HASIL CEK20_60010366

by 60010366 Cek 20

Submission date: 15-Dec-2021 11:34AM (UTC+0700)
Submission ID: 1730865869
File name: CEK20_60010366.pdf (1.09M)
Word count: 7733
Character count: 41539

Toxicology Letters 219 (2013) 77–84

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Effect of 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione, isolated from *Averrhoa carambola* L. (Oxalidaceae) roots, on advanced glycation end-product-mediated renal injury in type 2 diabetic KKAy mice

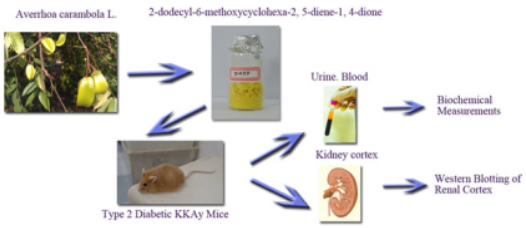
Ni Zheng, Xing Lin, Qingwei Wen, Kintoko, Shijun Zhang, Jianchun Huang, Xiaohui Xu, Renbin Huang*

Department of Pharmacology, Guangxi Medical University, Nanning 530021, PR China

HIGHLIGHTS

- DMDD attenuates AGEs expression in renal tissue.
- DMDD blocks NF-κB/TGF-β1 pathway in renal tissue.
- DMDD relieves renal damage in KKAy mice.
- DMDD plays an antihyperglycemic effect.
- DMDD exerts renoprotection in KKAy mice.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 February 2013
Received in revised form 27 February 2013
Accepted 2 March 2013
Available online 13 March 2013

Keywords:
2-Dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione
Diabetic nephropathy
Advanced glycation end products
Transforming growth factor-β1
Nuclear factor-κB
Renoprotection

ABSTRACT

The roots of *Averrhoa carambola* L. (Oxalidaceae) have a long history of medical use in traditional Chinese medicine for treating diabetes and diabetic nephropathy. 2-Dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione (DMDD) was isolated from the tuberous roots of *A. carambola* L. The purpose of this study was to investigate the beneficial effect of DMDD on the advanced glycation end-product-mediated renal injury in type 2 diabetic KKAy mice with regard to prove its efficacy by local traditional practitioners in the treatment of kidney frailties in diabetics. KKAy mice were orally administrated DMDD (12.5, 25, 50 mg/kg body weight/d) or aminoguanidine (200 mg/kg body weight/d) for 8 weeks. Hyperglycemia, renal AGE formation, and the expression of related proteins, such as the AGE receptor, nuclear factor-κB, transforming growth factor-β1, and N^{ε}-(carboxymethyl)lysine, were markedly decreased by DMDD. Diabetes-dependent alterations in proteinuria, serum creatinine, creatinine clearance, and serum urea-N and glomerular mesangial matrix expansion were attenuated after treatment with DMDD for 8 weeks. The activities of superoxide dismutase and glutathione peroxidase, which are reduced in the kidneys of KKAy mice, were enhanced by DMDD. These findings suggest that DMDD may inhibit the progression of diabetic nephropathy and may be a therapeutic agent for regulating several pharmacological targets to treat or prevent of diabetic nephropathy.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Diabetes is a disorder of chronic hyperglycemia, and glucose participates in diabetic complications such as atherosclerosis, cardiac dysfunction, and nephropathy (Cooper, 2004). Among diabetic complications, diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in developed countries and is a major cause of morbidity and mortality in patients with diabetes. DN is a major microvascular complication of both type 1 and

* Corresponding author at: Department of Pharmacology, Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, PR China. Tel.: +86 771 5339805; fax: +86 771 5358272.
E-mail address: huangrenbin064@163.com (R. Huang).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.03.001

type 2 diabetes mellitus, and its associated risk factors are high blood glucose, high blood pressure, and high cholesterol levels. Therefore, metabolic and hemodynamic factors should be controlled to prevent the occurrence of DN (Cooper, 2001).

DN is characterized histologically by glomerular basement thickening and mesangial expansion related to the loss of renal function and clinically progressive albuminuria followed by a gradual decline in renal function (Mauer et al., 1984). Measures to prevent the appearance and progression of diabetic nephropathy should therefore be instituted as early as possible. Although adequate control of blood glucose levels may prevent the development of complications, it is difficult to achieve strict blood glucose control; thus, there is a yearly increase in the number of patients with diabetes (LeRoith and Rayfield, 2007). In addition to glucose, other metabolic factors, and in particular advanced glycation end products (AGEs), have been shown to be involved in the development of diabetic kidney disease. AGEs promote the deposition of extracellular matrix (ECM) in the glomeruli via the receptor for advanced glycation end products and abnormal glomerular remodeling in the kidney (Hori et al., 1996; Raptis and Viberti, 2001; Vlassara et al., 1994). The interaction between AGEs and the receptor for advanced glycation end products (RAGE) is important in the pathogenesis of DN because RAGE upregulation and activation might be involved in the accumulation of AGEs (Yonekura et al., 2005). Moreover, AGEs or high levels of glucose lead to increased production of extracellular matrix (ECM) proteins such as collagen IV via transforming growth factor β1 (TGF-β1) (Umezono et al., 2006). TGF-β1 takes part in the uptake of albumin by renal proximal tubule cells and in the development of albuminuria (Russo et al., 2007). TGF-β1 also stimulates the synthesis of matrix components such as collagen IV (Tahara et al., 2008), which is thought to be the major collagenous component of GBM and ECM (Dong et al., 2004). To prevent the development and progression of DN, effective therapies directed toward key molecular targets are required (Sharma and Srinivasan, 2009).

Natural products derived from plants have long been used in folk medicine, making the compounds derived from these plants good candidates for new therapeutic strategies (Cordell and Colvard, 2005; Kobayashi et al., 2010; Padmavathi et al., 2005). *Averrhoa carambola* L., commonly known as star fruit or carambola, is a plant originally from Asia that has become acclimatized to many tropical countries (Moresco et al., 2012). This plant is a small bush that can grow to 4–6 m in height (Vasconcelos et al., 2008). The leaves and fruits of *A. carambola* are commonly used to treat headaches, vomiting, coughing and hangovers (Carolino et al., 2005). Furthermore, it is used as an appetite stimulant, a diuretic, and as an antidiarrheal and febrifugal agent. Additionally, the extract obtained by decocting the leaves of *A. carambola* has been used in the treatment of diabetes (Ferreira et al., 2008). The dry root of *A. carambola* L., known as Yang Tao Gen in China, has also been used in traditional Chinese medicine for diabetic mellitus and its complications because it invigorates the kidney and reinforces yang. In a previous study, we reported that an alcoholic extract of the *A. carambola* L. root (ACLR) has a therapeutic potential in diabetes (Huan and Huan, 2009). Thus, we hypothesized that 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione (DMDD), which can be isolated from ACLR, may have a beneficial effect on the progression of DN. To the best of our knowledge, the mechanisms of DMDD against glucose-associated metabolic disorders in diabetes have yet to be explored. KKAy mice are considered to be a good animal model for the early pathological changes associated with diabetes (Chen et al., 2002). To determine whether DMDD has a strong effect on AGE formation in diabetes and/or diabetic nephropathy, we examined the effect of DMDD in type 2 diabetic KKAy mice and compared it with the effect of aminoguanidine, an inhibitor of AGE formation.

2. Materials and methods

2.1. Plant material and preparation of extract

DMDD were prepared as previously described (Wen et al., 2012) contained partial improvement. A powder from air-dried roots of *A. carambola* L. (12 kg) was extracted with 60% aq. EtOH under reflux 3 times (3 × 96 L, 1 h for each). The ethanol solution was concentrated under vacuum conditions to yield a syrup-like extract, which was suspended in H_2O and then extracted with cyclohexane (3 × 20 L), EtOAc (3 × 20 L) and n-BuOH (3 × 20 L).

