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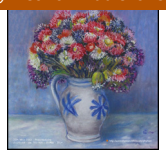

Kolaviron, a biflavonoid complex of *Garcinia kola* seeds modulates apoptosis by suppressing oxidative stress and inflammation in diabetes-induced nephrotoxic rats

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ABSTRACT

Diabetic nephropathy is a complex disease that involves increased production of free radicals which is a strong stimulus for the release of pro-inflammatory factors. We evaluated the renal protective effect of kolaviron (KV) – a *Garcinia kola* seed extract containing a mixture of 5 flavonoids, in diabetes-induced nephrotoxic rats. Male Wistar rats were divided into 4 groups: untreated controls (C); normal rats treated with kolaviron (C + KV); untreated diabetic rats (D); kolaviron treated diabetic rats (D + KV). A single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) was used for the induction of diabetes. Renal function parameters were estimated in a clinical chemistry analyzer. Markers of oxidative stress in the kidney homogenate were analyzed in a Multiskan Spectrum plate reader and Bio-plex Promagnetic bead-based assays was used for the analysis of inflammatory markers. The effect of kolaviron on diabetes-induced apoptosis was assessed by TUNEL assay. In the diabetic rats, alterations in antioxidant defenses such as an increase in lipid peroxidation, glutathione peroxidase (GPX) activity and a decrease in catalase (CAT) activity, glutathione (GSH) levels and oxygen radical absorbance capacity (ORAC) were observed. There was no difference in superoxide dismutase (SOD) activity. Diabetes induction increased apoptotic cell death and the levels of interleukin (IL)-1β and tumor necrosis factor (TNF)-α with no effect on IL-10. Kolaviron treatment of diabetic rats restored the activities of antioxidant enzymes, reduced lipid peroxidation and increased ORAC and GSH concentration in renal tissues. Kolaviron treatment of diabetic rats also suppressed renal IL-1β. The beneficial effects of kolaviron on diabetes-induced kidney injury may be due to its inhibitory action on oxidative stress, IL-1β production and apoptosis.

Introduction

Diabetes mellitus has dramatically increased globally. Diabetes can result in a progressive decline in glomerular filtration rate characterized by glomerular hyperfiltration, glomerular and tubular epithelial hypertrophy, increased urinary albumin excretion, increased basement membrane thickness and mesangial expansion with the accumulation of extracellular matrix (ECM) proteins (Jain 2012). The progression of renal injury often leads to end-stage renal disease affecting 20–40% of diabetic patients (Hakim and Pflueger 2010). Oxidative stress induced by the hyperglycemic milieu drives the development of complications including diabetic nephropathy (Kawahito et al. 2009). The initial cellular response to high glucose challenge also rapidly induces apoptosis (Park et al. 2001). Furthermore, the recruitment of inflammatory cells from the circulation into renal tissue is a critical feature of diabetic nephropathy (Navarro-González et al. 2011). Streptozotocin is selectively toxic to insulin-secreting beta cells of pancreatic islets inducing beta cell necrosis and impairs glucose oxidation and insulin release (Hayden and Tyagi 2002; Merzouk et al. 2000) and, in rats, mimics human diabetes (Weir et al. 1981) therefore it is a good experimental model for diabetic renal injury.

Garcinia kola seeds (Family: Guttiferae) are consumed in west and central Africa and contain high biflavonoid levels which have several pharmacokinetic advantages over simple monomeric flavonoids as they survive first-pass metabolism (Iwu and Igboko 1986). Kolaviron (KV) is a mixture of flavonoids extracted from

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the seeds of *Garcinia kola* which has numerous therapeutic effects. Although, kolaviron has been reported to produce a hypoglycemic and hypocholesterolemic effects in diabetic animals (Iwu et al. 1990; Adaramoye and Adeyemi 2006) and to protect against oxidative stress induced by toxins in experimental animal models (Akintonwa and Essien 1990; Farombi 2000), its efficacy in modulating the complex responses associated with oxidative stress and inflammation in diabetic kidney damage remains to be elucidated. In this study, we hypothesized that supplementation of kolaviron protects against diabetic kidney damage resulting from hyperglycemia-induced oxidative stress, inflammation and apoptosis in rats.

Materials and methods

Animals

The study protocol was approved by the Faculty of Health and Wellness Sciences Research Ethics Committee of the Cape Peninsula University of Technology (Ethics Certificate no: CPUT/HW-REC 2012/AO4). All the animals received humane care in accordance with the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health (Publication no. 80-23, revised 1978). Male Wistar rats (11–12 weeks, 270 ± 25 g) were used for the study. Experiments were performed at the animal facility of the Medical Research Council (MRC) and strictly adhered to the standard operating procedures (SOPs). All animals were housed individually at room temperature (22 ± 2 °C) with 55 ± 5% humidity and an automatically controlled cycle of 12 h light and 12 h dark. A standard laboratory diet and water were provided *ad libitum* and rats were habituated to the experimental conditions one week prior to experimentation.

Induction of diabetes

To induce diabetes in rats, a single intraperitoneal injection of freshly prepared solution of 50 mg/kg of STZ (Sigma–Aldrich, SA) in citrate buffer (0.1 M, pH 4.5) was administered to overnight fasted rats. Diabetes was confirmed after a tail prick by stable hyperglycemia (>18 mmol/l) five days post-STZ injection using a portable glucometer (Accu-Chek, Roche, Germany).

