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Azadirachta excelsa Improves Renal Morphology and Function in Streptozotocin Induced-Diabetic Sprague Dawley Rats

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Abstract

Long term diabetes mellitus (DM) is associated with serious complications such as nephropathy. Previous studies revealed the ability of *A. excelsa* leaf extract treatment to reduce fasting blood glucose (FBG) in streptozotocin (STZ)-induced diabetic rats. The aim of this study was to determine the effect of *A. excelsa* extract in delaying the progression of diabetic nephropathy by evaluating the kidney structure and function. The effects were compared with 2 positive controls, which were metformin (standard drug) and quercetin (plant active compound). Induction of diabetic conditions was conducted by the intraperitoneal (IP) injection of STZ (60 mg/kg bwt) in male Sprague Dawley rats. The experimental animals were grouped into: 1) normal control (NC, saline); 2) diabetic control (DC, saline); 3) metformin-treated diabetic rats (DMET, 1000 mg/kg bwt); 4) quercetin-treated diabetic rats (DQ, 40 mg/kg bwt), and 5) *A. excelsa*-treated diabetic rats (DAE, 250 mg/kg bwt). All treatments were given once daily for 8 weeks through oral gavage. The inter-relation between the changes in the fasting blood glucose and kidney oxidative stress, structure, and function was evaluated. The results showed a significant increase ($p < 0.05$) of MDA and SOD level and a decrease ($p < 0.05$) of GPx levels, plus distortion of renal morphology among the DC and DMET groups. Meanwhile, both DQ and DAE groups showed significant reduction ($p < 0.05$) of MDA levels and elevation ($p < 0.05$) of SOD and GPx levels. The quercetin and *A. excelsa* treatments also improved the kidney function parameters and morphological changes of the diabetic rats. These findings indicate that quercetin and *A. excelsa* possess renal therapeutic effects.

Keywords: Nephropathy, Streptozotocin, MDA, GPx, SOD, Kidney function parameters

Introduction

One of the most common endocrine and metabolic disorders seen in developing countries is diabetes mellitus (DM). Diabetes has taken on epidemic proportions, with global prevalence estimates of 382 million people [1]. DM is one of the main causes of nephropathy, rising creatinine levels, and Chronic Kidney Disease (CKD), and ultimately leads to End-Stage Renal Disease (ESRD) [2,3]. CKD is a common condition that is estimated to affect over 154 million people worldwide [4]. In Malaysia, there has been an increasing trend in dialysis provision for end-stage renal disease, from 96 in 2002 to 182 cases per million population in 2011 [5]. CKD resulting from diabetes has been termed "Diabetic Kidney Disease" (DKD). It is important to note that, in most of cases, CKD or DKD is often present at the time of diabetes diagnosis [6]. One of the most important complications in which end organ injuries occur is diabetic nephropathy, which manifests in the long term. End-organ injuries occurring in DM decrease quality of life, while highly increasing medical costs and mortality rates. Kidney injury is the most common pathological disorder predisposing end-stage renal disease worldwide [7,8].

There is evidence to show a significant role of reactive oxygen species (ROS) in the increase of diabetic nephropathy pathogenesis [9,10]. Cellular damage begins to occur when the production of ROS exceeds the antioxidant capacity, through which macromolecules that exist within the cellular composition, such as proteins, lipids, and carbohydrates, are oxidized and damaged [11,12]. Overproduction of ROS causes insufficient antioxidant capacity which eventually disturbs the oxidant-antioxidant balance [13]. However, various studies have depicted the alleviation of renal damage with the administration of antioxidant-rich agents [14,15].

One of the molecules researched in recent studies due to its antioxidant property is quercetin [16]. Quercetin, a powerful antioxidant bioflavonoid, attenuates pancreas and renal dysfunction in long-term experimental diabetes mellitus [17,18]. A previous study also recorded that quercetin prevented diabetic kidney injury in rats [19]. A study by Zin and colleagues [20] indicated the highest composition of quercetin in the ethanolic extract of *A. excelsa* leaf as compared to kaempferol, isovitexin, vitexin, and homoorientin. Therefore, this study was carried out to assess the effect of quercetin, the active compound of *A. excelsa*, in its therapeutic potential to alleviate DM nephropathy as compared with *A. excelsa* extract.