The cyclohexane extract (200 g) was subjected to open silica gel CC (3 × 80 cm, 200–300 mesh) via successive elutions with a gradient of cyclohexane/EtOAc (100:0–0:100, each 200 mL) to afford 7 fractions (Frs. 1–7). Fr. 4 (5.0 g) was further separated by open silica gel CC (3 × 80 cm, 200–300 mesh) via successive elutions with a gradient of cyclohexane/EtOAc (100:0–15:1, each 100 mL), producing 4 sub-fractions (Frs. 41–44). Fr. 44 was re-crystallized with MeOH to yield 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione (2350 mg). This compound was identified by FTIR spectroscopy using a SpectrumOne Perkin-Elmer spectrophotometer and by ^1H and ^{13}C NMR analysis on a Bruker AV 600; the results were further compared with the literature (Schmalle et al., 1988). As a yellow-colored water-soluble powder, DMDD was dissolved in distilled water (2.5 mg/ml) before administration to the mice.

2.2. Animals

The experimental procedures and protocols used in this investigation were approved by the Ethical Committee for the Experimental Use of Animals at Guangxi Medical University (Guangxi, China). Twelve-week-old male KKAy mice and age-matched C57BL/6J mice (C57BL) (The Experimental Animal Center, Chinese Academy of Medical Science, Beijing) were housed in individual cages under controlled temperature (23 ± 1 °C) and humidity (55 ± 5%) on a 12:12 h light–dark cycle and were given standard rodent chow and free access to water, unless otherwise noted.

2.3. Acute toxicology test

Acute toxicology test in mice was performed according to the revised UP and Down method (OECD, 2008). Ten male C57BL/6J mice were used for the test. The mice were fasted (16 h) overnight and the body weight (g) of each mouse was recorded prior to the test. A fixed dose of DMDD (5000 mg/kg body weight) was administered orally to each mouse and observed closely at 4 h initially, then every 6 h intervals for changes in behavioral (alertness, restlessness, irritability, recumbence, vomiting, and fearfulness), neurological (spontaneous activity, convulsion, gait, bleeding orifices, and touch/pain response), autonomic (defecation and micturition) profiles, and/or mortality (Whishaw and Kolb, 2004). The volume of the extract was adjusted to contain 500 mg/ml, and any significant morbidity or mortality within 24–72 h was recorded.

2.4. Experimental design

The mice were divided into the following groups:

Group I ($n = 10$): C57BL/6J mice administered distilled water: normal control.

Group II ($n = 10$): C57BL/6J mice administered DMDD (50 mg/kg body weight) by oral gavage once a day for 8 weeks: DMDD control.

Group III ($n =$): KKAy mice administered distilled water: vehicle-treated KKAy.

Group IV ($n =$): KKAy mice administered DMDD (12.5 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD12.5.

Group V ($n = 10$): KKAy mice administered DMDD (25 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD25.

Group VI ($n = 10$): KKAy mice administered DMDD (50 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD50.

Group VII ($n = 10$): KKAy mice administered aminoguanidine (200 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + AG. The concentration of the aminoguanidine was adjusted to 10 mg/ml before administration to the mice.

2.5. Collection of blood, urine and tissues

Blood, urine and tissue biochemical parameters were determined after 8 weeks of DMDD treatment; 24 h urine samples were collected using metabolic cages, and whole blood was collected from the retro-orbital venous plexus with heparinized capillary tubes. The mice were killed humanely with an overdose of sodium pentobarbital (150 mg/kg), and the serum was immediately separated from the blood samples by centrifugation at 1300 × *g* for 10 min. The kidneys were surgically removed, washed with cold saline and stored at −80 °C until further analysis.

2.6. Biochemical index assays

Blood glucose levels were assessed using blood collected from the tail vein with a One-Touch Ultra blood glucose meter (Accu-check Performa, Roche, Germany). Blood urea nitrogen (BUN), albumin, total cholesterol, serum glycosylated protein,

serum creatinine (Cr), urary Cr, and 24-h urinary protein excretion were analyzed by the laboratory of the First Affiliated Hospital of Guangxi Medical University (Guangxi, China). Creatinine clearance (Ccr) was calculated using the following equation: Ccr (mL min^{-1} kg^{-1}) = [urinary Cr (mg dL^{-1}) × urinary volume (mL)/serum Cr (mg dL^{-1})] × [1000/body weight (g)] × [1/1440 (min)].

2.7. Renal histology assays

The renal samples were fixed in 4% paraformaldehyde, embedded with paraffin and cut into sections approximately 4 μm thick, followed by routine hematoxylin and eosin (HE) staining. The slides were examined under light microscopy with a magnification of 400× by a pathologist who was blind to the experimental profile.

2.8. Lipid peroxidation assays

MDA the renal cortex was determined using commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China) according to the methods described by Jain et al. (1989). Data are expressed as expressed as nmol/mg protein.

2.9. Assays for renal antioxidant enzyme activity

Total superoxide dismutase (SOD, EC 1.15.1.1) activity in kidney homogenates was evaluated using a commercial kit (Jiancheng Institute of Biotechnology, Nanjing, China) that measures the ability of the dismutase to inhibit the photochemical reduction of tetrazolium salt. The amount of inhibition was measured by monitoring the absorbance at 450 nm. The activity of GSH-Px (E.C.: 1.11.1.9) in kidney homogenates was determined using a commercial assay (Jiancheng Institute of Biotechnology, Nanjing, China) that is based on the oxidation of NADPH to NAD+, catalyzed by a limiting concentration of glutathione reductase. The progress of the reaction was monitored by measuring absorbance at 340 nm. Data are expressed as units (U) per mg of protein as compared with the standard.

2.10. Renal advanced glycation end product (AGE) assays

The renal AGE level was determined according to a previously described method with slight modifications (Nakayama et al., 1993). Specifically, minced kidney tissue was de-lipidated with chloroform and methanol (2:1, v/v) overnight. After washing, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000 × g for 15 min at 4 °C. The amount of AGEs in these alkali-soluble samples was determined by measuring the fluorescence at an emission wavelength of 440 nm and an excitation wavelength of 370 nm using a fluorescence spectrophotometer (F-4500, Hitachi, Japan). A native BSA preparation (1 mg/ml in 0.1 N NaOH) was used as a standard, and its fluorescence intensity was defined as one unit of fluorescence. The fluorescence of the samples was measured at a protein concentration of 1 mg mL^{-1} and expressed in AUs compared with the fluorescence of the native BSA preparation.

2.11. Protein extraction and Western blot analysis

The mice from all of the groups were sacrificed by cervical dislocation, and the kidneys were promptly removed. Protein extraction was performed as follows. The samples were homogenized in ice-cold lysis buffer (pH 7.5) (20 mM Tris–HCl, 137 mM NaCl, 1% Tween-20, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor mixture in DMSO). After centrifugation (2000 × g at 4 °C) to ensure equal loading among lanes, the protein concentration of each tissue lysate was determined by using a protein assay reagent (Bio-Rad). BSA was used as the standard. The lysates were then subjected to immunoblotting. To determine the amounts of RAGE, NF-κBp65, TGF-β1, and N$^{\varepsilon}$-(carboxymethyl)lysine (CML), 30 mg of each sample was electrophoresed through 8, 10, and 15% sodium dodecyl sulfate–polyacrylamide gels (SDS-PAGE). The separated proteins were transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 1 h, and incubated with primary antibodies against RAGE, NF-κBp65, TGF-β1, CML, and β-actin overnight at 4 °C. After the blots were washed, they were incubated with a goat anti-rabbit and/or goat anti-mouse horseradish peroxidase conjugated secondary IgG (Boster Biotechnology) for 90 min at room temperature. Photo-density analysis was performed with a gel image analysis system (UVP) after staining with diaminobenzidine, and densities were determined by Scion image software (Scion Corp., Frederick, MD) and quantified as the ratio to β-actin. The mean levels of these proteins in control mice were represented as 1, and the corresponding levels in KKAy mice were expressed as the ratios of these values.

2.12. Statistical analysis

All group values are expressed as the mean ± SE data were evaluated using the Sigma Stat (version 13.0) statistical analysis program (SPSS Inc, Chicago, IL, USA). Differences between groups were assessed using a one-way analysis of variance (ANOVA) with the Tukey's test for post hoc multiple comparisons. A P-value of less than 0.05 was considered statistically significant.

