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Effects of *ficus exasperata* vahl. (moraceae) leaf aqueous extract on the renal function of streptozotocin-treated rats

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The present study was undertaken to evaluate the possible reno-protective effect of Ficus exasperata leaf aqueous extract (FEE) in a rat experimental paradigm of diabetes mellitus. Forty Wistar rats (weighing 200–230 g) were divided into four (A, B, C, and D) groups, each group consisting of 10 rats. Group A rats served as 'control' animals and received citrate buffer (pH 6.3) solution in quantities equivalent to intraperitoneally-administered volumes of streptozotocin (STZ) and FEE. Diabetes mellitus was induced in Groups B and C rats by intraperitoneal injections of STZ (75 mg/kg). Group C rats were additionally treated with FEE (100 mg/kg/ /day, p.o.) 4 weeks post STZ injections, for 4 consecutive weeks. Group D rats received FEE (100 mg/kg/day p.o.) only for 4 weeks. Post-euthanisation, kidney tissues were excised for histopathological evaluation and processed for light microscopy. Plasma malondialdehyde and tissue nitric oxide were determined. Serum creatinine, blood urea nitrogen, nitrite, and albumin concentrations were measured for the evaluation of renal function. The diabetic rats significantly lost more weight and their blood glucose levels were significantly elevated as compared to the 'control' group of animals. Renal dysfunction was evidenced by kidney hypertrophy, decreased renal blood flow, and increased serum creatinine and nitrite concentrations. Furthermore, vascular dysfunction, as evidenced by decreased carotid blood flow, was observed in the diabetic rats. FEE treatment positively ameliorated the alterations in the biochemical variables in the STZ + FEE-treated rats. In conclusion, our findings suggest that FEE treatment ameliorates STZ-induced nephrotoxicity. (Folia Morphol 2012; 71, 1: 1–9)

Key words: kidney, creatinine, blood urea nitrogen, nitrite, albumin, streptozotocin, diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a major global health problem, which is now becoming an epidemic. The latest WHO publication (global burden of disease) estimates DM in adults to be around 200 million, and about two thirds of diabetics live in developing countries. It is a metabolic disease characterised by high blood glucose levels, resulting from defects in insulin secretion, insulin action, or both [14]. The prevalence of DM is increasing and it is expected to

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increase further by approximately 5.4% by 2025. An increase in sedentary lifestyle, consumption of an energy-rich diet, and obesity are some of the factors accountable for the rise in the number of diabetics. However, Asia and Africa are identified as regions with the greatest potential, where the number of diabetics could rise to two- or three-fold above the present level. In traditional African societies, phytotherapy is highly valued and widely utilised for various human ailments, including DM. Indeed, the majority of the African populations use plant materials as their only source of primary healthcare [5].

Diabetes is the most common cause of kidney failure, accounting for nearly 44% of new cases of kidney disorders [23]. Even when diabetes is controlled, the disease can lead to chronic kidney disease (CKD) and kidney failure. However, most people with diabetes do not develop CKD that is severe enough to progress to kidney failure. As the disease progresses, more albumin leaks into the urine, a situation known as "macroalbuminuria" or "proteinuria". As the amount of albumin in the urine increases, the kidney's filtering function usually begins to drop. The body then starts to retain various waste products as filtration falls. As kidney damage develops, blood pressure often rises as well.

Streptozotocin (STZ) is a broad-spectrum antibiotic with oncogenic and diabetogenic properties. The diabetogenic action is mediated by selective destruction of pancreatic beta cells, which are known as insulin producing cells. Consequently, STZ has been widely utilised as a method for inducing DM in experimental animals, and for the treatment of malignant beta cell tumours and other neoplasms in humans [22]. To produce diabetes, STZ is conventionally administered as a single injection. STZ is cleared from the blood stream rapidly (with serum half-life of 15 min). Beta cell necrosis can be detected by electron microscopy within hours after STZ injection. Elevated blood glucose levels are demonstrable within 1–2 days, while dissolution and phagocytosis of necrotic cells are observable histologically after three days [3].