Azadirachta excelsa, (Jack) Jacobs, is a marrango or Philippine neem tree of congenric species in Southeast Asia. Originating in Borneo, *A. excelsa* grows naturally in southern Thailand, peninsular Malaysia, and the Palawan island of the Philippines. In Malaysia, *A. excelsa* is a typical wild plant known as the marrango tree or sentang [21]. Various studies revealed the health-promoting potential of *Azadirachta* species, which include antioxidant, diuretic, antiviral, antiulcer, anti-malarial, anti-inflammatory, and cytotoxic effects [22]. A study indicated that a GC-MS chromatogram of *A. excelsa* leaves was associated with higher plant constituents of flavonoids and moderate amounts of tannins, triterpenes, and steroids [23]. The presence of various antioxidant-rich compounds might explain the therapeutic property of *A. excelsa*. Even though the ability of *A. excelsa* to alleviate diabetes among diabetic animal models has been proven previously, its ability to ameliorate or delay the progression of secondary kidney complication remains elusive. Hence, this study aimed to evaluate the effect of *A. excelsa* on diabetic-associated kidney disease.

Materials and methods

Collection and authentication of plant specimen

The leaves of *A. excelsa* plant were collected in January, 2015, from the Forest Research Institute of Malaysia (FRIM), Kepong, Kuala Lumpur. The plant was authenticated by Mr. Sani Miran as *A. excelsa* with voucher number UKMB-40314, and the specimen was deposited at the Herbarium of Universiti Kebangsaan Malaysia (UKMB), Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia, Bandar Baru Bangi, Selangor Darul Ehsan, Malaysia.

Preparation of plant extract

The leaves of the *A. excelsa* plant was washed, sliced into small pieces, and oven-dried at 37 ± 5 °C. Next, they were soaked in 70 % ethanol at 1:10 ratio (100 g powder: 1000 mL ethanol) for 3 days at 27 °C [23,24]. The ethanolic extract was collected in a conical flask. The mixture was filtered by using a vacuum pump, Buchner funnel, and filter papers. The filtrate obtained was evaporated by using a rotary evaporator at 40 °C [25]. A dark semi-solid paste obtained was stored at 4 °C until further use.

Experimental animals

Twenty-four male Sprague Dawley rats, with body weights ranging between 150 - 200 g, aged 4 weeks old, were used in this study. The rats were attained from Chenur Supplier Sdn. Bhd., Serdang, Selangor. They were housed at the Laboratory Animal Facility and Management (LAFAM), Universiti Teknologi MARA, Puncak Alam, Selangor. The animals were acclimatized upon arrival for a week and were housed in groups of 6 and fed with a standard commercial rodent diet (Gold Coin Feedmills, Gold Coin Holdings, Malaysia) and plain water *ad libitum*. Experimental protocols involving the use of rats, as well as internationally-accepted practices for the usage and maintenance of laboratory animals as

contained in the guidelines, were strictly applied. The approval reference number is UiTM CARE, 112/2015.

Induction of diabetes mellitus in rats

Hyperglycemia conditions were induced at 60 mg/kg bwt in the overnight fasting male Sprague Dawley rats by conducting a single intraperitoneal (i.p.) injection of 0.5 mL Streptozotocin (STZ) (Sigma-Aldrich, Deisenhofen, Germany). The STZ was freshly dissolved in saline (0.9 % sodium chloride) [26]. Conversely, the age-matched normal control (NC) group received an equivalent volume of saline. Soon after the injection of STZ, the animals were given free access to food and water. In order to prevent drug-induced hypoglycemic shock, 5 % glucose water was given to the rats orally by using oral gavage, repeated the next day [27]. Hyperglycemia was confirmed 1 week post STZ injection by measuring the tail vein blood glucose level with an Accu-Check glucometer. The rats with FBG levels reaching more than 11 mmol/L (Day 0) were considered diabetic [28,29]. Those that failed to develop diabetes were excluded from the study.

The reason for using the specific high-dose treatment of STZ was to induce a more rapid onset of diabetes [30]. It is known that an intraperitoneal injection of STZ leads to β-cell destruction in the pancreatic islets [31]. This consequently produces an insulin deficiency and increased blood glucose levels [32,33]. In addition, similar doses of STZ, have been employed by many researchers in other related studies.

Experimental design

The rats were divided into five groups, with six rats each (**Table 1**). All of the treatments were given orally once daily for a duration of 8 weeks (56 days). Quercetin was purchased from Sigma-Aldrich USA. The use of 1000 mg/kg bwt metformin for DMET group was based on a study which revealed the robust ability of this drug to improve glucose tolerance in STZ-induced diabetic rats as compared to 100 mg/kg bwt and 300 mg/kg bwt [34]. Meanwhile, 40 mg/kg bwt of quercetin has shown a protective effect in a diabetic rat study [35]. In addition, the dose of *A. excelsa* extract was selected on the basis of our previous acute oral toxicity study. Therefore, according to the study by Nurdiana [24], 250 mg/kg of *A. excelsa* was enabled to reduce fasting blood glucose (FBG) as compared to a low dose of 100 mg/kg.

