kg of diet.

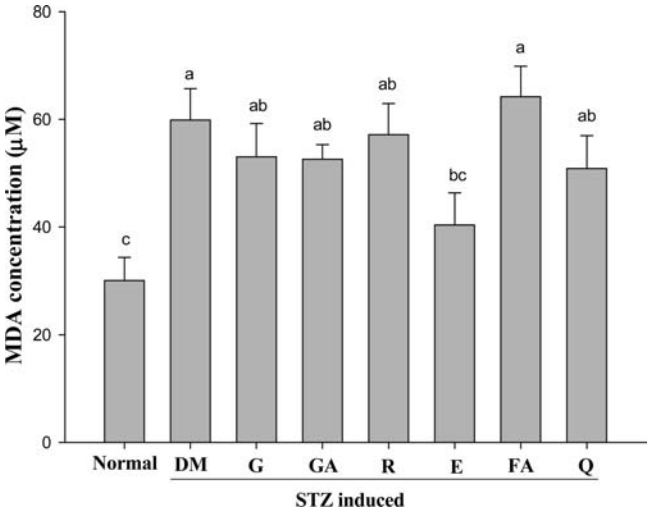

Figure 4. Serum MDA levels in experimental rats after 28 weeks of treatment. Each value represents mean ± SD ($n = 6$). Different letters indicate significant difference ($p < 0.05$). Normal, control group; DM, diabetic; G, glibenclamide (600 μg/kg); GA, gallic acid; R, rutin; E, EGCG; FA, ferulic acid; Q, quercetin. All phytoantioxidants were incorporated at 70 mg/kg of diet.

acid-, and rutin-treated groups (**Figure 6c**−**e**). In contrast, the islets were totally destroyed in the quercetin-treated rats (**Figure 6h**), and a nodular structure was apparently found in the interstitial connective tissues (**Figure 6h**).

Malignant Tumors of Higher Severity Occurred in the Ferulic Acid- and Quercetin-Treated Rats. As shown in **Figure 7**, huge cortical tumor nodules were found in all treated groups (**Figure 7c**−**h**). The most affected were the glibenclamide- (**Figure 7c**), rutin- (**Figure 7e**), ferulic acid- (**Figure 7g**), and quercetin-treated groups (**Figure 7h**). Alternatively, kidneys of the STZ-DM and gallic acid-treated groups were severely dehydrated (**Figure 7**, panel **a** vs panels **b** and **d**). HE stain revealed the incidence of renal cancer to be quite common in all STZ-DM rats after a long-term treatment with PAO (**Figure 8b**−**h**). Astonishingly, although STZ alone was able to induce renal carcinoma (**Figure 8b**), the most malignant tumors were found in the quercetin- and ferulic acid-treated rats (**Table 1**; **Figure 8g**,**h**). The degree of severity was seen in the manifestation showing slight to moderate multiple proximal

Table 3. Incidence Rate of Cataract in Different Rat Groups[a]

	group							
	N	D	G	GA	R	E	FA	Q
incidence rate of cataract	0/6	3/6	3/6	3/6	3/6	3/6	3/6	1/6

[a] Each group had six rats. N, control group; D, STZ-DM control group; G, glibenclamide control group; GA, gallic acid group; R, rutin group; E, EGCG group; FA, ferulic acid group; Q, quercetin group. Each phytoantioxidant was given at 70 mg/kg of diet. The dose of glibenclamide was 600 μg/kg.

tubular hydropic degeneration and focal adenoma of clear cells. Lesser degrees of severity were seen in other groups (**Figure 8b**−**f**; **Table 1**). In addition, focal papillary carcinoma and extensive chromophobe carcinoma occurred in the glibenclamide-, rutin-, ferulic acid-, and quercetin-treated rats (**Figure 8c**,**e**,**g**,**h**), among which the glibenclamide- and rutin-treated groups exhibited only moderate degrees of lesions, compared to the severe to high degrees of lesions found in the ferulic acid- and quercetin-treated rats (**Figure 8g**,**h**; **Table 1**).

DISCUSSION

Metabolic Syndrome Can Interfere with Body Weight Gain. Compared to the control, body weights were significantly reduced in the DM control and DM + PAO groups, indicating that STZ treatment severely suppressed anabolism (*4*). Amazingly, in the normal control, we found slight cardiac necrosis and focal cardiac fibrosis that was not found in other STZ-DM groups, suggesting that overweight may evoke oxidative stress, resulting in cardiac fibrosis and necrosis (**Figure 2**).