Ficus exasperata (Vahl) is known locally as "sand paper tree". It grows wild uncultivated in the rainforest belt of tropical Africa. It is a medicinal plant used for treating different human diseases. The water in which the stem-bark is boiled is usually given to cows to hasten afterbirth [7]. The sap is used to arrest bleeding in Ghana [1]. The viscid, non-milky sap is used for treating sores, eye trouble, and stomach pains in Ivory Coast [2]. The sap is also used by traditional birth attendants in Congo to ease childbirth. In South Africa, scrapings of the bark are used in an embrocation for body pains, and also as a stimulant [2]. In Zaire, a leaf poultice is used in a medication for ringworm [2]. Nimenibo-Uadia [12] reported that administration of aqueous extracts of *F. exasperata* resulted in decreased plasma triacylglycerol and β-OH-butyrate levels in alloxan-treated diabetic rats.

Several *in vivo* and *in vitro* studies have demonstrated marked down-regulation of nitric oxide (NO) production, and NO synthase (NOS) abundance in diabetic animals as well as in cultured endothelial and mesangial cells subjected to simulated hyperglycaemia [4]. The present study was designed to: (i) investigate the hypoglycaemic effect of *F. exasperate* leaf aqueous extract (FEE), (ii) explore the extract's possible role in NO production as well as in renal NOS expression, and (iii) examine the plausible reno-protective effect of FEE in STZ-treated Wistar rats.

MATERIAL AND METHODS

Animal care and monitoring

This study was carried out in healthy, male and female, young adult, Wistar rats (*Rattus norvegicus*) weighing 250–300 g. The animals were housed under standard laboratory conditions of light, temperature (21 ± 1°C), and relative humidity (55 ± 5%) and were fed with standard rat pellets and drinking tap water *ad libitum*. The rats were randomly divided into four (A, B, C, and D) experimental groups. The maintenance and treatment of the animals were in accordance with the principles of the "Guide for the care and use of laboratory animals in research and teaching" prepared by the National Institute of Health (NIH) [publication 86–23, revised in 1985].

Experimental design

The animals were divided into four groups of 10 rats each. Group A rats were treated with citrate buffer (pH 6.3) solution in quantities equivalent to the administered volumes of STZ and FEE intraperitoneally. The rats in groups B and C were treated with STZ (intraperitoneal injections of STZ, 75 mg/kg). Group C rats were additionally treated with FEE (100 mg/kg/day, p.o.) 4 weeks post STZ injection, for 4 consecutive weeks. Group D rats received FEE (100 mg/kg/day p.o.) only for 4 weeks. The mice were used for acute toxicity testing of the plant's crude extract, while the rats were used for hypoglycaemic and histopathological evaluations of the plant's extract. All the animals were fasted for 16 hours, but still allowed free access to drinking tap water, before the commencement of our experiments. At the end

of our experimental period of 8 weeks, the animals were sacrificed by cervical dislocation; serum/plasma samples and kidney tissue were collected and stored at –80°C for analysis.

Plant material

Fresh leaves of *F. exasperata* Vahl [family: Moraceae] (locally known as "sand paper tree" or "*Ipin*" in Yoruba language of Western Nigeria) were collected from suburban villages of the Ile-Ife metropolis in Osun State of Nigeria, between March and April 2009. The leaves were botanically identified by the taxonomist and curator of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. A voucher specimen of the plant has been deposited in the University's Botany Departmental Herbarium.

Preparation of *F. exasperata* leaf aqueous extract

Fresh leaves of *F. exasperata* were air-dried at room temperature. One kilogram (1 kg) of the air-dried leaves of the plant was milled into a fine powder in a Warring commercial blender. The powdered leaves were macerated in distilled water and extracted twice, on each occasion with 2.5 L of distilled water at room temperature for 48 h. The combined filtered aqueous extract solubles were concentrated to dryness under reduced pressure at 60 ± 1°C in a rotary evaporator. The resulting aqueous extract was freeze-dried; finally giving 46.18 g (i.e. 4.618% yield) of a dark-green, powdery, crude leaf aqueous extract of *F. exasperata* (FEE). Aliquot portions of the crude extract residue were weighed and dissolved in distilled water for use on each day of our experiment.