Table 1 Treatment groups.

Group	Treatment	Dosage
Normal control (NC)	Saline	-
Diabetic control (DC)	Saline	-
Diabetic rats + Metformin (DMET)	Metformin	1,000 mg/kg bwt [34]
Diabetic rat + Quercetin (DQ)	Quercetin	40 mg/kg bwt [35]
Diabetic rat + *A. excelsa* (DAE)	*A. excelsa* extract	250 mg/kg bwt [25]

Preparation of materials for treatment group

The dosage for each treatment was freshly prepared with saline. The respective dosages of metformin, quercetin, and A. excelsa extract were vortexed to mix with saline. Treatments were then administered to the respective group with volume of 1 mL/ 100 g bwt. A similar volume of saline was given to NC and DC, which were the control groups.

Determination of fasting blood glucose (FBG)

The general health and behavior of the animals were monitored during the entire period of the study. The FBG was recorded at the beginning and the end of the experiment, and the data presented the percentage change of FBG after 8 weeks of the treatment period.

Urine collection

Urine samples were collected once a week using a timely collection method (4 h). A timed collection method was used to measure the concentration and volume of urine over a specified length of time. In this experiment, urine was collected within 4 h. This method was adopted from Kimura *et al.* [36] with minor changes. Urine specimens were stored at −80 °C.

Serum collection

At the end of the treatments, rats were fasted overnight and anesthetized using diethyl ether. Then, blood samples (10 - 15 mL) were collected through cardiac puncture into plain red-top sterilized centrifuge tubes (BD Vacutainer®, USA) containing no anticoagulants. Serum was suppurated at 4000 rpm for 15 min and stored at –80 °C until analysis.

Preparation of kidney tissue homogenate

Kidney tissue samples were homogenized in 5 - 10 g/mL of phosphate buffered saline (PBS) (4 °C, pH 7.6; Sigma Chemicals, USA). It was then centrifuged at 3000 rpm for 15 min at 4 °C (Centrifuge 5417R; Eppendorf, Germany). The supernatant was then collected and stored at −80 °C until use.

Estimation of malondialdehyde (MDA) level

The levels of MDA equivalents were determined in the homogenate tissue of the kidney by using a TBARS assay kit (Cayman, MI, USA), as described previously [37]. The absorbance was determined at a wavelength of 540 nm using a spectrophotometer (Epoch 2 microplate spectrophotometer, BioTek Instruments Inc, Vermont, USA).

Antioxidant enzymes activity assessment

Glutathione peroxidase (GPx) activity was evaluated using an assay kit (Cayman, MI, USA). The experimental procedures were carried out according to the manufacturer's instructions [38]. The measurement of GPx activity was based on the rule of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydroperoxide by GPx is recycled to its reduced state by GR in the presence of NADPH. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GPx is defined as the quantity of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25 °C.

Superoxide dismutase (SOD) activity was determined using an assay kit (Cayman, MI, USA). This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to produce 50 % dismutation of superoxide radical.

Glomerular filtration rate (GFR) measurement

Glomerular filtration rate (GFR) is based on creatinine clearance and was determined using urine and serum creatinine assay kits (Cayman Chemicals, Ann Arbor, MI) and urine output levels. GFR was calculated using the following formula:

$$\mathrm{GFR}\left(\frac{\mathrm{ml}}{\mathrm{min}}\right)=\frac{\text{Urinary creatinine}\left(\frac{\mathrm{mg}}{\mathrm{ml}}\right)\times\text{Urine volume (ml)}\times 1000\,\mathrm{(g)}}{\text{Serum creatinine}\left(\frac{\mathrm{mg}}{\mathrm{ml}}\right)\times\text{body weight (g)}\times 240\,\mathrm{min}} \quad (1)$$

Urine creatinine determination

Urine creatinine (UCRE) was determined quantitatively using Enzyme-Linked Immunosorbent Assays (Cayman, MI, USA). Each ELISA test was conducted in triplicate and performed according to the manufacturer's protocols.

Measurement of renal parameters in serum

The levels of serum creatinine, albumin, urea, uric acid, and total bilirubin were determined spectrophotometrically using commercial diagnostic kits (Boehringer-Mannheim Diagnostic Systems) and analyzed by an auto-analyzer (Hitachi-917, Germany).