Plant materials

Fresh seeds of *Garcinia kola* were purchased from Bodija market in Ibadan, Oyo State, Nigeria and authenticated by Professor E. A Ayodele at the Department of Botany, University of Ibadan. A voucher specimen (FHI-109777) is available at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan.

Extraction of kolaviron

Garcinia kola seeds were peeled, sliced and air-dried (25–28 °C). Kolaviron was isolated according to the method of Iwu et al. (1990). The powdered seeds (600 g) were defatted with 800 ml of light petroleum ether (bp 40–60 °C) in a soxhlet for 24 h. The defatted powder was spread in thin layers on trays and air dried at room temperature for 24 h, repacked in the soxhlet and extracted with acetone (500 ml) at a temperature of 40 °C. The extract was concentrated and diluted twice its volume with distilled water and extracted with ethylacetate (6 ml × 300 ml). The concentrated ethylacetate yielded kolaviron.

Liquid chromatography–mass spectrometry (LC–MS) analysis of Garcinia kola seed extract

LC–MS was performed on a Dionex HPLC system (Dionex Softron, Germering, Germany) equipped with a binary solvent manager and autosampler coupled to a Brucker ESI Q-TOF mass spectrometer (Bruker Daltonik GmbH, Germany) as previously described (Ayepola et al. 2013). Kolaviron was separated by reversed phase chromatography on a Thermo Fischer Scientific C18 column 5 μm; 4.6 mm × 150 mm (Bellefonte, USA) using gradient elution with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as solvent at a flow rate of 1.0 ml min^{-1}, an injection volume of 10 μl and an oven temperature of 30 °C. MS spectra were acquired in negative mode using the full scan and auto MS/MS (collision energy 25 eV) scan modes with dual spray for reference mass solution. Electrospray voltage was set to +3500 V. Dry gas flow was set to 9 l min^{-1} with a temperature of 300 °C and nebulizer gas pressure was set to 35 psi.

Study design

The rats were randomly assigned into four groups ($n = 10$ per group): control (C), KV-treated control (C + KV), diabetic (D) and KV-treated diabetic (D + KV). Treatment commenced on the sixth day post STZ injection and continued for 6 weeks. The 100 mg/kg dose of kolaviron was selected based on our preliminary investigation. This 100 mg/kg kolaviron dose was a more effective dose among the doses (100 and 200 mg/kg) investigated in our preliminary study. Kolaviron (100 mg/kg body weight), dissolved in dimethylsulphoxide (DMSO) was administered by gastric gavage five times weekly. The dosage of kolaviron was adjusted weekly according to changes in body weight to standardize the dosages over the duration of the study.

Blood and tissue collection

After treatment, the rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). Blood was collected in tubes without anticoagulant for serum separation. Blood samples were centrifuged at 4000 × *g* for 10 min at 4 °C. Aliquots of the supernatant were stored at −80 °C for biochemical analysis. The left kidney was rapidly excised, washed in ice-cold phosphate buffered saline, blotted, frozen in liquid nitrogen and stored at −80 °C until use whereas the right kidney was excised and fixed in 10% (v:v) neutral buffered formalin.

Determination of relative kidney weight

Hypertrophy of the kidney was estimated by comparing the kidney weight to the body weight (relative kidney weight).

$$\text{Relative kidney weight(mg/100 g body weight)} = \frac{\text{Kidney weight(g)}}{\text{Total body weight(g)}} \times 100$$

Analysis of renal function parameters

Serum uric acid, blood urea nitrogen (BUN) and albumin concentrations were analyzed with diagnostic kits in an automated clinical chemistry analyzer (Medical Cooperation, Bedford, MA, USA).

Oxygen radical absorbance capacity (ORAC)

The ORAC assay kinetically measured the peroxyl radical scavenging activity in kidneys with trolox as the antioxidant

standard according to the method of Ou and Hampsch-Woodill Prior (2001). The fluorescence of the reaction mixture was monitored and recorded every minute (excitation = 485 nm and emission = 535 nm) for 2 h with a Fluoroscan Ascent plate reader (Thermo Fischer Scientific, Waltham, MA, USA). Results were determined with a regression equation relating trolox concentrations and the net area under the kinetic fluorescein decay curve ($y = ax2 + bx + c$). The ORAC value was expressed in micromoles of trolox equivalents per gram of tissue (μmol TE/g).

Measurement of antioxidant enzyme activities

Activities of antioxidant enzymes were determined in a clear 96-well plate using a Multiskan Spectrum plate reader (Thermo Fisher Scientific, USA). Catalase (CAT) activity was determined by the method of Aebi (1984). Superoxide dismutase (SOD) was determined by the method of Crosti et al. (1987). Glutathione Peroxidase (GPX) activity was determined according to the method of Ellerby and Bredesen (2000). The protein level in the kidney was estimated with a bicinchoninic acid (BCA) kit (Pierce, IL, USA).

Determination of glutathione (GSH) concentrations

The concentration of reduced glutathione (GSH) in the kidney was estimated by the method of Jollow et al. (1974).

Measurement of lipid peroxidation

Kidney malondialdehyde (MDA) was determined by high performance liquid chromatography (HPLC) using a method adapted from Khoschsorur et al. (2000). Fluorometric detection was performed with excitation at 532 nm and emission at 552 nm. The peak of the MDA-TBA adduct was calibrated with the MDA standard.