Induction of experimental diabetes

Diabetes mellitus was induced in Groups B and C 'test' rats by intraperitoneal (i.p.) injections of STZ (75 mg/kg/body weight), freshly dissolved in 0.1 mol/L citrate buffer [17]. Group A 'control' rats received i.p. volumes of citrate buffer solution (pH 6.3) equivalent to the volumes of STZ administered. The 'test' animals in groups B and C became diabetic within 48 h after STZ administration. Diabetes was allowed to develop and stabilise in these STZ-treated rats over a period of 3–5 days. Group C rats additionally received oral administrations of FEE (100 mg/kg/day) daily from the 4th week post STZ treatment. All the animals in groups A, B, C, and D were kept and maintained under laboratory conditions of light, humidity, and temperature. Before the commencement of our experiments, both the normal (normoglycaemic) 'control' and STZ-treated diabetic (hyperglycaemic) 'test' rats were fasted for 16 h but still allowed free access to drinking tap water throughout. At the end of the 16-h fasting period — taken as 0 time (i.e. 0 h) — blood glucose levels (initial glycaemia, G_0) of the fasted normal and STZ-treated diabetic rats were determined and recorded.

Blood glucose determination

Blood samples (0.02 mL) were obtained from each rat by repeated needle puncture of the tail tip vein. Blood samples were taken 1 day before STZ- and FEE--treatments and also on various days after the induction of DM and the commencement of our experiment.

Blood glucose concentrations were determined by means of Bayer's Elite® Glucometer and compatible blood glucose test strips. Fasted STZ–treated rats with blood glucose concentrations ≥ 18 mmol/L were considered to be diabetic and were used in this study. The test compound (i.e. *Ficus exasperata* leaf aqueous extract [FEE, 100 mg/kg/day p.o.]) was administered orally by intragastric intubation to fasted Groups B and C rats. In both Groups B and C rats, administration of FEE (100 mg/kg) commenced as from the end of the 4th week post STZ injection, and continued for the next 4 consecutive weeks.

Common carotid arterial blood flow measurement

Under pentobarbital (50 mg/kg i.p.) anaesthesia, rats were placed in dorsal recumbency, and through a midline cervical incision the left common carotid artery was identified and carefully separated from adhering connective tissues. The carotid artery was cleared from the vagal nerve and a Transonic© flow probe (0.3 mm 1RB, Transonic Systems Inc., Itheca, NY) was carefully placed around the artery. The probe was manually positioned so that the artery was centred within its window, and the probe was held in position. Through a sterile 10 cc syringe loaded with sterile K-Y brand lubricating jelly (Johnson & Johnson, Arlington, TX), the acoustical window within the flow probe was filled while avoiding any air bubbles.

Renal arterial blood flow measurement

Under pentobarbital (50 mg/kg i.p.) anaesthesia, rats were placed in dorsal recumbency, and through a midline abdominal skin incision the left renal artery was carefully separated from renal vein and a 0.5 VB Transonic© flow probe was placed around it. The probe was manually positioned so that the artery was centred within the window and the probe was held in position. The acoustical window within the flow probe was filled with sterile

K-Y brand lubricating jelly (Johnson & Johnson, Arlington, TX). Renal and carotid artery blood flows were measured using Transonic© flow meter T206 attached to MP100 System of Biopac Systems Inc., CA, via analogue-to-digital conversion. The MP100 System was calibrated with the minimum and maximum flow capacity of the individual probe connected to the flow meter. Sampling of data was carried out at 1000 Hz and recorded by a dedicated computer using AcqKnowledge™ software.

Determination of the concentration of nitric oxide by-products (NOx)

The concentrations of endogenous NOx (nitrite + + nitrate) were determined in plasma and kidney cortex and medulla. Blood (500 μL) was collected into a heparinised specimen bottle. The blood sample was decanted and stored at –20°C until ready for use.

Renal samples were harvested from each rat and immediately homogenised with cold phosphate buffered saline (PBS) on ice, which inhibited the activity of nitric oxide synthase *ex-vivo*. The homogenate was centrifuged (3000 × g, 5 min) and the supernatant was collected. The supernatant obtained from tissues and plasma was passed through a 1.2 μm multiscreen filter plate. Plasma and tissue nitrite/nitrate levels were determined by converting the nitrate to nitrite, using enzyme nitrate reductase (Boehriger Mannheim) followed by the addition of Griess Reagent to colourimetrically quantify the nitrite concentration [18]. The plasma was diluted 1:5 in PBS before a 25-μL aliquot was added to a mixture of 25 μL nitrate reductase (1.5 U/mL) and 25 μL of NADPH (0.134 mg/mL), both prepared in 40 mM Tris, pH 7.6. The samples were thereafter incubated at room temperature for 3 h. Following this incubation period, 100 μL of Griess Reagent (1:1 mixture of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl-ethylenediamine) was added and incubated for a further 10 min at room temperature.