Cataract Incidence Cannot Be Prevented by Phytoantioxidants. The retinas of approximately 50% of DM rats had become opaque due to long-term hyperglycemia (figure not shown). Quercein exhibited the slightest prevalence (figure not shown, incidence rate 16.7%),which can be attributed to its potent hypoglycemic effect (**Table 2**). Diseased small blood vessels in the posterior side of eyes in DM patients are always attacked, resulting in leakage of protein and blood into the retina, forming microaneurysms, eliciting neovascularization and impairing vision (*3*) (**Figure 5**). As a result, blurry vision, cataract, and glaucoma are very common among diabetics (*3*) (figure not shown). Approximately 50−80% of patients with diabetes will develop some degree of diabetic retinopathy after 10−15 years of diabetes (*3*). In terms of the 3 year life span for rats, a 28 week experimental duration would be correspondingly as long as 11.7 years in human life. On this basis, we estimate the occurrence of cataract in humans having a hyperglycemic level > 300 mg/dL (**Table 2**) would take about 12 years, being consistent with Horton (*3*). More severely, the retinopathy in STZ-induced diabetic mice may elicit neuron death in ganglia by taking the apoptotic pathway (*18*).

Accumulating evidence indicates that all of these PAO actually failed to inhibit the progress of renal carcinoma in STZ-DM rats, especially ferulic acid and quercetin. Currently, the carcinogenic action mechanism of ferulic acid is still lacking. As for quercetin, accumulating findings including ours point to the following pharmacological action.

STZ Inherently Induced Renal Carcinoma. Horton demonstrated that the prevalence rate of renal tumors in DM Wistar male rats reached 44.0% (36/80) (*3*). The epithelial tumors correspond to the renal adenomas and adenocarcinomas seen in man (*3*), and the mesenchymal tumors were composed of either undifferentiated spindle cells or a mixture of poorly differentiated mesenchyme and epithelial glands. Microscopically, the mixed tumors resembled the nephroblastomas seen in man; both elements appeared to be malignant, similar to our findings (**Figure 6**).

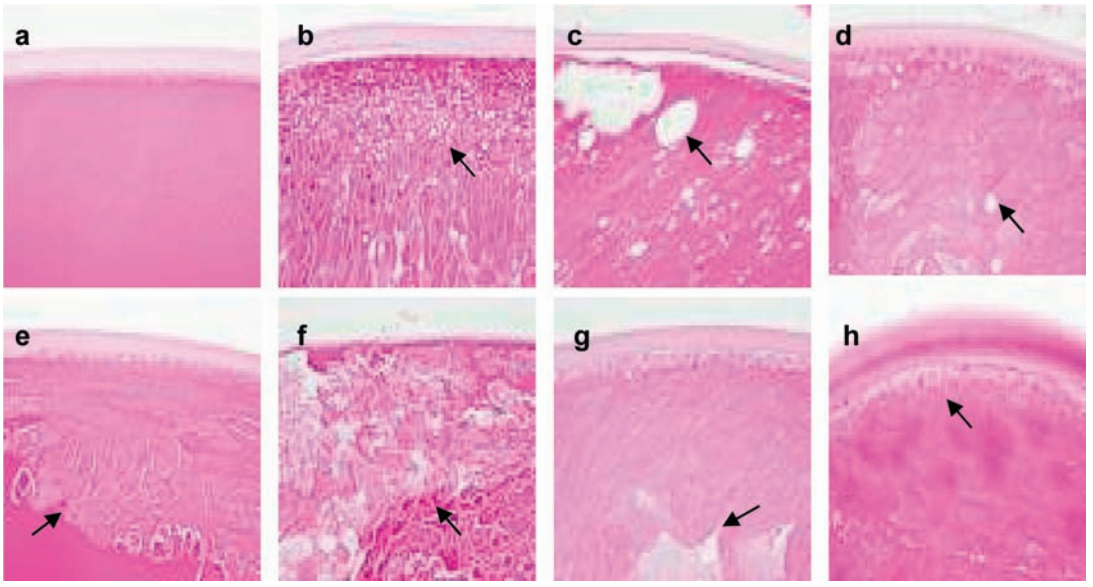

Figure 5. Light micrographs of rat lens tissues with hematoxylin−eosin (HE) staining (magnification ×400): (**a**) the control normal group appeared to be transparent; the other groups showed aneurythm crystalline structure in lens (arrows), (**b**) diabetic; (**c**) glibenclamide (600 μg/kg); (**d**) gallic acid; (**e**) rutin; (**f**) EGCG; (**g**) ferulic acid; (**h**) quercetin. All phytoantioxidants were incorporated at 70 mg/kg of diet. The experimental period was 28 weeks.