Determination of renal interleukin (IL)-1β, IL-10 and TNF-α concentrations

The levels of IL-1β, IL-10 and TNF-α were determined in the rat kidney homogenate using Bio-plex Promagnetic bead-based assay (Bio-Rad Laboratories, Hercules, USA) on the Bio-plex platform (Bio-Rad). Following previous optimization, undiluted samples were blindly evaluated. Samples were reacted with a mixture of fluorescent polystyrene beads bound with specific anti-cytokine primary antibodies, resulting in binding of the cytokines to the beads with the corresponding antibody. The biotinylated anti-cytokine secondary antibodies were then added and allowed to bind to the cytokine-bead complex followed by the addition of fluorescent phycoerythrin-conjugated streptavidin. Bio-Plex Manager software version 6.0 was used for bead acquisition and analysis.

Assessment of apoptosis

The right kidney was excised, fixed in 10% (v:v) neutral buffered formalin and dehydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin. Apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) using ApopTag fluorescein *in situ* Apoptosis Detection Kit (#S7110; Millipore, Temecula, CA) according to the manufacturer's instruction without modification. Paraffin-embedded kidney sections (5 μm) were layered onto glass slides, dewaxed in xylene and rehydrated in graded dilutions of ethanol. Sections were treated with freshly diluted protein digesting enzyme, proteinase K (20 μg/ml) for 15 min at room temperature. Equilibration buffer was applied directly on the kidney sections followed by incubation with the TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT), digoxigenin nucleotide and unlabeled nucleotide for 1 h in a humidified chamber at 37 °C. Antidigoxigenin conjugate was applied directly to the slides and sections were counter stained with propidium iodide (PI), mounted and viewed on a Carl Zeiss LSM 780 confocal microscope using a Plan-Neofluar 40X objective. Samples were excited with an argon mutline laser with excitation at 488 nm (for green) and 561 nm (for red). Images were acquired with a GaAsP detector set up for standard FITC and PI detection and optimized with the Carl Zeiss ZEN 2011 (Germany) software package. Total cell population and apoptotic cells were counted using the Image J image analysis software. TUNEL positive cells were expressed as percentage of total cells.

Histology

Kidney tissue was fixed in 10% (v:v) neutral buffered formalin and embedded in paraffin wax. Fixed kidney tissues were cut into 5 μm slices. After being deparaffinized using xylene and ethanol dilutions and rehydration, tissue sections were stained with hematoxylin and eosin (H&E). Mounted slides were examined in a masked fashion under a light microscope and photographed using a digital camera.

Statistical analysis

Data were expressed as the means ± standard deviations. Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA) with MedCalc software. Data not normally distributed was log transformed and analyzed using the Kruskal–Wallis one-way ANOVA on ranks hypotheses. Differences were considered significant at $p < 0.05$.

Results

LC–MS analysis of Garcinia kola seed extract

LC–MS analysis of *Garcinia kola* seed extract shows molecular ion peaks $[M-H]^-$ at (1) *m/z* 573.1023, (2) *m/z* 557.1074, (4) *m/z* 557.1072 (3) *m/z* 587.1178, (5) *m/z* 541.1123 and (6) *m/z* 555.0909 (Fig. 1). The molecular mass corresponds respectively to the active constituents (Fig. 2); *Garcinia* biflavonoid 2 (*m/z* 573.1023 = $C_{30}H_{22}O_{12}$), *Garcinia* biflavonoid 1 (*m/z* 557.1074, 557.1072 = $C_{30}H_{22}O_{11}$), kolaflavanone (*m/z* 587.1178 = $C_{31}H_{24}O_{12}$) and kolaflavones (*m/z* 555.0909 = $C_{30}H_{21}O_{11}$) which have been previously identified in kolaviron (Iwu et al. 1990). The mass spectrum of peak 5 shows the ion peak $[M-H]^-$ at *m/z* 541.1123. On the basis of this data and database searching, we deduce that this compound (peak 5) is binaringenin, a biflavanone which has been reported in *Garcinia* species (Waterman 1983).

Kolaviron's effect on weight parameters and renal function in normal and STZ-induced diabetic rats

There was an increase in the relative kidney weight of the diabetic rats compared to control rats (Table 1). Elevated serum levels of blood urea nitrogen and uric acid and a decrease in albumin levels were also observed in the diabetic rats compared to control rats. Treatment of diabetic rats with kolaviron reduced the relative kidney weight and the serum levels of kidney function biomarkers, *viz.*, uric acid, blood urea nitrogen and increased albumin concentration. Administration of kolaviron to non-diabetic control rats had no adverse effect on the kidney.