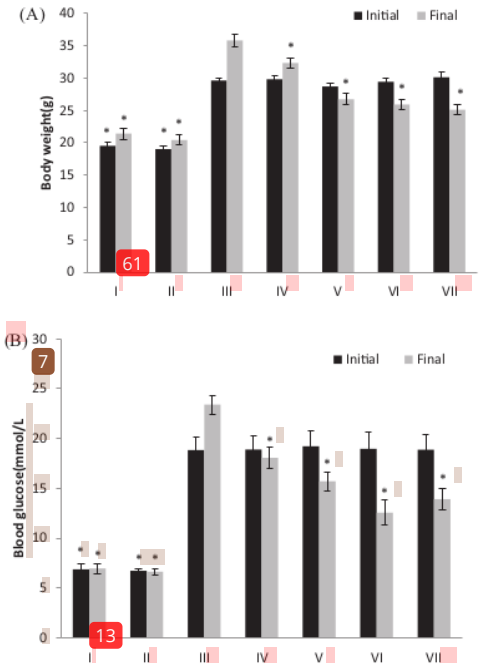


Fig. 1. Effect of DMDD on body weight (A), random blood glucose level (B), both were evaluated before (12 weeks) and at the end of 8-week (20 weeks) DMDD administration. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy + AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day, to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *P < 0.05 vs. vehicle-treated KKAy mice at the same age.

3. Results

3.1. Acute toxicology test of DMDD in mice

Acute toxicology test of DMDD was evaluated in mice at a single large dose of 5000 mg/kg body weight p.o. administered for 48 h. DMDD did not cause behavioral changes and no death was observed. The oral LD50 value of DMDD was greater than 5000 mg/kg bodyweight in mice and considered to be a practically non-toxic substance.

3.2. Body weight and blood glucose

The effects of DMDD on body weight and blood glucose levels in the animals are shown in Fig. 1. The body weight (Fig. 1A) of KKAy mice was significantly higher than that of C57BL/6J mice at 12 weeks of age, and the KKAy mice gained significantly more weight throughout the 8-week experimental period. Administration of DMDD to KKAy mice resulted in a significant decrease in weight gain. The effects were similar to those in AG group. Although the reduction in DMDD treated KKAy mice was dose dependent, it was not statistically significant. Whereas the weight gain in the DMDD treated C57BL/6J mice was not significantly different from the normal controls.

As shown in Fig. 1B, the fasting blood glucose (FBG) levels in C57BL/6J mice maintained constant during the experimental period

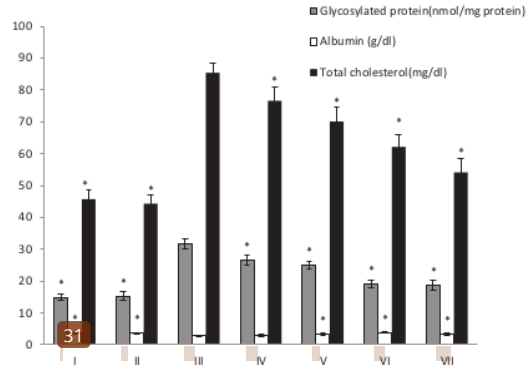


Fig. 2. Effect of DMDD treatment on serum parameters. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy+AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day, to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *$P < 0.05$ vs. vehicle-treated KKAy mice.

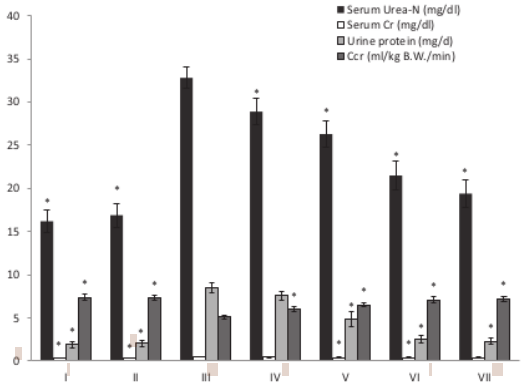


Fig. 3. Effect of DMDD treatment on renal function parameter. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy+AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day, to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *$P < 0.05$ vs. vehicle-treated KKAy mice.

and was significantly lower than KKAy mice before experiment at the beginning of the treatment, The degree of hyperglycemia in the KKAy mice increased significantly during the 8-week study period. DMDD treatment markedly attenuated the hyperglycemia in KKAy mice, and the effect appeared to be dose dependent. However, it did not affect the blood glucose level of the C57BL/6J mice.

3.3. Serum constituents

The effect of DMDD on the serum constituents of the mice are shown in Fig. 2. The levels of glycosylated protein and cholesterol in plasma were markedly higher and the albumin level was significantly lower in vehicle-treated KKAy mice than in C57BL/6J mice. After 8 weeks of administration, the levels of glycosylated protein in the plasma of the DMDD and aminoguanidine-treated KKAy mice were significantly lower than those in the vehicle-treated KKAy group. The albumin levels in the KKAy mice treated with DMDD at 12.5 mg/kg body weight/d for 8 weeks were somewhat higher than the corresponding value for the vehicle-treated group, but this difference was not statistically significant; however, the albumin levels of the KKAy mice treated with DMDD at 25 and 50 mg/kg body weight/d were significantly higher than those of the vehicle-treated KKAy mice. The total cholesterol level in the plasma of the DMDD and aminoguanidine treated KKAy mice were significantly lower than those in the vehicle-treated KKAy group. DMDD had no effect on the basal glycosylated protein, cholesterol and the albumin.

3.4. Renal function

The serum levels of Urea-N, Cr and 24-h urine protein were significantly higher and the Ccr level was significantly lower in the KKAy mice than in the C57BL/6J mice. The results showed that the serum levels of Urea-N, Cr and 24-h urine protein were obviously decreased after treatment with DMDD (Fig. 3); in addition, Ccr level was markedly increased after treatment. There were no significant differences in the levels of all previously listed renal functional parameters between the DMDD control group and normal control group.

3.5. Oxidant/antioxidant enzyme levels in the renal cortex

Oxidative stress was observed in the vehicle-treated KKAy mice, as indicated by a significant increase in MDA and reduced SOD and GSH-px activity in the renal cortex. Administration of variable doses of DMDD(12.5, 25, 50 mg/kg body weight/d, respectively) for 8 weeks lead to dose dependent elevation of plasma antioxidant activity in comparison to vehicle-treated KKAy group (Fig. 4). There were no significant differences in the activities of MDA, SOD and GSH-px between the DMDD control group and normal control group.

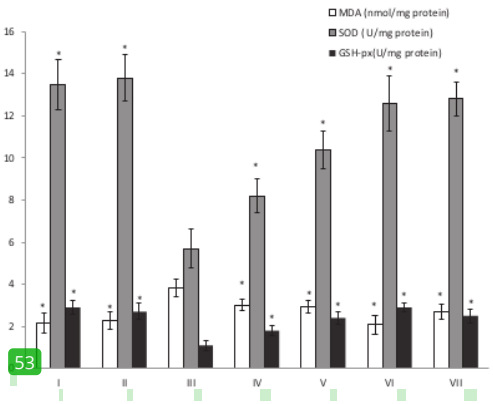


Fig. 4. Effect of DMDD treatment on oxidant/antioxidant enzyme activities in renal cortex. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy+AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day, to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *$P < 0.05$ vs. vehicle-treated KKAy mice.

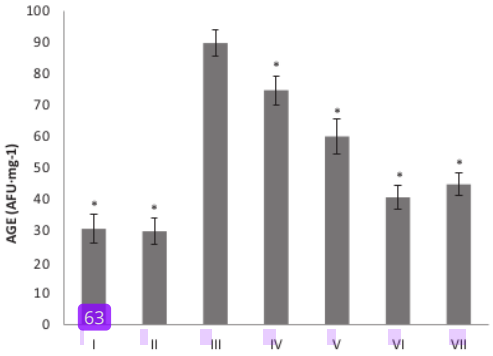


Fig. 5. Effect of DMDD treatment on the AGE level in renal cortex. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy+AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *$P < 0.05$ vs. vehicle-treated KKAy mice.

3.6. Renal AGE levels

The renal AGEs levels in the vehicle-treated KKAy mice were significantly higher than in the C57BL/6J mice, but they were effectively lowered by aminoguanidine and by DMDD. Additionally, DMDD reduced the renal AGE levels in a dose-dependent manner (Fig. 5). However, it did not affect the renal AGEs levels of the C57BL/6J mice.