Kidney histological assessment

The kidney tissues were fixed in 4 % formaldehyde embedded in paraffin blocks, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). All slides were examined using light microscopy (Motic BA410, Wetzlar, Germany) equipped with a digital camera (Moticam Pro 285A, Wetzlar, Germany) under a magnification of 400×.

Statistical analysis

All data were analyzed with the Statistical Package for the Social Sciences (SPSS) version 21.0 software. Data are expressed as means ± standard error of the means (SEM) for each group of treatment. A 2-way repeated measure Analysis of Variance (ANOVA) was conducted to compare means of the time dependent parameters in the FBG. Meanwhile, 1-way ANOVA analysis was conducted for the kidney MDA, SOD, and GPx levels, GFR, and the kidney function parameters. Significant difference at $p < 0.05$ were further analyzed using Duncan's multiple test.

Results

Fasting blood glucose

The means and standard errors of the means (SEM) for fasting blood glucose (FBG) in the entire groups of animals are given in **Table 2**. Administration of STZ had significantly higher levels of FBG by 38.70 % ($p < 0.05$) in the DC group as compared to the NC group throughout the 8 weeks period, with 20.88 mmol/L from week 0 to 28.96 mmol/L in week 8. On the contrary, the NC group had a relatively constant blood glucose, ranging from 4.66 to 5.66 mmol/L. Meanwhile, treatment with metformin significantly reduced the FBG level by 34.59 % from 27.06 to 17.70 mmol/L, while the quercetin and *A. excelsa* groups showed significant decreases of ($p < 0.05$) of FBG, by 20.83 and 27.57 %, respectively.

Table 2 Means of weekly fasting blood glucose level (FBG) (mmol/L) among groups throughout experimental period.

Group	Mean fasting blood glucose (mmol/L) ± SEM		
	Week 0	Week 8	% FBG change
NC	$4.66 \pm 0.13^{a,x}$	$5.16 \pm 0.16^{a,x}$	+ 10.73 % [b]
DC	$20.88 \pm 1.37^{b,y}$	$28.96 \pm 1.35^{c,z}$	+ 38.70 [c]
DMET	$27.06 \pm 1.82^{c,z}$	$17.70 \pm 1.77^{b,y}$	- 34.59 [a]
DQ	$28.04 \pm 1.54^{c,z}$	$22.20 \pm 1.55^{b,y}$	- 20.83 [a]
DAE	$28.58 \pm 1.41^{c,z}$	$20.70 \pm 2.19^{b,y}$	- 27.57 [a]

Values are means ± SEMs. Superscripts ^{a,b,c,d} indicate significant differences ($p < 0.05$) within same column, while superscripts ^{x,y,z} indicate significant differences ($p < 0.05$) between week 0 and week 8 within same row. NC = Normal control rats, DC = Diabetic control rats, DMET = Diabetic rats treated with metformin, DQ = Diabetic rats treated with quercetin, DAE = Diabetic rats treated with *A. excelsa*. (+ = addition, - = reduction).

Kidney oxidants and antioxidants

The results for the effects of the treatments on kidney lipid peroxidation and the activities of antioxidant enzymes are tabulated in **Table 3**. There was a significant increase ($p < 0.05$) in the MDA levels of the DC, DMET, and DAE, except for the DQ, as compared with the NC group. The results reflected nephrotoxicity associated with STZ-induction. The DMET group marked the second highest MDA value, with 19.76 nmol/mg proteins, after the DC group, which was 19.96 nmol/mg proteins. Conversely, the DQ group indicated the significantly lowest kidney MDA value (0.31 nmol/mg proteins). Treatment with *A. excelsa* significantly decreased ($p < 0.05$) kidney MDA value to 1.92 nmol/mg proteins, which was not significantly different to those of the DQ and NC groups. On the other hand, the DC group showed a significant decrease ($p < 0.05$) in the GPx level, to 83.11 U/mg proteins, and an increase in SOD level to 11.11 mU/mg, as compared with the NC group (242.09 U/mg and 4.24 mU/mg protein, respectively). Meanwhile, SOD level was markedly increased in the DMET group (12.79 mU/mg proteins) as compared to the DQ group, with 13.65 mU/mg proteins being the highest. It also was noted that both quercetin and *A. excelsa* treatments significantly increased the GPx concentrations (150.73 and 167.21 U/mg proteins, respectively), which were higher than that of the DMET group (137.38 ± 0.62 U/mg proteins).