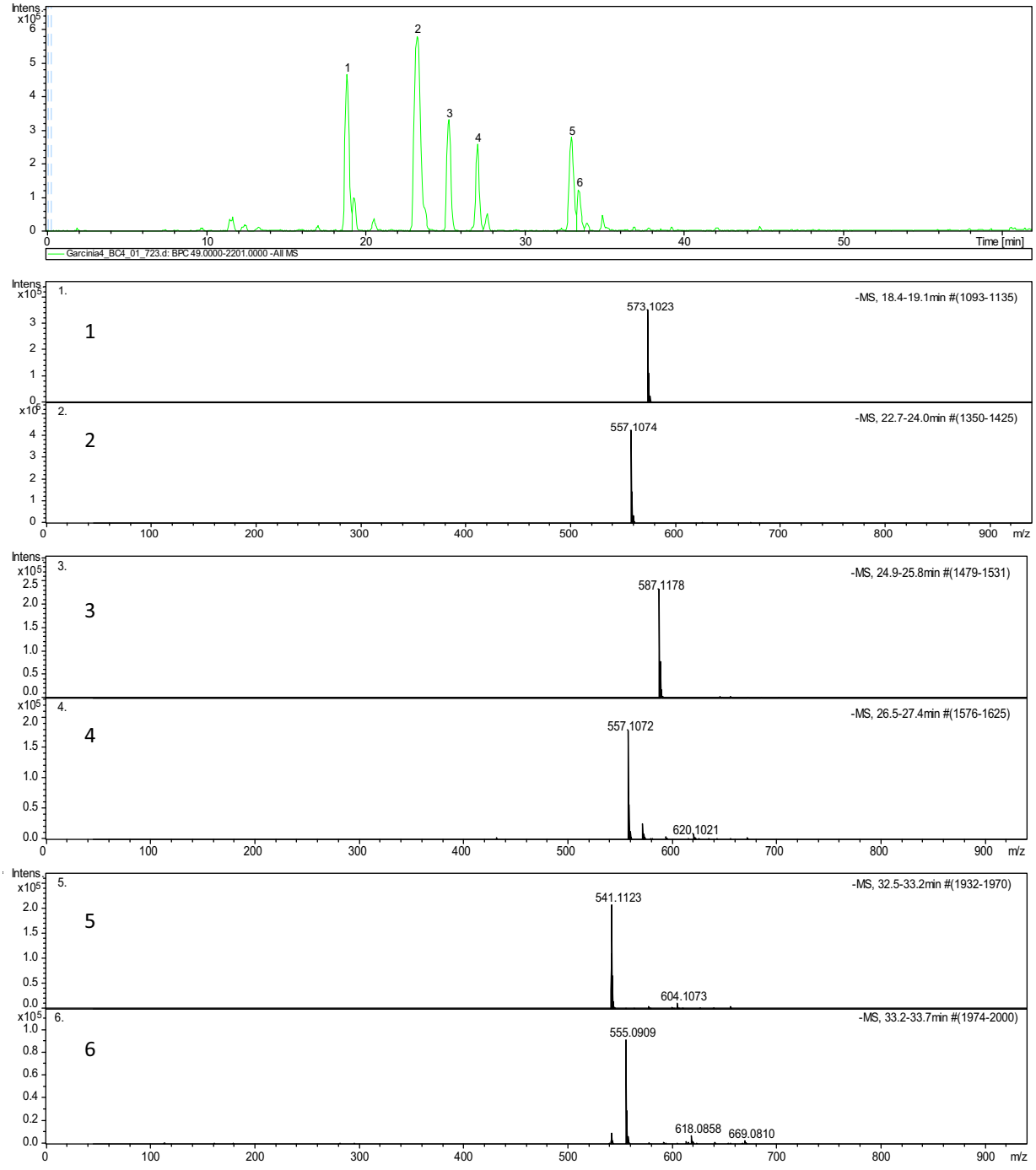

Fig. 1. LC–MS analysis of *Garcinia kola* seed extracts (kolaviron). (1) *Garcinia* biflavonoid 2 ($C_{30}H_{22}O_{12}$, *m*/*z* 573.1023); (2) *Garcinia* biflavonoid 1 ($C_{30}H_{22}O_{11}$, *m*/*z* 557.1074); (3) kolaflavanone ($C_{31}H_{24}O_{12}$, *m*/*z* 587.1178); (4) *Garcinia* biflavonoid 1 ($C_{30}H_{22}O_{11}$, *m*/*z* 557.1072); (5) X ($C_{30}H_{22}O_{10}$, 541.1123), deduced to be binaringenin; (6) Kolaflavone ($C_{30}H_{21}O_{11}$, *m*/*z* 555.0909). According to the LC–MS analysis, the approximate ratios of the single compounds in kolaviron are; 0.18: 0.38: 0.14: 0.11: 0.12: 0.06 respectively.

Table 1
Effect of kolaviron on weight parameters and serum markers of kidney damage.

	C	C + KV	D	D + KV
Change in body weight (%)	+33.34	+33.84	−3.52[a]	+17.30[b]
Kidney weight (g)	1.34 ± 0.09	1.37 ± 0.07	1.28 ± 0.09	1.38 ± 0.09
Relative kidney weight (g/100 g)	0.33 ± 0.02	0.35 ± 0.04	0.5 ± 0.03[a]	0.43 ± 0.04[b]
Uric acid (unit)	48.83 ± 9.87	49.27 ± 7.06	82.8 + 11.35[a]	55.29 ± 12.34[b]
BUN (unit)	6.46 ± 0.77	6.17 ± 0.5	18.35 ± 2.90[a]	8.28 ± 1.21[b]
Albumin (unit)	30.84 ± 0.91	30.18 ± 0.62	26.23 ± 0.72[a]	27.4 ± 0.98[b]

Data are presented as means ± S.D. C, non-diabetic control rats; C + KV, kolaviron-treated control rats; D, untreated diabetic rats; D + KV, KV-treated diabetic rats; BUN, blood urea nitrogen.

[a] Values differ from control rats ($p < 0.05$).

[b] Values differ from diabetic rats ($p < 0.05$).