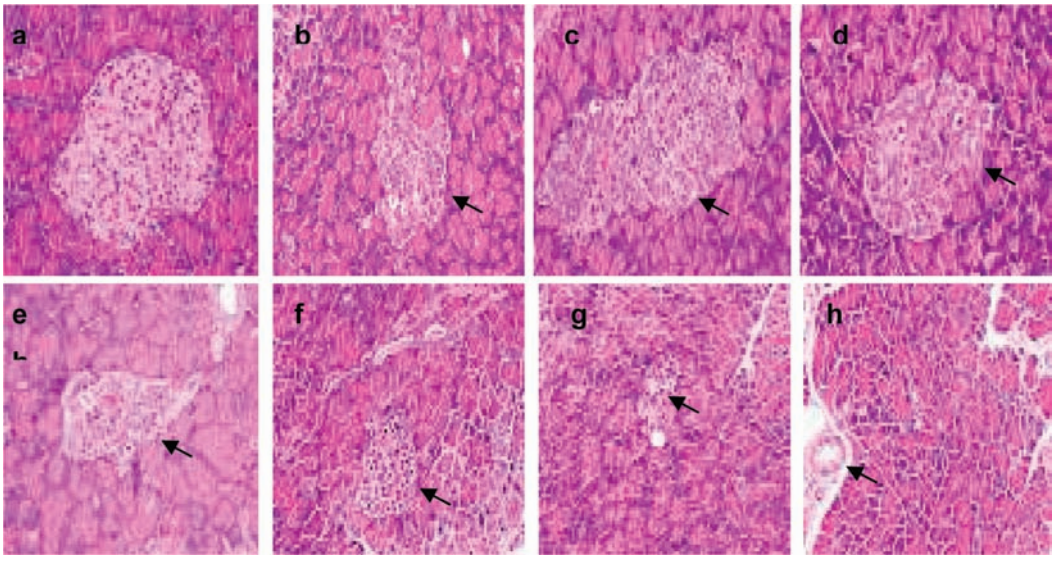

Figure 6. Light micrographs of rat pancreas tissues with hematoxylin−eosin staining (magnification ×400): (**a**) the control group had intact islet structure deep in red and appearing to be solid-like; arrows indicate atrophy or destruction of islet structures or the decreased number of pancreatic β-cells in other groups, (**b**) diabetic; (**c**) glibenclamide (600 μg/kg); (**d**) gallic acid; (**e**) rutin; (**f**) EGCG; (**g**) ferulic acid; (**h**) quercetin. All phytoantioxidants were incorporated at 70 mg/kg of diet. The experimental period was 28 weeks.

Diabetic Hyperglycemia Is the Crucial Risk Factor of Nephrocarcinogenesis. Although accumulating literature has demonstrated the plausible hypoglycemic bioactivity of PAO such as ferulic acid, gallic acid, (−)-epigallocatechin-3-gallate (EGCG), rutin, and quercetin (*19*), nonetheless, all of these hypoglycemics have been shown to be totally ineffective in suppressing the serum glucose level in long-term DM (**Table 2**). This can be due to the occurrence of multiple pathological changes at the late stage of DM, which no longer can be secured by PAO. This evidence strongly supports our hypothesis (**Figure 1**).

Literature elsewhere indicated that nephrocarcinogenesis in diabetic rats results from sustained hyperglycemia, which leads to an adaptive metabolic response, altered growth factor signaling, and subsequent neoplastic transformation of the tubular epithelial cells. Moreover, a close relationship indicating a common origin and a precursor−product of Armanni−Ebstein lesions (AEL) and renal cell carcinoma (RCC) was reported (*4*).