3.7. Renal histopathology

H&E staining of the kidneys of KKAy mice revealed glomerular hypertrophy and expansion of the mesangial area and ECM. After the 8-week treatment with DMDD, glomerular hypertrophy and mesangial matrix accumulation in the KKAy mice were significantly inhibited (Fig. 6). In addition, the DMDD control group exhibited minimal variation in these histological changes in comparison to normal controls. In tandem, the histological studies of the kidneys of treated animals revealed less matrix expansion and glomerular basement membrane thickening compared to the KKAy mice.

3.8. Western blotting of renal cortex proteins

Fig. 7 depicts the RAGE, NF-κB, TGF-β1, and CML protein levels in the renal cortex. Based on the band densities, renal RAGE, NF-κB, TGF-β1 and CML were significantly elevated in the diabetic KKAy mice compared with the C57BL/6J mice. However, the altered expression of these proteins was significantly normalized in the KKAy mice by the oral administration of either DMDD or aminoguanidine. DMDD alone had no significant effect on the basal expression of RAGE, NF-κB, TGF-β1, and CML.

4. Discussion

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes and is a leading cause of end-stage renal disease (Fleetz and King, 2002). The pathogenesis of DN is multifactorial, and chronic hyperglycemia plays a crucial role (Kikkawa et al., 2003). During the course of diabetes, supraphysiological glucose increases the formation of AGEs and the production of free radicals by the mitochondria. Cell death and renal dysfunction occur as a result.

Consistent with an earlier report (Hayase et al., 1996), we found that the KKAy mice developed non-insulin-dependent diabetes mellitus spontaneously at 12 weeks of age. The KKAy mice were bigger and weighed significantly more than the C57BL/6J mice; the blood glucose level in the KKAy mice was significantly higher than in the C57BL/6J mice. In this study, we demonstrated that treatment with DMDD or aminoguanidine for 8 weeks significantly reduced hyperglycemia and body weight in type 2 diabetic KKAy mice. We conclude that DMDD has an anti-hyperglycemic effect that is correlated with a decrease in body weight.

Excretion of albumin in the urine has been demonstrated to be a good clinical predictor of renal lesions in DN (Parving et al., 2001; Viberti and Wheeldon, 2002). Many studies have shown that urine protein levels are associated with a graded increase in the risk of progression to end-stage renal disease and cardiovascular events (Lea et al., 2005). We observed an increase in the concentration of urinary albumin in the KKAy mice in the present study. This increase corresponds to the degree of hyperglycemia. Additionally, serum Cr and BUN levels and creatinine clearance, which are generally considered to be markers of renal function, were higher in the type 2 diabetic KKAy mice than in the C57BL/6J mice, implying the presence of diabetic kidney disease with renal hyperfiltration. DMDD-treated KKAy mice showed significant improvements in renal function, as indicated by urine protein, Serum Urea-N, and Ccr levels. However, it did not affect all previously listed renal functional parameters of the C57BL/6J mice.

A number of studies by important authors have shown that oxidative stress is a key pathogenic factor in the development of diabetic complications, including nephropathy (Busch et al., 2010; Giacco and Brownlee, 2010). It has been suggested that severe glucose-induced renal damage is associated with excessive levels of reactive oxygen species produced under hyperglycemic conditions (Jiang et al., 2010). Oxidants have direct biological effects that are connected to diabetic nephropathy (Koya et al., 2003). Thus, strategies to reduce oxidative stress in diabetes mellitus may have a favorable effect on the progression of diabetic glomerulosclerosis (Koya et al., 2003; Prabhakar et al., 2007). MDA content is a good index of increased oxidative stress in the tissues, as it reflects enhanced peroxidation processes (Kedziora-Kornatowska et al., 2002). Among the antioxidative enzymes, SOD catalyzes dismutation of the superoxide anion into hydrogen peroxide, whereas GSH-Px both detoxifies hydrogen peroxide and converts lipid hydroperoxides to nontoxic alcohols. In this study, the production of MDA was significantly enhanced, indicating an oxidative stress state in the type 2 diabetic KKAy mice. At the same time, SOD and GSH-Px activity were markedly reduced in the KKAy mice compared with the C57BL/6J mice. Furthermore, we found for the first time that this oxidative damage is suppressed by DMDD treatment in a dose-dependent manner. However, there were no significant differences in the activities of MDA, SOD and GSH-px between the DMDD control group and normal control group. These changes were attenuated by DMDD, suggesting that DMDD could be used to protect renal tissues against oxidative damage in diabetic nephropathy.

Much attention has been focused on exploring the mechanisms related to the development of DN. At present, several growth factors have been proposed to be involved in mediating the development of diabetic renal hypertrophy; among them, the multifunctional cytokine TGF-β1 is known to be upregulated in diabetic kidneys (Reeves and Andreoli, 2000). TGF-β1 plays an important role in ECM metabolism. Several studies have demonstrated that stimuli such as hyperglycemia, AGEs, and oxidative stress increase TGF-β1 expression (Forbes et al., 2007). TGF-β1 is believed to have a prominent role in the proliferation of mesangial cells and ECM

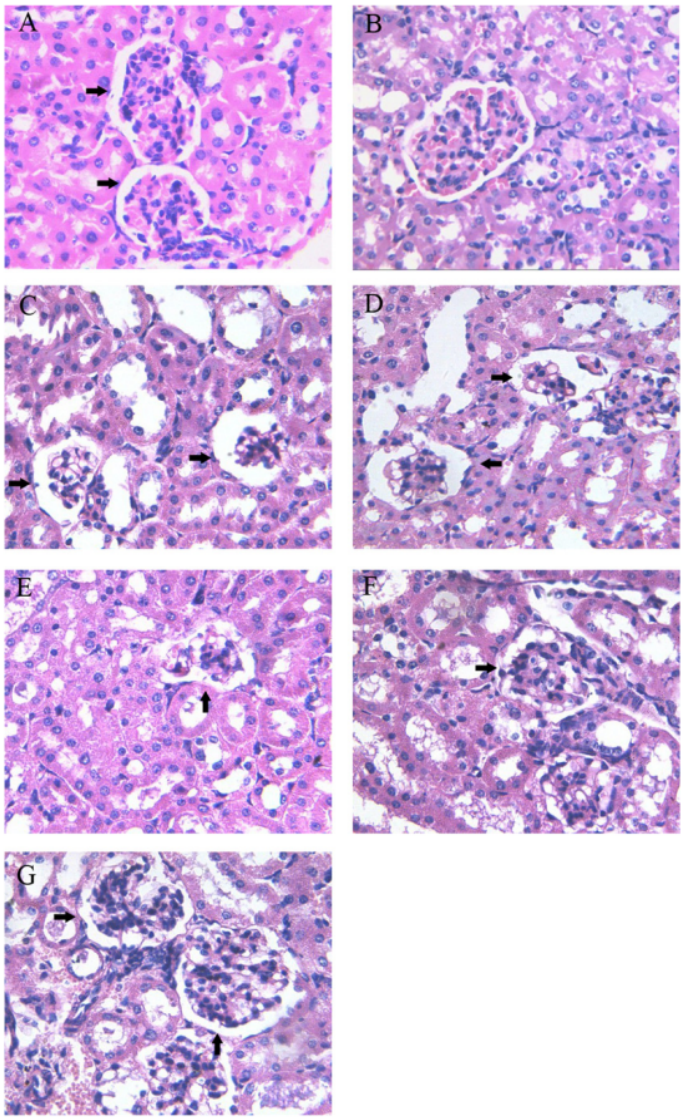


Fig. 6. Renal sections of the mice. Histology of kidneys was characterized by staining with hematoxylin–eosin staining (magnification: 400×). (A) normal control; (B) DMDD control; (C) vehicle-treated KKAy; (D) KKAy + DMDD12.5; (E) KKAy + DMDD25; (F) KKAy + DMDD50; and (G) KKAy + AG.

production, which are the major pathological changes in early diabetic nephropathy (Reeves and Andreoli, 2000). Thus, TGF-β1 has been considered as a therapeutic target in fibrotic diseases such as diabetic nephropathy and other chronic kidney diseases (McGowan et al., 2004). In this study, the expression of renal TGF-β1 was markedly inhibited by DMDD in KKAy mice. The accumulation of TGF-β1 in KKAy mice was inhibited by DMDD at 8 weeks after starting treatment. The finding that DMDD improved renal function in type 2 diabetic KKAy mice and that this beneficial effect was associated with an inhibitory effect of DMDD on kidney TGF-β1 overexpression is novel. Additionally, histological examination of the kidneys revealed that DMDD significantly attenuated diabetes-induced mesangial expansion.