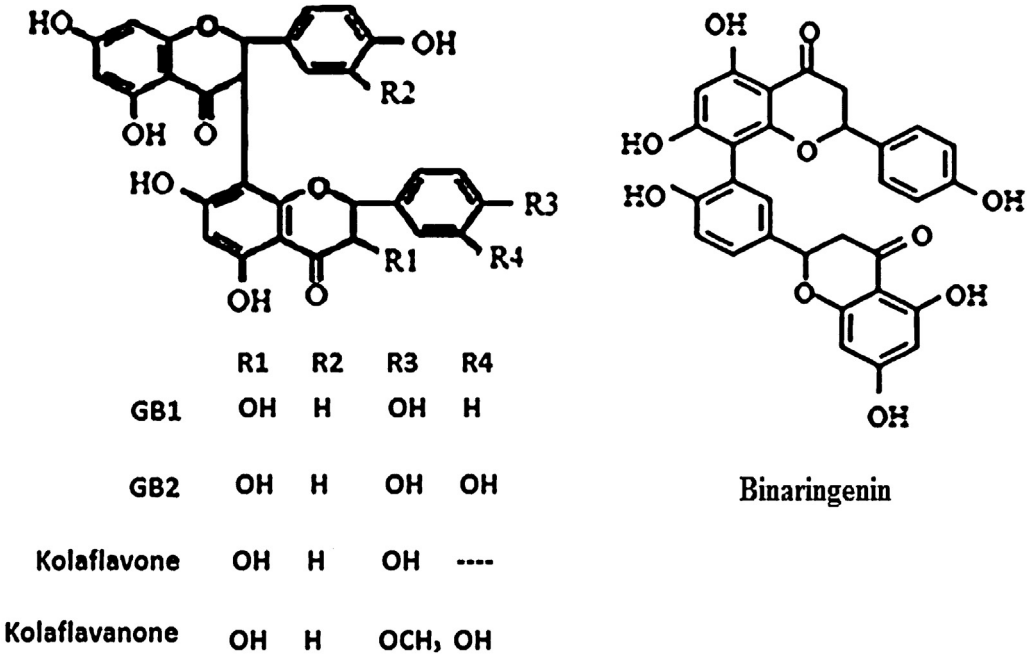

Fig. 2. Kolaviron, a complex mixture of flavonoids from *Garcinia kola* seeds.

Effect of kolaviron on kidney antioxidant enzymes (CAT, SOD, GPX) and non-enzyme (GSH) in normal and STZ-induced diabetic rats

Table 2 illustrates the effect of kolaviron on glutathione concentration and activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the control and diabetic rat kidney. The induction of diabetes with STZ reduced kidney glutathione (GSH) concentration and CAT activity in the diabetic rats. GPX activity was elevated in diabetic rats compared to control rats with no differences in SOD activities among the groups. Kolaviron treated diabetic rats demonstrated increased GSH concentration and CAT activity compared to diabetic controls. GPX activity was also normalized after kolaviron administration to diabetic rats. Kolaviron also increased GSH concentration in renal tissues of non-diabetic rats.

Oxygen radical absorbance capacity of kolaviron and its effect on lipid peroxidation in the kidney of normal and STZ-induced diabetic rats

As shown in Table 3, malondialdehyde (MDA; lipid peroxidation marker) levels were elevated in the kidneys of diabetic control rats whereas oxygen radical absorbance capacity (ORAC) values were reduced compared to non-diabetic control. Kolaviron treatment reduced MDA levels and increased ORAC values in diabetic rats compared to diabetic control rats. Kolaviron-treated control rats also had increased ORAC values compared to untreated control rats.

Effect of kolaviron on pro-inflammatory cytokines [interleukin (IL)-1β, tumor necrosis factor (TNF-α)] and anti-inflammatory cytokine (IL-10) in normal and STZ-induced diabetic rats

The induction of diabetes increased renal TNF-α and IL-1β concentrations (Fig. 3). The increase in IL-1β was inhibited after the administration of kolaviron although no statistically significant effect was observed in the renal concentration of TNF-α in the diabetic rats after kolaviron treatment. No significant difference was observed in the levels of IL-10 among the groups.

Effect of kolaviron on apoptosis in kidney of normal and STZ-induced diabetic rats

Examination of kidney apoptosis with TUNEL and PI staining revealed an increase in TUNEL positive cells in the kidney sections of diabetes control group as shown in Fig. 4. Kolaviron reduced apoptotic cells in the kidney of diabetic rats.

Table 2
Effect of kolaviron on renal antioxidant status.

	C	C + KV	D	D + KV
CAT	1.32 ± 0.26	1.31 ± 0.24	0.95 ± 0.24[a]	1.16 ± 0.19
SOD	401.70 ± 40.32	386.85 ± 43.53	423.68 ± 28.56	391.87 ± 39.17
GPX	4.76 ± 0.37	3.93 ± 0.61	6.09 ± 0.89[a]	4.70 ± 0.90[b]
GSH	1.19 ± 0.07	1.33 ± 0.10[a]	0.96 ± 0.14[a]	1.10 ± 0.13[b]

Data are presented as mean ± S.D. C, non-diabetic control rats; C + KV, kolaviron-treated control rats; D, untreated diabetic rats; D + KV, kolaviron-treated diabetic rats; CAT, catalase; μmol H_2O_2 consumed/min/mg protein; SOD, superoxide dismutase; U/mg protein, GPX, glutathione peroxidase; μmol NADPH oxidized/min/μg protein; GSH, reduced glutathione; μmol/g tissue.

[a] Values differ significantly from control ($p < 0.05$).

[b] Values differ significantly from diabetic control ($p < 0.05$).

Table 3
Effect of kolaviron on oxygen radical and lipid peroxidation in the kidney of diabetic and non-diabetic rats.