Quercetin Is a Potent Prooxidant Capable of Accelerating Nephrocarinogenesis. Of note, different PAO can exhibit different bioactivities. Quercetin at a dose ≥200 μm significantly stimulated the production of superoxide anion levels in human lymphocytes, reaching a level of 9 nM in cytoplasma. The samples at 200 μM showed that the generation of superoxide anions was approximately 4.87-, 2.49-, 1.70-, and 1.66-fold for quercetin, morin, naringenin, and hesperetin, respectively, that of the control (**Figure 9**) (*15*, *20*). Quercetin and hesperetin had no significant effect ($p < 0.05$) on the activity of glutathione reductase. Conversely, morin and naringenin inhibited its activity (*15*). The glutathione *S*-transferase activity was only slightly decreased (*15*). The damage to DNA induced by these flavonoids was ascribed to

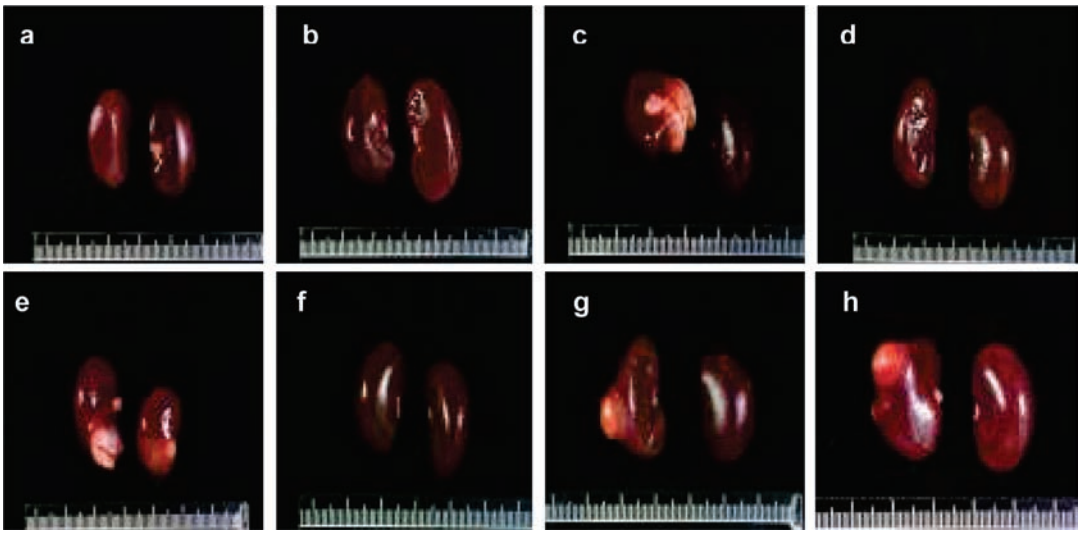

Figure 7. Photographs of kidneys. Rat groups **c**, **e**, **g**, and **h** apparently show nodular tumors on the renal cortical layer. The control group had kidneys normal in size and texture (**a**). The other groups apparently show renal cell tumors in groups diabetic (**b**), glibenclamide (600 μg/kg) (**c**), gallic acid (**d**), rutin (**e**), EGCG (**f**), ferulic acid (**g**), and quercetin (**h**) or adenocarcinoma in ferulic acid (**g**) and quercetin (**h**). All phytoantioxidants were incorporated at 70 mg/kg of diet. The experimental period was 28 weeks.