Table 3 Kidney oxidative stress marker and antioxidant enzymes among experimental groups.

Group	Oxidative stress marker	Antioxidant enzymes	
	MDA (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
NC	1.40 ± 0.03^{a}	242.09 ± 0.09^{e}	4.24 ± 0.39^{a}
DC	19.96 ± 0.83^{b}	83.11 ± 0.41^{a}	11.11 ± 0.50^{c}
DMET	19.76 ± 0.51^{b}	137.38 ± 0.62^{b}	12.79 ± 0.20^{d}
DQ	0.31 ± 1.60^{a}	150.73 ± 0.27^{c}	13.65 ± 0.20^{e}
DAE	1.92 ± 0.55^{a}	167.21 ± 0.40^{d}	8.47 ± 0.60^{b}

Values are presented as means ± SEM. Superscripts ^{a,b,c,d,e} in a column differ significantly at $p < 0.05$. NC = Normal control rats, DC = Diabetic control rats, DMET = Diabetic rats treated with metformin, DQ = Diabetic rats treated with quercetin, DAE = Diabetic rats treated with *A. excelsa*.

GFR and kidney function parameters

In order to determine the effects of the treatments on the kidney, the glomerulus filtrate rate (GFR) was measured. As depicted in **Table 4**, the GFR values in all groups decreased dramatically ($p < 0.05$) compared to the value in the NC group. The DC group showed lower GFR (0.98 ± 0.05 mL/min) than that in the NC group, (2.62 ± 0.14 mL/min). Among the treatment groups, the *A. excelsa* treatment gave the highest values of GFR, which was (1.60 ± 0.25 mL/min), compared with the DQ group (1.28 ± 0.05 mL/min) and the DMET group (1.34 ± 0.20 mL/min). However, their values were not significantly different to the DC group, but significantly lower than that of the NC group (2.62 ± 0.14 mL/min).

Table 4 also shows a significant increase in serum creatinine (92.50 ± 3.54 µmol/L), but a significant decrease in urinary creatinine (6.81 ± 0.13 mg/dL) in the DC group, as compared to the NC group (63.00 ± 1.50 µmol/L, 94.09 ± 2.31 µmol/L, respectively). Meanwhile, serum creatinine of the DMET, DQ, and DMET groups (72.00 ± 1.00 µmol/L, 69.50 ± 1.50, 66.50 ± 2.50 µmol/L µmol/L, respectively) were significantly different to that of the NC group (63.00 ± 1.50 µmol/L). In addition, total bilirubin of the diabetic groups returned to near normal levels in response to the treatments. Interestingly, *A. excelsa* improved the uric acid levels of diabetic rats to near normal volume, which was 146.14 ± 5.45 µmol/L.

Table 4 Changes of GFR and kidney function parameters of experimental groups.

Group	Parameter						
	GFR (mL/min)	**Serum creatinine (µmol/L)**	**Urinary creatinine (mg/dL)**	**Albumin (g/L)**	**Urea (mmol/L)**	**Uric acid (µmol/L)**	**Total bilirubin (µmol/L)**
NC	2.62 ± 0.14^{a}	63.00 ± 1.50^{a}	94.09 ± 2.31^{c}	40.05 ± 0.75^{b}	9.90 ± 0.30^{a}	145.65 ± 2.75^{a}	1.05 ± 0.95^{a}
DC	0.98 ± 0.05^{b}	92.50 ± 3.54^{b}	6.81 ± 0.13^{a}	36.15 ± 3.25^{ab}	15.85 ± 0.95^{c}	241.90 ± 2.7^{b}	2.40 ± 0.20^{b}
DMET	1.34 ± 0.20^{b}	72.00 ± 1.00^{a}	9.77 ± 0.26^{a}	27.80 ± 3.30^{a}	13.55 ± 0.15^{bc}	242.25 ± 0.15^{b}	0.80 ± 0.20^{a}
DQ	1.28 ± 0.09^{b}	69.50 ± 1.50^{a}	26.22 ± 0.01^{b}	28.15 ± 0.85^{ab}	11.60 ± 0.90^{ab}	224.40 ± 4.30^{b}	1.65 ± 0.15^{ab}
DAE	1.60 ± 0.25^{b}	66.50 ± 2.50^{a}	8.48 ± 0.01^{a}	28.10 ± 5.30^{ab}	12.85 ± 0.45^{ab}	146.15 ± 5.45^{a}	1.25 ± 0.15^{a}

Values are presented as mean ± SEM. Superscripts ^{a,b,c} in a column differ significantly at $p < 0.05$. NC = Normal control rats, DC = Diabetic control rats, DMET = Diabetic rats treated with metformin, DQ = Diabetic rats treated with quercetin, DAE = Diabetic rats treated with *A. excelsa*.