Hyperglycemia, a chronic metabolic glucose disorder, results in irreversible tissue damage because of protein glycation, which leads to the formation of glycosylated protein and AGEs (Kanwar et al., 2008). The increase in glycosylated protein in the serum is caused by the reaction glucose and other reducing sugars such as ribose and fructose with the amino residues of proteins to form Amadori products such as glycosylated hemoglobin (HbA1c). Oxygen is also generated in the process of AGE formation (Daroux et al., 2010). AGE accumulation occurs earlier and at an accelerated rate in diabetes mellitus patients than it does in non-diabetic individuals (Schleicher et al., 1997). AGE formation appears to be synergistic with other pathogenic pathways in diabetes, including oxidative stress, hypertension, and activation of the renin-angiotensin system. Each of these pathways may be activated by AGEs, and each may promote the formation of AGEs (Thomas et al., 2005). AGEs can contribute to renal aging. Through AGE accumulation, in situ glycation and RAGE activation, glycation could enhance the

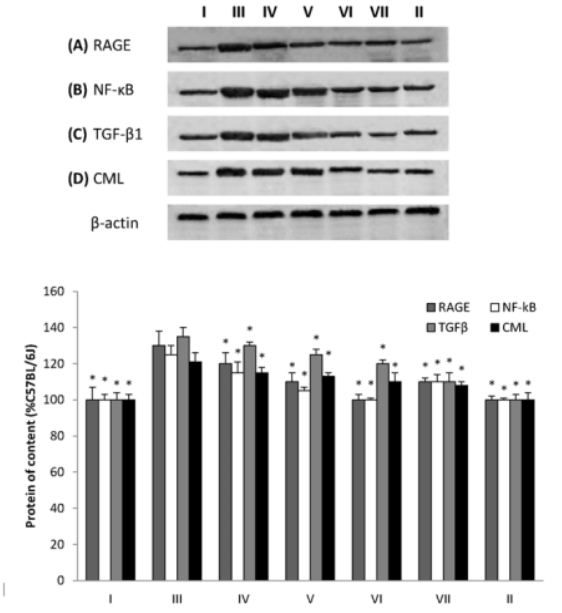


Fig. 7. Effect of DMDD and aminoguanidine on RAGE (A), NF-κB (B), TGF-β1 (C), and CML (D) protein expression in renal cortex. Group I: normal control, C57BL/6J mice administered distilled water; Group II: DMDD control, C57BL/6J mice administered DMDD (50 mg/kg body weight); Group III: vehicle-treated KKAy, KKAy mice administered distilled water; Groups IV–VI: DMDD treatment, KKAy mice administered DMDD (12.5, 25, 50 mg/kg body weight); and Group VII: KKAy + AG: KKAy mice administered aminoguanidine (200 mg/kg body weight). DMDD or aminoguanidine were given by oral gavage at the indicated dosage, once a day, to the separate groups of the mice. Results are expressed as means ± SE for 10 animals in each group. *$P < 0.05$ vs. vehicle-treated KKAy mice.

physiological and pathological effects of renal aging (Daroux et al., 2010). It has been reported that AGEs trigger the activation of NF-κB via interaction with the AGE receptor (RAGE), leading to its translocation to the nucleus, where it induces transcription (Yan et al., 1994). Additionally, the promoter region of the RAGE gene contains NF-κB binding sites (Li and Schmidt, 1997), potentially producing a self-perpetuating pathway. Moreover, the AGE-RAGE interaction activates TGF-β1 signaling pathways and subsequently induces mesangial cell hypertrophy and glomerular sclerosis by ECM synthesis (Kanwar et al., 2008). The interactions between AGEs and RAGE induce the activation of oxidative stress and stimulate the production and release of cytokines, which amplify tissue damage (Wautier and Guillausseau, 2002). Therefore, AGE accumulation in the kidney has been regarded as an index of progressive renal damage in diabetic nephropathy. In the present study, not only the overexpression of AGEs and RAGE but also the higher levels of NF-κB and TGF-β1 in the kidneys of type 2 diabetic KKAy mice were attenuated by DMDD treatment for 8 weeks. It seems that DMDD influenced not only AGE-RAGE signaling but also the NF-κB-TGF-β1-dependent pathway to some extent, thus leading to an attenuation of renal damage caused by protein glycation.

CML, pentosidine, and methylglyoxal derivatives are among the well-characterized compounds that are commonly used as markers of AGE (Chappey et al., 2003). CML is not only a glycoxidation product that is similar to pentosidine, but it is also formed during the metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein (Fu et al., 1996). Therefore, CML could serve as a general biomarker of oxidative stress resulting from carbohydrate and lipid oxidation reactions. We found that treatment of type 2 diabetic KKAy mice with DMDD for 8 weeks not only lowered the level of renal CML but also decreased the accumulation of lipid peroxidation products in kidney. The results indicate that the beneficial effect of DMDD on diabetic nephropathy may be linked to the reduction of oxidative stress.

In conclusion, we discovered that 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione had an antidiabetic effect via a reduction in hyperglycemia, which attenuated AGE expression and downregulated the NF-κB-TGF-β1 pathway in diabetic glomeruli, consequently decreasing ECM deposition in renal tissues. Hence, DMDD could be used as dietary supplement in the management of renal impairment associated with chronic diabetes infirmities.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81160533); Guangxi Natural Science Foundation (Nos. 2012GXNSFAA053106 and 0832013Z); and Education Innovation Plan Program for Postgraduate in Guangxi Zhuang Autonomous Region (No. 2011105981002D27).

References

Busch, M., Franke, S., Rüster, C., Wolf, G., 2010. Advanced glycation end-products and the kidney. European Journal of Clinical Investigation 40 (8), 742–755.
Carolino, R.O.G., Beleboni, R.O., Pizzo, A.B., Vecchio, F.D., Garcia-Cairasco, N., Moyses-Neto, M., Santos, W.F., Coutinho-Netto, J., 2005. Convulsant activity and neurochemical alterations induced by a fraction obtained from fruit *Averrhoa carambola* (Oxalidaceae: Geraniales). Neurochemistry International 46 (7), 523–531.
Chappey, O., Dosquet, C., Wautier, M.P., Wautier, J.L., 2003. Advanced glycation end products, oxidant stress and vascular lesions. European Journal of Clinical Investigation 27 (2), 97–108.
Chen, L., Li, X., Huang, L., Li, Y., Duan, L., Zhang, X., 2002. The early pathological changes of KKAy mice with type 2 diabetes (Zhongguo yi xue ke xue yuan xue bao). Acta Academiae Medicinae Sinicae 24 (1), 71.
Cooper, M., 2001. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 44 (11), 1957–1972.
Cooper, M.E., 2004. Importance of advanced glycation end products in diabetes-associated cardiovascular and renal disease. American Journal of Hypertension 17, 31S–38S.
Cordell, G.A., Colvard, M.D., 2005. Some thoughts on the future of ethnopharmacology. Journal of Ethnopharmacology 100 (1), 5–14.
Daroux, M., Prévost, G., Maillard-Lefebvre, H., Gaxatte, C., D'Agati, V., Schmidt, A., Boulanger, E., 2010. Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. Diabetes & Metabolism 36 (1), 1–10.
Dong, F., Li, H., Cai, W., Tao, J., Li, Q., Ruan, Y., Zheng, F.P., Zhang, Z., 2004. Effects of pioglitazone on expressions of matrix metalloproteinases 2 and 9 in kidneys of diabetic rats. Chinese Medical Journal-Beijing-English Edition 117 (7), 1040–1044.
Ferreira, E.B., Fernandes, L.C., Galende, S.B., Cortez, D.A.G., Bazotte, R.B., 2008. Hypoglycemic effect of the hydroalcoholic extract of leaves of *Averrhoa carambola* L. (Oxalidaceae). Revista Brasileira de Farmacognosia 18 (3), 339–343.
Forbes, J., Fukami, K., Cooper, M., 2007. Diabetic nephropathy: where hemodynamics meets metabolism. Experimental and Clinical Endocrinology and Diabetes 115 (2), 69–84.
Fu, M.X., Requena, J.R., Jenkins, A.J., Lyons, T.J., Baynes, J.W., Thorpe, S.R., 1996. The advanced glycation end product, N-(carboxymethyl) lysine, is a product of both lipid peroxidation and glycoxidation reactions. Journal of Biological Chemistry 271 (17), 9982–9986.
Giacco, F., Brownlee, M., 2010. Oxidative stress and diabetic complications. Circulation Research 107 (9), 1058–1070.
Hayase, M., Ogawa, Y., Katsuura, G., Shintaku, H., Hosoda, K., Nakao, K., 1996. Regulation of obese gene expression in KK mice and congenic lethal yellow obese KKAy mice. American Journal of Physiology-Endocrinology and Metabolism 271 (2), E333–E339.
Hori, O., Du Yan, S., Ogawa, S., Kuwabara, K., Matsumoto, M., Stern, D., Schmidt, A.M., 1996. The receptor for advanced glycation end-products has a central role in mediating the effects of advanced glycation end-products on the development of vascular disease in diabetes mellitus. Nephrology Dialysis Transplantation 11 (Suppl. 5), 13–16.
Huang, G., Huang, R., 2009. Effects of alcoholic extracts of *Averrhoa carambola* L. root on blood glucose level and lipid peroxidation in diabetic mice. Lishizhen Medicine and Materia Medica Research 11, 038.