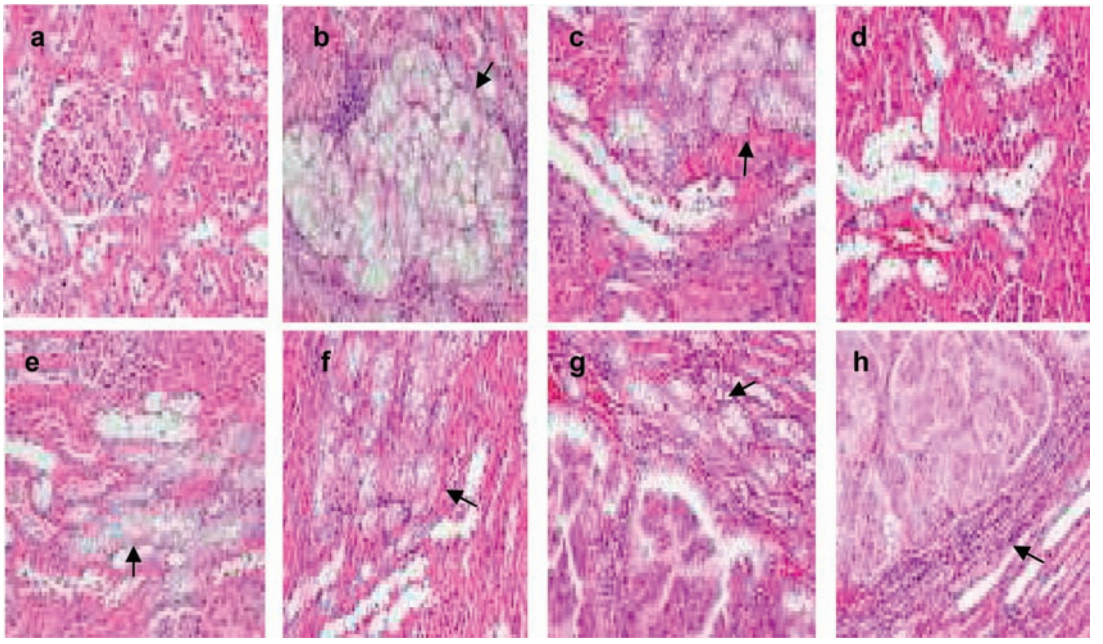

Figure 8. Light micrographs of rat renal cell carcinoma with hematoxylin-eosin staining (magnification $\times$400). Figures reveal that the control group had kidneys normal in size and texture (**a**). The other groups apparently show renal cell tumor (adenoma) in groups diabetic (**b**), glibenclamide (600 μg/kg) (**c**), gallic acid (**d**), rutin (**e**), and EGCG (**e**), ferulic acid (**g**), and quercetin (**h**), whereas the other groups show adenocarcinoma in ferulic acid (**g**), and quercetin (**h**). All phytoantioxidants were incorporated at 70 mg/kg of diet. The experimental period was 28 weeks.

the stimulation of oxidative stress in the lymphocytes, which modulated the antioxidative enzyme activities (*15*) (**Figure 9**).

When the superoxide anion accepts a proton, it forms the hydroperoxyl radical ($^{\bullet}HO_2$), which is able to cross a membrane and then conceivably create damage (*30*). In addition, the superoxide anion is converted to H_2O_2 and O_2 by superoxide dismutase, and then, in the presence of transition metal ions, H_2O_2 forms $OH^{\bullet}$. It is well-known that superoxide anions produced in significant quantity can directly or indirectly damage biomacromolecules by forming hydrogen peroxide, hydroxyl radicals, peroxynitrite, or singlet oxygen during pathophysiological events such as ischemia-reperfusion injury (*20*). Most phenolic compounds have prooxidant activity at low concentrations (*20*). Quercetin and gallic acid exhibited significant ($p < 0.05$) prooxidant effects on total oxidation in the pH 3.0 emulsions (*21*, *22*). Normally, polyphenolics containing a phenol ring are generally more prooxidant than polyphenolics containing a catechol ring (*22*).

Pretreatment of animals with gallic acid (10 and 20 mg/kg of body weight) resulted in a significant decrease in the levels of the parameters involving renal glutathione content, glutathione metabolizing enzyme, and antioxidant enzyme levels ($p < 0.001$). The enhanced reduced glutathione level and enzyme activities involved in xenobiotic metabolism and maintaining antioxidant status of cells are suggestive of a chemopreventive efficacy of gallic acid against Fe-NTA-mediated oxidative stress, toxicity, and cell proliferative response in Wistar rats (*23*). Moreover,

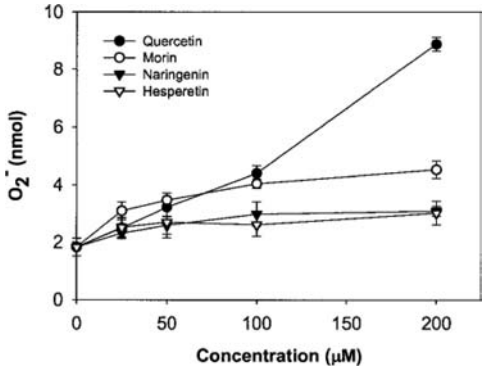

Figure 9. Production of the superoxide anion in human lymphocytes treated with flavonoids. The concentration of the superoxide anion was calculated by using a molar absorption coefficient of 15000 M^{-1} cm^{-1}. Data are expressed as means ± SD ($n \geq 3$) (depicted from ref *15*).

natural polyphenolics are good inhibitors of human dihydrofolate reductase (DHFR). Quercetin glucuronides bind to DHFR. This binding is dependent on the sugar residue, with quercetin-3-xyloside being the stronger inhibitor of DHFR, implicating the prophylactic role for certain forms of cancer and opening a possibility for the use of polyphenolics in cancer therapy. Conversely, the inhibitory effect of DHFR could also contribute to the teratogenic or further tumorigenicity, if any, of polyphenolics (*24*, *25*).