Kidney histology

Figure 1 reveals the histological assessment of the kidneys of the treated rats. The kidney tissue section of the NC group represents a normal tissue-architecture of the glomeruli and kidney tubules (**Figure 1A**). However, the kidney structure of the DC group showed marked morphological disturbances. There were varying degrees of deterioration in the histological architecture, with apparent increase in glomeruli sizes, including congested capillary vessels, vacuolar degeneration of the tubules, hyaline casts, distorted cellular boundaries of tubules, and areas of extravasated blood cells. Severe tubular degeneration and tubular necrosis were also noted (**Figure 1B**). Similarly, all of the treated rats had kidneys with shrunken glomeruli. **Figure 1C** shows equally severe changes in the proximal convoluted tubules, which consisted of cytoplasmic vacuolation of tubular epithelial cells and cellular swelling were markedly observed in the DMET group. The rats of this group displayed no observable improvement in the histological alterations of the glomeruli and tubules. Some irregular capillaries, in part attached to Bowman's capsule, were noted among the DQ group (**Figure 1D**). However, it was observed that the degree of necrosis of the tubular epithelium of the kidney was minimal in the DAE group. The diabetic rats treated with *A. excelsa* presented near-normal glomeruli and tubules (**Figure 1E**). The DAE group revealed nearly-normal renal architectures with minimal alterations, without hyaline casts, no extravasated blood cells, and reduced glomerular congestion.