Jain, S.K., McVie, R., Duett, J., Herbst, J.J., 1989. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 38 (12), 1539–1543.
Jiang, T., Huang, Z., Lin, Y., Zhang, Z., Fang, D., Zhang, D.D., 2010. The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. Diabetes 59 (4), 850–860.
Kanwar, Y.S., Wada, J., Sun, L., Xie, P., Wallner, E.I., Chen, S., Chugh, S., Danesh, F.R., 2008. Diabetic nephropathy: mechanisms of renal disease progression. Experimental Biology and Medicine 233 (1), 4–11.
Kedziora-Kornatowska, K., Szram, S., Kornatowski, T., Szadujkis-Szadurski, L., Kedziora, J., Bartosz, G., 2002. The effect of verapamil on the antioxidant defence system in diabetic kidney. Clinica Chimica Acta; International Journal of Clinical Chemistry 322 (1–2), 105.
Kikkawa, R., Koya, D., Haneda, M., 2003. Progression of diabetic nephropathy. American Journal of Kidney Diseases 41 (3; Suppl. 1), 19–21.
Kobayashi, H., Ishii, M., Takeuchi, S., Tanaka, Y., Shintani, T., Yamatodani, A., Kusunoki, T., Furue, M., 2010. Efficacy and safety of a traditional herbal medicine. Hochu-ekki-to in the long-term management of Kikyo (Delicate Constitution) patients with atopic dermatitis: a 6-month, multicenter, double-blind, randomized, placebo-controlled study. Evidence-based Complementary and Alternative Medicine 7 (3), 367–373.
Koya, D., Hayashi, K., Kitada, M., Kashiwagi, A., Kikkawa, R., Haneda, M., 2003. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. Journal of the American Society of Nephrology 14 (Suppl. 3), S250–S253.
Lea, J., Greene, T., Hebert, L., Lipkowitz, M., Massry, S., Middleton, J., Rostand, S.G., Miller, E., Smith, W., Bakris, G.L., 2005. The relationship between magnitude of proteinuria reduction and risk of end-stage renal disease: results of the African American study of kidney disease and hypertension. Archives of Internal Medicine 165 (8), 947.
LeRoith, D., Rayfield, E.J., 2007. The benefits of tight glycemic control in type 2 diabetes mellitus. Clinical Cornerstone 8, S19–S29.
Li, J., Schmidt, A.M., 1997. Characterization and functional analysis of the promoter of RAGE, the receptor for advanced glycation end products. Journal of Biological Chemistry 272 (26), 16498–16506.
Mauer, S.M., Steffes, M.W., Ellis, E.N., Sutherland, D., Brown, D.M., Goetz, F.C., 1984. Structural–functional relationships in diabetic nephropathy. Journal of Clinical Investigation 74 (4), 1143.
McGowan, T.A., Zhu, Y., Sharma, K., 2004. Transforming growth factor-beta: a clinical target for the treatment of diabetic nephropathy. Current Diabetes Reports 4 (6), 447–454.
Moresco, H.H., Queiroz, G.S., Pizzolatti, M.G., Brighente, I., 2012. Chemical constituents and evaluation of the toxic and antioxidant activities of *Averrhoa carambola* leaves. Revista Brasileira de Farmacognosia 22 (2), 319–324.
Nakayama, H., Mitsuhashi, T., Kuwajima, S., Aoki, S., Kuroda, Y., Itoh, T., Nakagawa, S., 1993. Immunochemical detection of advanced glycation end products in lens crystallins from streptozocin-induced diabetic rat. Diabetes 42 (2), 345–350.
OECD, 2008. Organization for Economic Co-operation and Development (OECD). OECD Guidelines for the Testing of Chemicals. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure (October 2008) Available at http://lysander.sourceoecd.org/. (accessed 15.8.09).
Padmavathi, B., Rath, P.C., Rao, A.R., Singh, R.P., 2005. Roots of Withania somnifera inhibit forestomach and skin carcinogenesis in mice. Evidence-based Complementary and Alternative Medicine 2 (1), 99–105.
Parving, H.H., Lehnert, H., Bröchner-Mortensen, J., Gomis, R., Andersen, S., Arner, P., 2001. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. New England Journal of Medicine 345 (12), 870–878.
Prabhakar, S., Starnes, J., Shi, S., Lonis, B., Tran, R., 2007. Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. Journal of the American Society of Nephrology 18 (11), 2945–2952.
Raptis, A., Viberti, G., 2001. Pathogenesis of diabetic nephropathy. Experimental and Clinical Endocrinology and Diabetes 109, 424–437.
Reeves, W.B., Andreoli, T.E., 2000. Transforming growth factor β contributes to progressive diabetic nephropathy. Proceedings of the National Academy of Sciences 97 (14), 7667–7669.
Russo, L.M., del Re, E., Brown, D., Lin, H.Y., 2007. Evidence for a role of transforming growth factor (TGF)-β1 in the induction of postglomerular albuminuria in diabetic nephropathy. Diabetes 56 (2), 380–388.
Schleicher, E.D., Wagner, E., Nerlich, A.G., 1997. Increased accumulation of the glycoxidation product N (epsilon)-(carboxymethyl) lysine in human tissues in diabetes and aging. Journal of Clinical Investigation 99 (3), 457.
Schmalle, H.W., Hanggi, G., Dubler, E., 1988. Acta Crystallographica Section C: Crystal Structure Communications. Structure of hypoxanthine 44 (4), 732–736.
Sharma, A.K., Srinivasan, B., 2009. Triple verses glimepiride plus metformin therapy on cardiovascular risk biomarkers and diabetic cardiomyopathy in insulin resistance type 2 diabetes mellitus rats. European Journal of Pharmaceutical Sciences 38 (5), 433–444.
Sheetz, M.J., King, G.L., 2002. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. JAMA: Journal of the American Medical Association 288 (20), 2579–2588.
Tahara, A., Tsukada, J., Tomura, Y., Yatsu, T., Shibasaki, M., 2008. Vasopressin increases type IV collagen production through the induction of transforming growth factor-beta secretion in rat mesangial cells. Pharmacological Research 57 (2), 142–150.
Thomas, M.C., Forbes, J.M., Cooper, M.E., 2005. Advanced glycation end products and diabetic nephropathy. American Journal of Therapeutics 12 (6), 562–572.
Umezono, T., Toyoda, M., Kato, M., Miyauchi, M., Kimura, M., Maruyama, M., Honma, M., Yagame, M., Suzuki, D., 2006. Glomerular expression of CTGF, TGF-beta 1 and type IV collagen in diabetic nephropathy. Journal of Nephrology 19 (6), 751.
Vasconcelos, C.M.L., Gondim, A.N.S., Cruz, J.S., Mafra, R.A., Silva, B.A., Conde-Garcia, E.A., 2008. Aqueous leaf extract of *Averrhoa carambola* L. (Oxalidaceae) reduces both the inotropic effect of BAYK 8644 on the guinea pig atrium and the calcium current on GH3cells. Revista Brasileira de Farmacognosia 18 (4), 539–543.
Viberti, G., Wheeldon, N.M., 2002. Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus. Circulation 106 (6), 672–678.
Vlassara, H., Bucala, R., Striker, L., 1994. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Laboratory Investigation; A Journal of Technical Methods and Pathology 70 (2), 138.
Wautier, J., Guillausseau, P., 2002. Advanced glycation end products, their receptors and diabetic angiopathy. Journal of the Peripheral Nervous System 7 (2), 138.
Wen, Q., Lin, X., Liu, Y., Xu, X., Liang, T., Zheng, N., Huang, R., 2012. Phenolic and lignan glycosides from the butanol extract of *Averrhoa carambola* L. root. Molecules 17 (10), 12330–12340.
Whishaw, I.Q., Kolb, B., 2004. The Behavior of the Laboratory Rat: A Handbook with Tests. Oxford University Press, USA.
Yan, S.D., Schmidt, A.M., Anderson, G.M., Zhang, J., Brett, J., Zou, Y.S., Pinsky, D., Stern, D., 1994. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. Journal of Biological Chemistry 269 (13), 9889–9897.
Yonekura, H., Yamamoto, Y., Sakurai, S., Watanabe, T., Yamamoto, H., 2005. Roles of the receptor for advanced glycation endproducts in diabetes-induced vascular injury. Journal of Pharmacological Sciences 97 (3), 305–311.