Quercetin and Ferulic Acid Were Potent Insulin Secretagogues, Which Induced Hyperinsulinemia To Enhance Nephrocarcinoma. The management of the diabetic state cannot influence the incidence of tumors, but insulin appears to enhance tumor growth (*6*). The introduction of *m*-hydroxy- and *p*-methoxy-substituted groups in cinnamic acid structure (ferulic acid) displayed the most potent insulin-secreting agent among the cinnamic acid derivatives. In particular, the stimulatory insulin-secreting activities of ferulic acid were associated with a rise of the cyplasmic $[Ca^{2+}]_i$. In the present study, we found that among the PAO used, only quercetin was revealed to be a strong insulin secretagogue, inconsistent with the citings for ferulic acid (**Figure 3**). Hyperinsulinemia has been shown to be closely correlated with tumorigenicity (*3*). Especially, hyperinsulinemia most likely favors cancer in diabetic patients. As insulin is a growth factor with preeminent metabolic but also mitogenic effects, its action in malignant cells is favored by mechanisms acting at both the receptor and postreceptor levels (*3*).

By Inhibiting O-Methyltransferase, Quercetin Enhanced the Tumorigenicity of Estradiol. Inhibition of catechol *O*-methyltransferase-catalyzed O-methylation of 2- and 4-hydroxyestradiol by quercetin raised the accumulated level of 2- and 4-hydroxyestradiol in kidney, which could be otherwise normally excreted into urine (**Figure 10**). The over-accumulated hydroxyestradiols are substantially tumorigenic to the renal cells (*12*, *13*).

As often cited, obesity, hyperglycemia, and increased oxidative stress may also contribute to the increased cancer risk in diabetics. Besides, quercetin is a neurotoxin in vitro (*26*).

Some researchers believe quercetin should not be used by healthy people (for prevention) until it can be shown that quercetin does not itself cause cancer (*27*, *28*). In in vitro studies on cell model, quercetin produces biochemical changes that are also produced by many carcinogens that cause cancer (*27*, *28*). However, in this study, we report quercetin aggravated, at least, if not directly caused, nephrocarcinoma in rats. Until the present, the U.S. Food and Drug Administration has not approved any health claims for quercetin (US FAD, 2007). A current early-stage clinical research on quercetin addressing its safety and efficacy

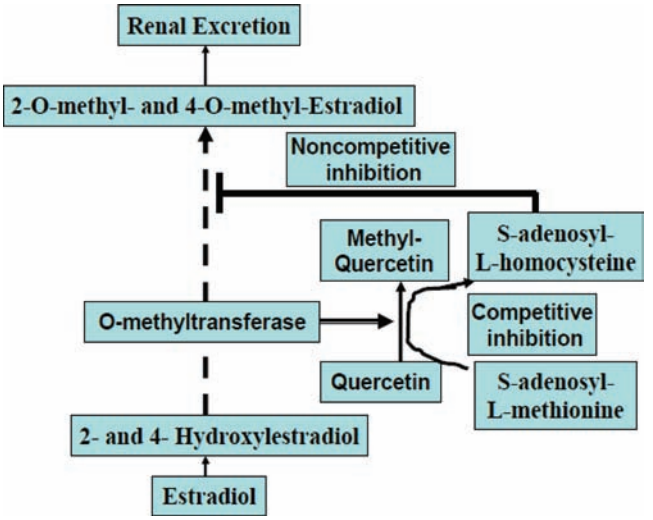

Figure 10. Inhibition of *O*-methyltransferase by quercetin enhances estradiol-induced tumorigenesis (reconstructed in this paper according to refs *12* and *13*).