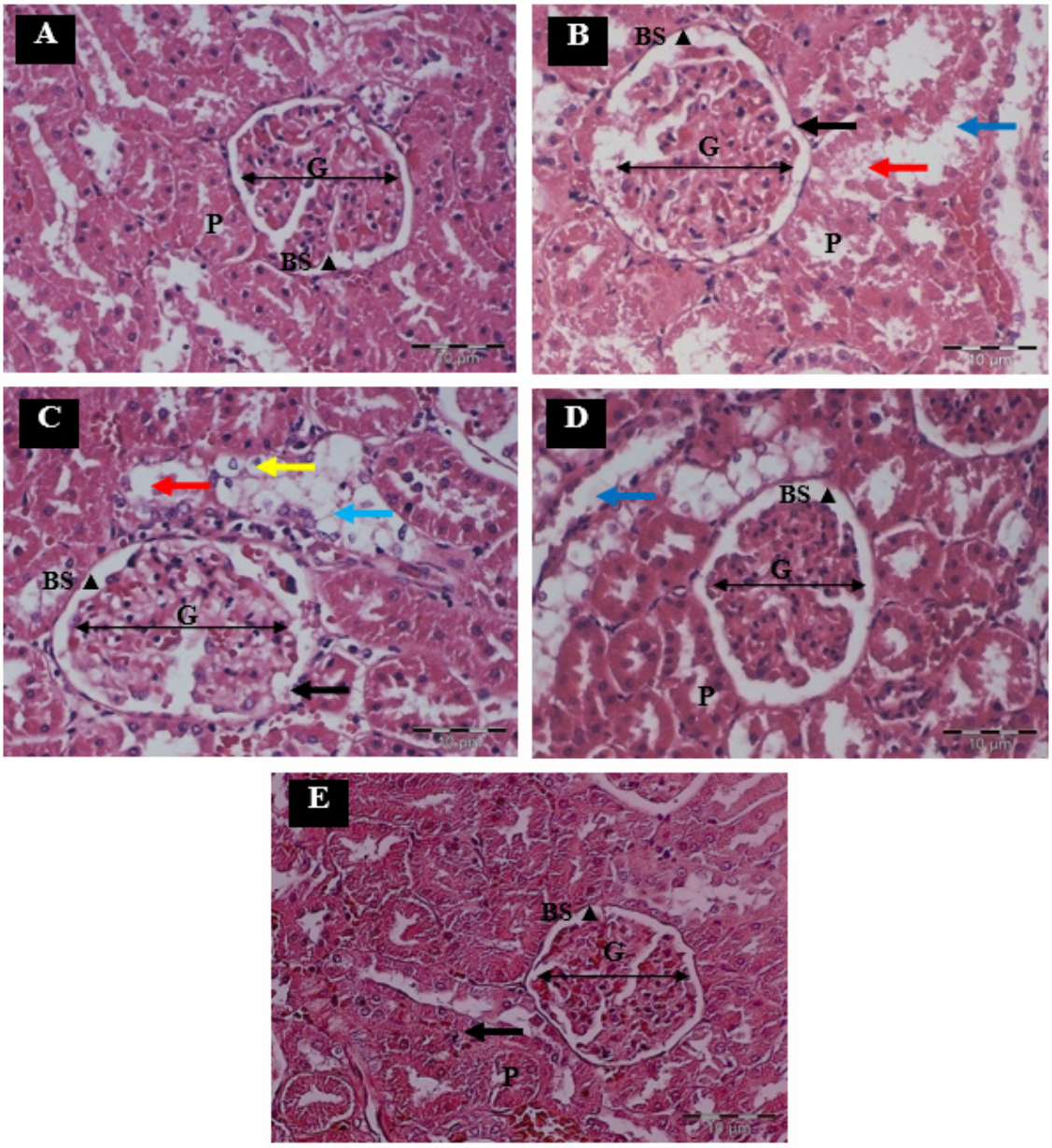

Figure 1 Light photomicrographs of glomeruli and renal tubules in rat kidneys. (A) The NC group showing normal glomeruli [**G**] with Bowman space [BS▲] and proximal convulated tubules [**P**], (B) the DC group depicting a glomerulus [**G**] with irregular capillaries [black arrow] and tubular necrosis [red arrow], (C) the DMET group showing the lumina of the tubules was closed with swollen epithelial cells [yellow arrow], (D) the DQ group revealing the degree of necrosis of the tubular epithelium was lessened by quercetin extract, but capillary loops were poorly defined [the blue arrow], and (E) the DAE group presenting near-normal glomeruli and tubules (magnification 400×).

Discussion

Fasting blood glucose

Hyperglycemia, or increased blood glucose, are the hallmark signs of DM. In this study, the DC group showed a significant increase in FBG levels (**Table 1**). The results herein confirmed that induction of STZ at 60 mg/kg bwt causes hyperglycemia in rats [39]. The FBG levels were decreased following treatment with quercetin and *A. excelsa*. The ability of quercetin to reduce blood glucose levels in rat models has been reported by Alnahdi *et al.* [40]. Moreover, it has been reported that quercetin delayed the digestion of carbohydrates in the small intestine [41,42]. Previous studies also documented that plant active compounds are very useful in achieving optimal blood glucose control [43,44]. Treatment with *A. excelsa* extract improved the FBG levels in the STZ-treated rats. Similar findings were reported by Nurdiana *et al.* [45] and Nurul`Izzati *et al.* [25]. The ability of *A. excelsa* in reducing FBG levels might be at least partially contributed to by its ability to increase plasma insulin levels [25,44]. Other contributing factors, including insulin sensitivity, reaction of glucose transporter, and glucose metabolism in the liver, require further evaluation.

Kidney oxidant and antioxidant levels

The study indicated that kidney MDA and SOD levels increased, whereas the concentration of GPx decreased, in the DC group (**Table 3**). The increase of SOD levels in kidneys of diabetic rats indicate the presence of antioxidant defense against oxidative stress. Other than that, previous studies also demonstrated that STZ treatment resulted in a lower anti-oxidant defense to protect kidney cells against ROS [46,47]. STZ is reported to promote lipid peroxidation production, which is a factor for renal injury and DN pathogenesis [10,19,48]. Results showed that the levels of SOD and GPx levels were significantly increased in the renal systems of both DMET and DQ treated groups. The DQ group indicated lower values of MDA compared with the NC group. Meanwhile, the MDA levels of the DAE group were reduced to the levels of the NC group. The findings implied that quercetin and *A. excelsa* extract improve antioxidant balance and have the ability to cope with renal oxidative stress. In agreement with the present findings, it was documented that plants can restrain lipid peroxidation, since they contain high levels of antioxidants [49]. The renal therapeutic ability of high antioxidant content of medicinal plants was also proven previously in xanthone derivatives from *Garcinia mangostana* [50]. Previous experimental work on nephrotoxicity confirmed the efficacy of quercetin in reducing oxidative injury [51]. The renal therapeutic activities in *A. excelsa* extract and quercetin treatments could also be due to this antioxidant mechanism.