HASIL CEK20_60010366

ORIGINALITY REPORT

19%	10%	14%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Jianchun Huang, Xudong Zhang, Feizhang Qin, Yingxin Li, Xiaoqun Duan, Jie Jian, Yongwen Li, Jian Chen, Renbin Huang. "Protective Effects of ***Millettia Pulchra*** Flavonoids on Myocardial Ischemia ***In Vitro*** and ***In Vivo***", Cellular Physiology and Biochemistry, 2015 Publication	1%
2	A. Tsuchida, T. Yamauchi, S. Takekawa, Y. Hada, Y. Ito, T. Maki, T. Kadowaki. "Peroxisome Proliferator-Activated Receptor (PPAR) Activation Increases Adiponectin Receptors and Reduces Obesity-Related Inflammation in Adipose Tissue: Comparison of Activation of PPAR , PPAR , and Their Combination", Diabetes, 2005 Publication	<1%
3	Submitted to Higher Education Commission Pakistan Student Paper	<1%
4	Rong Li, Tao Liang, Yongwen Li, Weizhe Jiang, Renbin Huang. "Effects of l-dopa methyl ester	<1%

on visual cortex injury induced by amblyopia and its underlying mechanism", Neuroscience Letters, 2012
Publication

---

5 Chao Guo, Tao Liang, Qiaoling He, Pingyuan Wei, Ni Zheng, Lingyuan Xu. "Renoprotective effect of ramulus mori polysaccharides on renal injury in STZ-diabetic mice", International Journal of Biological Macromolecules, 2013 <1%
Publication

---

6 Iwashima, Y.. "Advanced Glycation End Product-Induced Peroxisome Proliferator-Activated Receptor @c Gene Expression in the Cultured Mesangial Cells", Biochemical and Biophysical Research Communications, 19991022 <1%
Publication

---

7 www.pure.ed.ac.uk <1%
Internet Source

---

8 Shu-Dong Wei, Hui Chen, Ting Yan, Yi-Ming Lin, Hai-Chao Zhou. "Identification of antioxidant components and fatty acid profiles of the leaves and fruits from Averrhoa carambola", LWT - Food Science and Technology, 2014 <1%
Publication

Submitted to University of Liverpool

9	Student Paper	<1%
10	d-scholarship.pitt.edu Internet Source	<1%
11	estudogeral.sib.uc.pt Internet Source	<1%
12	Hiroki Kumagai, Naoto Kubota, Tetsuya Kubota, Takehiro Takahashi et al. "Combined treatment with low-dose pioglitazone and beraprost sodium improves glucose intolerance without causing body weight gain", Diabetology International, 2013 Publication	<1%
13	s3.amazonaws.com Internet Source	<1%
14	He-lin DING, Ying GUO, Ming-tong XU, Hai-yan LI, Zu-zhi FU. "Effect of angiotensin II receptor blocker on glucose-induced mRNA expressions of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in rat mesangial cells", Chinese Medical Journal, 2007 Publication	<1%
15	Yang Li. "Matrine Induces Apoptosis in Angiotensin II-Stimulated Hyperplasia of Cardiac Fibroblasts: Effects on Bcl-2/Bax	<1%

Expression and Caspase-3 Activation", Basic & Clinical Pharmacology & Toxicology, 7/2007
Publication

16	coek.info Internet Source	<1%
17	edepot.wur.nl Internet Source	<1%
18	www.researchpublish.com Internet Source	<1%
19	Chiu, J.. "Curcumin prevents diabetes-associated abnormalities in the kidneys by inhibiting p300 and nuclear factor-@kB", Nutrition, 200909 Publication	<1%
20	T. Jono. "N -(Carboxymethyl)lysine and 3-DG-Imidazolone Are Major AGE Structures in Protein Modification by 3-Deoxyglucosone", Journal of Biochemistry, 09/01/2004 Publication	<1%
21	Tunez, I.. "Protective effect of carvedilol on oxidative stress induced by okadaic acid in N1E-115 cells", Pharmacological Research, 200609 Publication	<1%
22	Ugochukwu, N.H.. "Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated	<1%

with extracts from Gongronema latifolium leaves", Clinica Chimica Acta, 200310
Publication

23	minerva-access.unimelb.edu.au Internet Source	<1 %
24	tobacco.cleartheair.org.hk Internet Source	<1 %
25	Ploj, K.. "Basal levels and alcohol-induced changes in nociceptin/orphanin FQ, dynorphin, and enkephalin levels in C57BL/6J mice", Brain Research Bulletin, 20000915 Publication	<1 %
26	Qing-Hua, G.. "The kidney expression of matrix metalloproteinase-9 in the diabetic nephropathy of Kkay mice", Journal of Diabetes and Its Complications, 200811/12 Publication	<1 %
27	Xue, W.. "High selenium status in individuals exposed to arsenic through coal-burning in Shaanxi (PR of China) modulates antioxidant enzymes, heme oxygenase-1 and DNA damage", Clinica Chimica Acta, 20100906 Publication	<1 %
28	Huang, Jianchun, Vanphuc Nguyen, Xiaojun Tang, Jinbin Wei, Xing Lin, Zefeng Lai, Vanminh Doan, Qiuqiao Xie, and Renbin Huang. "Protection from diclofenac-induced liver	<1 %

injury by Yulangsan polysaccharide in a mouse model", Journal of Ethnopharmacology, 2016.
Publication

29	Viberti, G.. "Thiazolidinediones-Benefits on microvascular complications of type 2 diabetes", Journal of Diabetes and Its Complications, 200505/06 Publication	<1 %
30	Ziqi Dong, Hui Gong, Yadan Chen, Hong Wu, Jun Wu, Yinghong Deng, Xinmao Song. "LH-21, A Peripheral Cannabinoid Receptor 1 Antagonist, Exerts Favorable Metabolic Modulation Including Antihypertensive Effect in KKAy Mice by Regulating Inflammatory Cytokines and Adipokines on Adipose Tissue", Frontiers in Endocrinology, 2018 Publication	<1 %
31	pie.net.pl Internet Source	<1 %
32	www.jci.org Internet Source	<1 %
33	Ming Xiang, Jing Tang, Xiao-Lei Zou, Zeng-Yu Zhao, Yun-Yang Wang, Sheng-Nan Xie. " β Cell Protecting and Immunomodulatory Activities of Mycelium in STZ Induced T1DM Mice ", The American Journal of Chinese Medicine, 2012 Publication	<1 %