	MDA (μmol/g tissue)	ORAC (μmol TE/g tissue)
C	2.09 ± 0.11	4.21 ± 0.86
C + KV	2.06 ± 0.11	7.05 ± 0.78[a]
D	2.26 ± 0.12[a]	3.62 ± 0.76[a]
D + KV	1.96 ± 0.14[b]	7.13 ± 0.81[b]

Data are presented as mean ± S.D. C, non-diabetic control rats; C + KV, kolaviron-treated control rats; D, untreated diabetic rats; D + KV, kolaviron-treated diabetic rats; ORAC, oxygen radical absorbance capacity; TE, trolox equivalent; MDA, malondialdehyde.

[a] Values differ significantly from control ($p < 0.05$).

[b] Values differ significantly from diabetic control ($p < 0.05$).

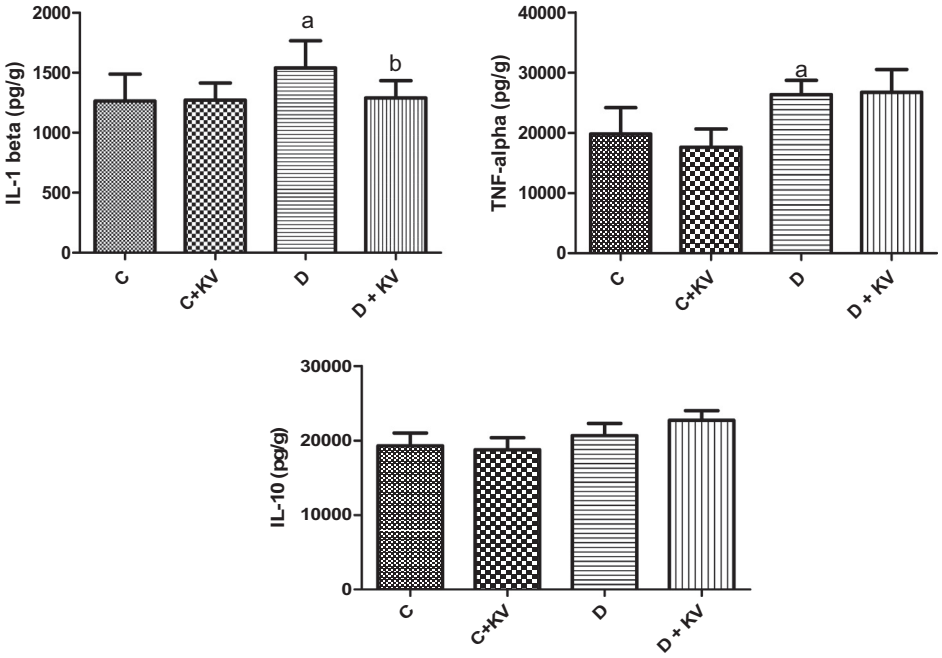

Fig. 3. Effect of kolaviron on renal inflammation in diabetic and non-diabetic rats. Data are presented as mean ± S.D. (a) Values differ significantly from non-diabetic control ($p < 0.05$). (b) Values differ significantly from diabetic control ($p < 0.05$). C, non-diabetic control rats; C + KV, control treated with kolaviron; D, untreated diabetic rats; D + KV, diabetic rats treated with kolaviron.