GFR and kidney function parameters

The GFR is the best indicator for renal function [52]. The results showed that the DC group had a lower GFR (**Table 4**). This finding is important, because low GFR implies the progression of a kidney disease [53], as well as reflecting the retreated effects on tubular hyper-reabsorption, which is a potent cause of nephropathy injury [54]. Therefore, low GFR in the DC group indicated the existence of end-stage renal failure in STZ-treated rats [55]. Even though the results were not significant, the DMET, DQ, and DAE groups showed an increase in GFR levels as compared to the DC group, with the DAE group revealing the highest GFR among the 3 treated groups. This might suggest the potential of prolonged *A. excelsa* treatment in alleviating renal disease.

Renal function markers play important roles in the diagnosis of renal condition [56]. The results showed that the DC group had significantly increased serum creatinine and uric acid, as well as decreased serum albumin and urinary creatinine (**Table 4**). These observations support the idea that increased serum creatinine levels and decreased excretion of creatinine in the urine are indicators of the development of DN [57]. The results obtained in the DC group provided evidence of DN among the diabetic rats. However, the DC group also showed a significant increase in total bilirubin, which is a sign of overcoming the oxidative stress effect. Bilirubin has potent antioxidant properties, which were described by Zhu *et al.* [58]. Additionally, it was demonstrated that high bilirubin concentrations in serum were protective factors for the development of DN [59]. Other than that, significant increase of uric acid in the

DC group predicts the association of diabetes with 'gout' problem. A previous study already mentioned that, most commonly, kidney disease can cause gout [60,61]. However, gout itself may also lead to kidney disease [62].

Meanwhile, the level of urine creatinine was markedly increased in the DQ group as compared with the DC group. The novel finding of this study was that treatments with quercetin and *A. excelsa* caused significant decreases in serum creatinine as compared with the DC group. Additionally, treatments of quercetin and *A. excelsa* showed an increment in total bilirubin and were predicted to have renal protection effects from DN. In fact, the increase of total bilirubin and the decline of serum creatinine levels are evidence of renal therapeutic effects in STZ-induced DN [58,59]. It is also worth mentioning that the levels of uric acid were significantly reduced in the *A. excelsa* extract treatment group as compared with the DC and DMET groups. This finding implies that both treatments preserve renal therapeutic effects. Similar findings also reported the benefits of plant flavanoids in reinstating diabetic renal functions in rats [63].

Kidney histology

High doses of STZ with persistent hyperglycemia are well-known to result in renal failure [64]. The results demonstrated that the DC group is associated with structural damage in the kidney. Hence, the histology study reaffirmed structural damages, which include glomerulosclerosis, the swelling of renal cells with severe tubular degeneration, necrosis, and widening of Bowman's capsule (**Figure 1B**). Therefore, the presence of glomerulosclerosis was a major indicator of renal failure and diabetic nephropathy (DN). In fact, similar pathological changes have been described in renal tubules and glomeruli following STZ treatment [65]. The DQ group showed comparable renal morphology to those observed in the DAE group. Inconsistent with the results obtained by Bashir *et al.* [66], the quercetin-treated diabetic rats demonstrated attenuation of the kidney structure. Indeed, anti-apoptotic effects of quercetin on renal glomerular cell cultures have been reported previously [67,68]. The important finding of this study was that treatment with *A. excelsa* lessened the glomerular and tubular lesions. Thus, this study highlights the relevance of *A. excelsa* as an alternative therapeutic option for DN. Therefore, it is possible to suggest that *A. excelsa* and quercetin possess renal therapeutic effects.

Conclusions

The DC group showed disturbances in the levels of antioxidants, which were associated with decline in kidney function and structure. It is conceivable to claim that high FBG was responsible for renal injury and antioxidant imbalance in the DC group. Importantly, these findings were in support of the disruption of renal function parameters and renal morphology. Therefore, it shows that treatment with *A. excelsa* and quercetin were capable of managing oxidant and antioxidant balance in the kidney. Both of the DAE and DQ groups showed reduction in kidney MDA, with improvement of SOD and GPx levels. The *A. excelsa* treatment indicated high GPx levels after the NC group. However, the metformin treatment indicated high MDA levels, even though their SOD levels increased. Other than that, it can be concluded that antioxidant balance contributes to the preservation of renal function parameters and renal morphology in a diabetic rat model. The level of uric acid was higher in the DC and DMET groups, which indicates the possible risk of gout arthritis. Meanwhile, *A. excelsa* treatment enabled the reduction of uric acid levels to normal volume. Hence, it is suggested that the therapeutic potential of *A. excelsa*, especially in ameliorating kidney function, might be contributed to by the abundance of quercetin in the extract. Moreover, HPLC analysis in a previous study showed a high content of quercetin in *A. excelsa* extract [20].

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