34	Schlussman, S.D.. "Locomotion, stereotypy, and dopamine D"1 receptors after chronic ''binge'' cocaine in C57BL/6J and 129/J mice", Pharmacology, Biochemistry and Behavior, 200304 Publication	<1 %
35	Submitted to University of Sheffield Student Paper	<1 %
36	sasas.co.za Internet Source	<1 %
37	Hao Tan, Wenjie Xu, Xiaoqian Ding, Huayu Ye, Yun Hu, Xinyi He, Ye Ming, Leilei Zheng. "Notch/NICD/RBP-J Signaling Axis Regulates M1 Polarization of Macrophages Mediated by Advanced Glycation End Products", Research Square Platform LLC, 2021 Publication	<1 %
38	Submitted to Kaohsiung Medical University Student Paper	<1 %
39	Submitted to Naresuan University Student Paper	<1 %
40	Submitted to University of Cape Town Student Paper	<1 %
41	Submitted to University of Sydney Student Paper	<1 %
	journals.sagepub.com	

42	Internet Source	<1%
43	repositorio.chlc.min-saude.pt Internet Source	<1%
44	www.cell.com Internet Source	<1%
45	Jun Peng, Yi Huan, Qian Jiang, Su-juan Sun, Chun-ming Jia, Zhu-fang Shen. "Effects and Potential Mechanisms of Pioglitazone on Lipid Metabolism in Obese Diabetic KKAy Mice", PPAR Research, 2014 Publication	<1%
46	glycob.oxfordjournals.org Internet Source	<1%
47	repository.dl.itc.u-tokyo.ac.jp Internet Source	<1%
48	www.americasquarterly-digital.org Internet Source	<1%
49	Huang, Hong, Ying Jiang, Genxiang Mao, Fang Yuan, Hexin Zheng, Yuan Ruan, and Tianfeng Wu. "Protective effects of allicin on streptozotocin-induced diabetic nephropathy in rats", Journal of the Science of Food and Agriculture, 2016. Publication	<1%

50	Santosh Kumar. "Catecholamine-induced myocardial fibrosis and oxidative stress is attenuated by Terminalia arjuna (Roxb.)", Journal of Pharmacy and Pharmacology, 11/2009 Publication	<1 %
51	Wang, Jun-Feng, Yi-Hui Deng, Sheng-Hui Yang, Yan-Qun Liu, Yue-Hu Wang, Wei-Wei Pan, and Xiao-Jiang Zhou. "Characterization and biological evaluation of six new dimeric lignans with an unusual α,β-unsaturated ketone motif from Zanthoxylum simulans", Bioorganic & Medicinal Chemistry Letters, 2014. Publication	<1 %
52	cdn.publisher.gn1.link Internet Source	<1 %
53	scholarworks.uvm.edu Internet Source	<1 %
54	www.ifrj.upm.edu.my Internet Source	<1 %
55	www.researchsquare.com Internet Source	<1 %
56	Brian Siu, Jharna Saha, William E Smoyer, Kelli A Sullivan, Frank C Brosius. "Reduction in podocyte density as a pathologic feature in	<1 %

early diabetic nephropathy in rodents: Prevention by lipoic acid treatment", BMC Nephrology, 2006
Publication

---

57 Tahara, A.. "Vasopressin increases type IV collagen production through the induction of transforming growth factor-beta secretion in rat mesangial cells", Pharmacological Research, 200802
Publication

<1 %

---

58 W. D. Comper. "Disease-dependent mechanisms of albuminuria", AJP Renal Physiology, 06/25/2008
Publication

<1 %

---

59 Yan Yang, Jie Li, Lingqiang Zhang, Zeyuan Lin, Haiming Xiao, Xiaohong Sun, Meng Zhang, Peiqing Liu, Heqing Huang. "CKIP-1 acts downstream to Cx43 on the activation of Nrf2 signaling pathway to protect from renal fibrosis in diabetes", Pharmacological Research, 2020
Publication

<1 %

---

60 Yihui Liu, Luosheng Wan, Zuoqi Xiao, Jingjing Wang, Yonglong Wang, Jiachun Chen. " Antidiabetic Activity of Polysaccharides from Tuberous Root of var. in KKAy Mice ", Evidence-Based Complementary and Alternative Medicine, 2013
Publication

<1 %

61	discovery.ucl.ac.uk Internet Source	<1 %
62	journals.physiology.org Internet Source	<1 %
63	repository.medri.uniri.hr Internet Source	<1 %
64	ugspace.ug.edu.gh Internet Source	<1 %
65	www.biomed.cas.cz Internet Source	<1 %
66	www.onlinepbe.com Internet Source	<1 %
67	Cai, Y.. "Zhen-wu-tang, a blended traditional Chinese herbal medicine, ameliorates proteinuria and renal damage of streptozotocin-induced diabetic nephropathy in rats", Journal of Ethnopharmacology, 20100819 Publication	<1 %
68	D. Ziegler. "Current Evidence For Treating Diabetic Neuropathy", Journal of the Peripheral Nervous System, 2001 Publication	<1 %
69	Fenqin Chen, Ning Zhang, Xiaoyu Ma, Ting Huang, Ying Shao, Can Wu, Qiuyue Wang.	<1 %

"Naringin Alleviates Diabetic Kidney Disease through Inhibiting Oxidative Stress and Inflammatory Reaction", PLOS ONE, 2015
Publication

---

70 I Young. "The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat", Free Radical Biology and Medicine, 1992 <1%
Publication

---

71 John M. Ong, Nadia C. Zorapapel, Annette M. Aoki, Donald J. Brown, Anthony B. Nesburn, Kathryn A. Rich, M. Cristina Kenney. "Impaired electroretinogram (ERG) response in apolipoprotein E-deficient mice", Current Eye Research, 2009 <1%
Publication

---

72 Papandrea, D.. "Dissociation of seizure traits in inbred strains of mice using the flurothyl kindling model of epileptogenesis", Experimental Neurology, 200901 <1%
Publication

---

73 S Li. "Urocortin ameliorates diabetic nephropathy in obese db/db mice", British Journal of Pharmacology, 07/2008 <1%
Publication

---

74 Tina Soulis. "Effects of aminoguanidine in preventing experimental diabetic <1%

	nephropathy are related to the duration of treatment", Kidney International, 08/1996 Publication	
75	Zia A. Khan, Hana Farhangkhoee, Yousef P. Barbin, Paul C. Adams, Subrata Chakrabarti. "Glucose-induced regulation of novel iron transporters in vascular endothelial cell dysfunction", Free Radical Research, 2009 Publication	<1 %
76	abfmc.net Internet Source	<1 %
77	deepblue.lib.umich.edu Internet Source	<1 %
78	e-space.mmu.ac.uk Internet Source	<1 %
79	pesquisa.bvsalud.org Internet Source	<1 %
80	staff.usc.edu.eg Internet Source	<1 %
81	www.jicosh.gr.jp Internet Source	<1 %
82	www.nice.org.uk Internet Source	<1 %
83	www.unn.edu.ng Internet Source	<1 %

84	G BASTA. "Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes", Cardiovascular Research, 09/2004 Publication	<1 %
85	"AES Proceedings", Epilepsia, 08/20/2003 Publication	<1 %
86	"Handbook of dietary and nutritional aspects of bottle feeding", Wageningen Academic Publishers, 2014 Publication	<1 %
87	Chunxia Chen, Zhihuan Nong, Jiangchun Huang, Zhaoni Chen, Shijun Zhang, Yang Jiao, Xiaoyu Chen, Renbin Huang. "Yulangsan polysaccharide attenuates withdrawal symptoms and regulates the NO pathway in morphine-dependent rats", Neuroscience Letters, 2014 Publication	<1 %
88	Lei Lei, Shuainan Liu, Yongqiang Li, Huipeng Song, Lianchao He, Quan Liu, Sujuan Sun, Yan Li, Zhiqiang Feng, Zhufang Shen. "The potential role of glucokinase activator SHP289-04 in anti-diabetes and hepatic protection", European Journal of Pharmacology, 2018 Publication	<1 %

89	Li, W.. "Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus", Journal of Ethnopharmacology, 200405 Publication	<1%
90	www.imedpub.com Internet Source	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		