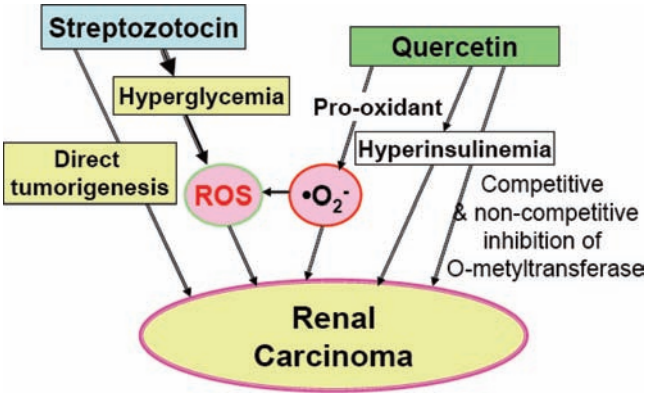

Figure 11. Proposed action mechanism to interpret the overall process of tumorigenesis induced by combined treatment with steptozotocin plus quercetin.

against sarcoidosis, asthma, and glucose absorption in obesity and diabetes was issued in February 2009 (www.clinicaltrials.gov, National Institutes of Health).

Alternatively, quercetin competitively binds to bacterial DNA gyrase (*28*). Whether this could contribute to its tumorigenicity is not entirely clear. Quercetin is also a potent inhibitor of CYP3A4 and CYP2C9, which are enzymes that break down most drugs in the body. As such, quercetin would be expected to increase the serum levels and, therefore, effects of drugs metabolized by this enzyme (*29*). On the contrary, whether this anti-CYP bioactivity may suppress the in vivo antioxidative ability, allowing an easier attack by ROS elicited elsewhere, requires further intensive investigation.

Recently, a bioavailability study showed that the actual physiological roles of quercetin, if they exist, involve quercetin in only minute amounts (*30*). In other words, the implication in the bioactivity of quercetin, either beneficial or harmful to humans, pharmacodynamically requires only a minute amount of quercetin and pharmacokinetically only a very transiently short action time. Thus, despite the preliminary indications of possible health benefits as above-mentioned, quercetin has been neither confirmed as a specific therapeutic for any condition nor approved by any regulatory agency.

In summary, quercetin can aggravate nephrocarcinoma. On the basis of the accumulated evidence, we have derived a proposed action mechanism to interpret how quercetin enhanced STZ-induced nephrocarcinoma (**Figure 11**). STZ is tumorigenic

in nature, which is able to directly induce nephrocarcinoma (*3*, *4*). Hyperglycemia has been shown to be closely related with obesity, diabetes, and carcinogenesis (*4*). Alternatively, quercetin is a potent prooxidant responsible for the production of huge amounts of superoxide anions (*15*). Biochemically, quercetin acts as an *O*-methyltransferase inhibitor, directly as well as indirectly inhibiting the transformation of 2- and 4-hydroxyestradiol into mono-2-*O*- or mono-4-*O*-methylestradiol (*12*, *13*). A long-term over-accumulation of hydroxyestradiol would elicit tumorigenicity in renal cells (*12*, *13*). Moreover, the hyperinsulinemic effect of quercetin can be an alternate risk factor to renal cell carcinoma (*3*) (**Figure 11**).

On the basis of the above, the discovery of an alternative DM inducer that would not cause renal carcinoma would be necessary for further research.

Conclusively, phytoantioxidant (PAO) therapy is ineffective for treatment of the renopathy and renal carcinoma resulting from long-term DM. Quercetin and ferulic acid aggravate the severity of renal cell carcinoma. The tumorigenic action mechanism of quercetin can be attributed to (i) the prooxidant effect, (ii) the insulin secretagogue bioactivity, and (iii) the competitive and noncompetitive inhibition of *O*-methyltransferase to enhance the estradiol-induced tumorigenesis. More importantly, it is time to reevaluate the detrimental tumorigenic effect of PAO, especially those exhibiting stronger prooxidant bioactivity.

ABBREVIATIONS USED

AEL, Armanni−Ebstein lesions; RCC, renal cell carcinoma; CAM, complementary alternative medicine; CYP3A4, cytochrome P450 3A4 (EC 1.14.13.97); CYP2C9, cytochrome P450 2C9; DHFR, dihydrofolate reductase; DM, diabetes mellitus; DM + PAO, STZ-induced DM rats treated with phytoantioxidants; EGCG, epigallocatechin gallate; MDA, malondialdehyde; PAO, phytoantioxidants; SOD, superoxide dismutase; STZ, streptozotocin.

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