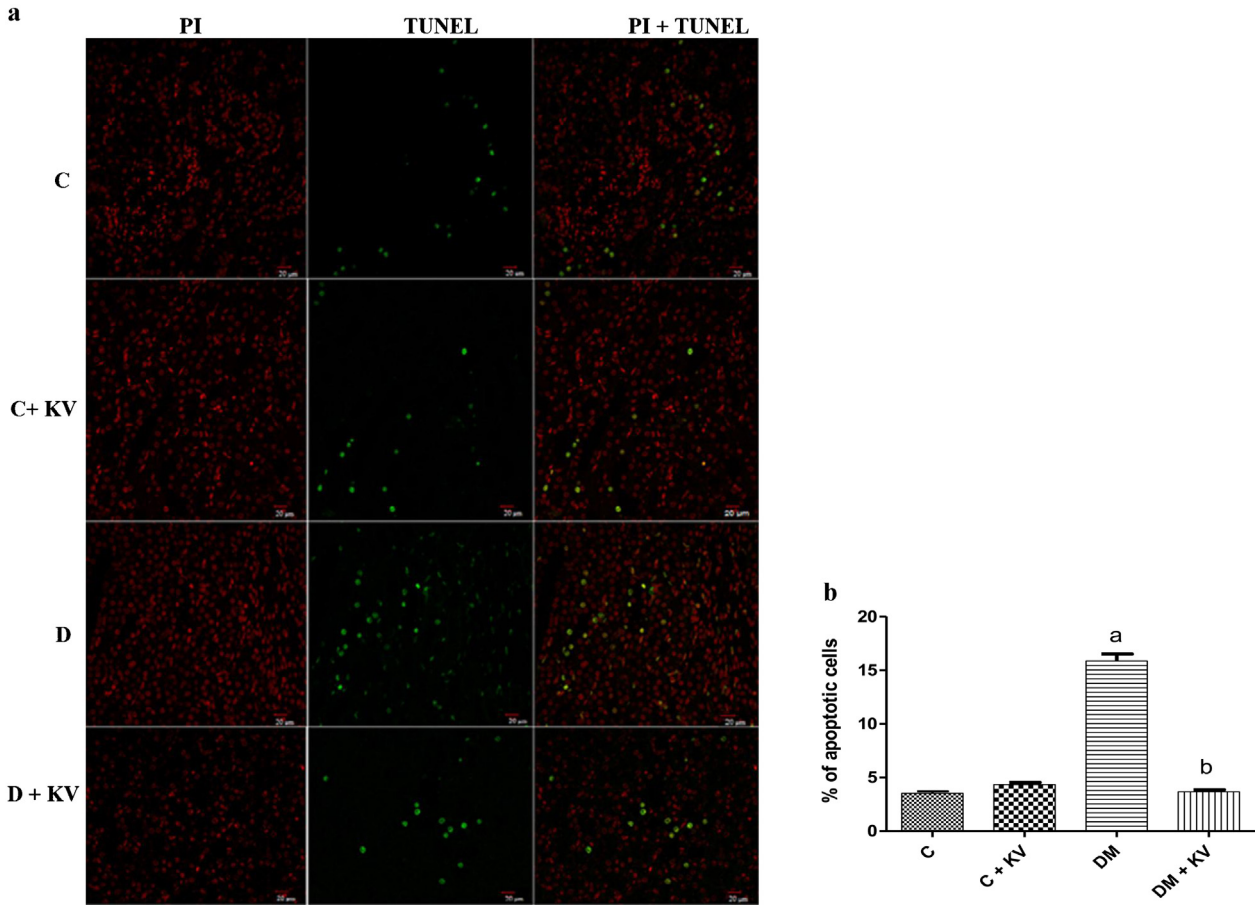

Fig. 4. (a and b) Effect of kolaviron on diabetic induced apoptosis. Representative photomicrographs showing TUNEL-positive cells at 40× magnification in experimental groups. The cells emitting a green signal are TUNEL-positive apoptotic cells. TUNEL; terminal deoxynucleotidyltransferase mediated dUTP-biotin nick end labeling, PI; propidium iodide. C, non-diabetic control rats; C + KV, control treated with kolaviron; D, untreated diabetic rats; D + KV, diabetic rats treated with kolaviron. Data are presented as mean ± S.D. (a) Values differ significantly from non-diabetic control ($p < 0.05$). (b) Values differ significantly from diabetic control ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

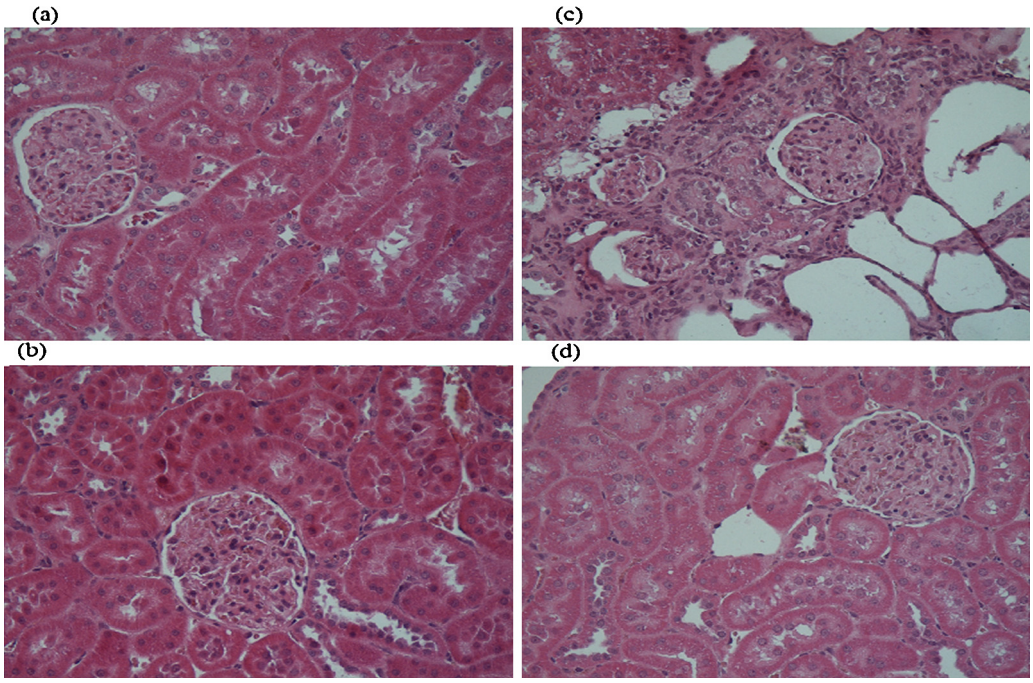

Fig. 5. Effects of kolaviron on renal structure of rats. Representative photomicrographs showing the renal structures of rats at 40× magnification in experimental groups. (a) Control; (b) kolaviron-treated control rats; (c) diabetic rats and (d) kolaviron-treated diabetic rats. Hematoxylin–eosin: 20×.

Effects of kolaviron on renal structure of rats

No pathological change was observed in kidney sections of rats treated with kolaviron and was comparable to those of control rats showing normal kidney architecture (Fig. 5a and b). STZ caused degeneration of the glomerulus (Fig. 5c) with increased interstitial matrix. Renal sections of diabetic rats treated with kolaviron showed preserved renal glomerulus (Fig. 5d).

Discussion

Oxidative stress results from an imbalance between oxidants and antioxidants in favor of oxidants thus leading to cell damage. Accumulation of reactive oxygen species (ROS) which causes oxidative stress in tissues such as the kidney results in damage or toxicity and oxidative stress has been singled as a major cause of diabetic complications including diabetic nephropathy (Rolo and Palmeira 2006). Cells maintain a variety of defenses in response to oxidative stress through the induction of antioxidant enzymes. Among these are endogenous enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In the present study, GPX activity was increased in the diabetic kidney whereas catalase activity was decreased. However, in kolaviron-treated diabetic rats, CAT and GPX activities normalized. GPX uses the cellular non-enzymatic antioxidant glutathione (GSH) as an electron donor to scavenge ROS generating products that can easily be excreted out of the body (Sahoo et al. 2008). Therefore a marked decline in renal GSH concentrations in diabetic rats may reflect an impaired antioxidant defense and thus increased susceptibility to oxidative stress. Kolaviron administered to diabetic and non-diabetic control rats increased kidney GSH concentration. The normalization of GSH in kolaviron-treated diabetic rats therefore suggests a protective effect of kolaviron against ROS overproduction in the diabetic rat kidney.

ROS degrade membrane polyunsaturated fatty acids through sequential peroxidation processes. The major products of lipid peroxidation are 4-hydroxylnonenal (4-HNE) and malondialdehyde (MDA) (Demir et al. 2011). MDA is a highly unstable aldehyde that induces oxidative stress by forming covalent protein adducts collectively referred to as advanced lipoxidation end-products (ALE) (Farmer and Davoine 2007). The production of MDA serves as a bio-marker to measure tissue oxidative stress levels. Extensive membrane lipid peroxidation impairs membrane fluidity and inactivates some membrane bound enzymes leading to cell death (Goel et al. 2005). From our investigation, kolaviron may prevent these deleterious effects by inhibiting the lipid peroxidation process thus reducing the formation of MDA. Previous studies identified some active compounds in kolaviron which include *Garcinia* biflavonoids (GB) 1, GB 2, kolaflavone and kolaflavanone (Iwu 1985). Recently, we also identified a new compound in kolaviron which may be another biflavonoid-binaringenin (Ayepola et al. 2013). The antioxidative actions of kolaviron may therefore be attributed to the complex mixture of the five *Garcinia* seeds flavonoids (Garcinia biflavonoid (GB) 1, GB2, kolaflavone kolaflavanone and binaringenin) as shown in Fig. 2.

Kolaviron-treated diabetic rats have reduced IL-1β concentrations with no effect on TNF-α concentrations. IL-1 and TNF-α are pro-inflammatory cytokines produced by a wide range of cell types and can exert their actions in a systemic, paracrine or autocrine manner. IL-1 and TNF-α regulate immunological and inflammatory responses and have been implicated in diabetic kidney disease through diverse biological activities including activation of growth and transcription factors, impairment of insulin secretion and induction of apoptosis and necrosis (Navarro-González et al. 2011; Banerjee and Saxena 2012). IL-10, however, is an anti-inflammatory cytokine which stimulates humoral immune responses and its elevation in the serum of patients with diabetic nephropathy suggests it plays a role in the pathogenesis of the disease (Wong et al. 2007; Wu et al. 2011). We did not find differences in IL-10 concentrations. The anti-inflammatory action of kolaviron in the diabetic kidney may therefore be related to the suppression of renal IL-1β production, independent of TNF-α.

Apoptosis is a process of physiological cell death that maintains tissue homeostasis; however, excessive or dysregulated apoptosis

may lead to various pathological processes including diabetes (Kim et al. 2011). Hyperglycemic-induced oxidative stress has been shown to result in pancreatic beta cell dysfunction and apoptosis (Donath et al. 1999). *In vitro* and *in vivo* diabetic kidney studies revealed that hyperglycemic-induced oxidative stress also triggers tubular and glomerular cells to undergo apoptosis (Brownlee 2001; Ha et al. 2008). In renal tubular epithelial cells, elevated glucose concentrations induce ROS-dependent apoptosis by an increased Bax protein expression causing mitochondrial permeability and subsequently releasing cytochrome C (Kang et al. 2003; Wagener et al. 2009). Apoptosis is also associated with inflammation in diabetic nephropathy (Kim et al. 2011). Our study demonstrated that renal apoptosis was induced in diabetic rats compared to the non-diabetic control rats while treatment of diabetic rats with kolaviron protected against the renal apoptosis. The ability of kolaviron to inhibit oxidative damage by boosting renal antioxidant status is a possible mechanism of its anti-apoptotic activity. IL-1β has been shown to induce Fas-triggered apoptosis through the activation of the nuclear transcription factor NF-κB. The inhibitory action of kolaviron on IL-1β in the present study potentially contributes to the anti-apoptotic effect of kolaviron.

The increased relative kidney weights of diabetic rats may be due to fatty infiltration, enlargement of tubular cells lining and lymphocyte infiltration in hyperglycemic rats. Elevated concentrations of uric acid and blood urea nitrogen are related to renal dysfunction in diabetes due to metabolic disturbances. Moreover, metabolic disturbances and muscle wasting in diabetes can also result in an increased release of purine, a major source of uric acid. Although uric acid is considered an antioxidant, its excessive generation could result from increased free radical production due to activation of xanthine oxidase (Madianov et al. 2000). Kolaviron treatment lowered blood urea nitrogen (BUN) and serum uric acid levels in diabetic rats compared to untreated diabetic rats suggesting an ameliorative effect on kidney function. We also observed reduced serum albumin concentrations in the diabetic rats compared to non-diabetic rats which may be due to glomerular basement membrane damage and increased urinary excretion of albumin which was normalized by kolaviron treatment.

Conclusion

The results of our study suggest that hyperglycemia caused renal dysfunction and cell death *via* oxidative stress and inflammation. We demonstrated the beneficial effects of kolaviron on renal dysfunction in diabetic rats *via* modulation of hyperglycemic-induced inflammation, oxidative damage and apoptosis. Kolaviron should be further explored as a potential candidate in the treatment of diabetic nephropathy.

Competing interests

The authors declare that they have no competing interests.

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