The absorbency of the samples was measured at 540 nm with a 650 nm reference. The concentration of nitrite/nitrate was determined from a standard curve of sodium nitrate, and calculated as μmol/g protein in tissues. Protein in the supernatant obtained from samples was determined using standard Lowry method [11].

Thiobarbituric acid reactive substances

The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) was measured by a modified method of Ohkawa et al. [13]. For each sample to be assayed, four tubes were set up containing 100, 150, 200, and 250 μL of plasma, 100 μL of 8.1% SDS, 750 μL of 20% acetic acid, and 750 μL of 0.8% aqueous solution of TBA. The volume was made up to 4 mL with distilled water, mixed thoroughly, and heated at 95°C for 60 min. After cooling, 4 mL of n-butanol was added to each tube, the contents mixed thoroughly, and then centrifuged at 3000 rpm for 10 min. The absorption of the clear, upper (n-butanol) layer was measured using a Shimadzu (Japan) UV-1601 spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5/ /cm/M, and was expressed in μmol TBARS/L.

Renal histopathology

Renal tissues were excised from sacrificed animals, individually weighed, and thin kidney slices were cut, fixed in 4% paraformaldehyde, and sequentially embedded in paraffin wax blocks. Tissue sections of 5 μm thickness were cut and stained with haematoxylin-eosin (H-E) and Masson's trichrome [20] for conventional morphological evaluation, and subsequently examined under light microscope (BX50; Olympus, Tokyo). Images were obtained by a digital camera system (Pixcera Co., Osaka, Japan) attached to the microscope. A minimum of 10 fields for each kidney slide were examined and scored semi-quantitatively for severity of changes by a pathologist unaware of the type of treatment. The scoring was done as none (–), mild (+), moderate (++), and severe (+++).

Determination of creatinine (Cr) and blood urea nitrogen levels and clearances (C_{cl})

Before sacrifice, the rats were kept individually in metabolic cages (TP-85M; Toyoriko, Tokyo). After 4–5 days of adaptation, urine samples were collected in ice-cooled glass flasks every 8 h for 24 h and stored at 4°C. Urine samples from each rat were combined and measured. After centrifugation, aliquots of the supernatant were frozen at –40°C. Rats were sacrificed (under light anaesthesia with halothane) by decapitation. The abdomen was opened by transverse incision, and the kidneys were excised for histological studies and enzymatic analysis. Blood samples were collected by cardiac puncture into heparinised containers. Blood urea nitrogen (BUN) values as indicators of renal damage were determined by NADH-coupled enzymatic method, using urease spectrophotometrically in an enzyme-based assay [8]. Urine and serum samples were assayed for creatinine using the spectrophotometric assay method of Junge et al. [10]. For the assay, urine

Table 1. Body and kidney weights of 'control', STZ-, STZ + FEE-, and FEE-treated rats and urinary volume per day

Parameter	Control	STZ	STZ + FEE	FEE
Body weight [g]	240.1 ± 2.0	210.3 ± 0.4*	232.2 ± 2.6	242.4 ± 3.2
Kidney weight [g]	0.94 ± 0.32	1.47 ± 0.91*	1.03 ± 0.14	0.96 ± 0.12
Urine volume [mL/day]	20.1 ± 2.3	38.2 ± 1.4*	24.4 ± 0.3	20.7 ± 3.2

Values are expressed as means ± SEM of 8–10 rats for all groups; *significant difference ($p < 0.05$) in the same row between various treatments and 'control' group A rats

was diluted 1:50 in distilled water. Three microliters of plasma or diluted urine and 300 μL picric acid reagent (consisting of saturated aqueous picric acid diluted 5-fold in 0.25 M NaOH) were dispensed in duplicate, using a MicroLab dilutor (Hamilton, Reno, NV) into wells of a microtiter plate. After incubation for 15 min at room temperature, absorbance was read at 515 nm in a microplate reader. Creatinine clearance was calculated according to the Cockcroft-Gault formula (Cockcroft and Gault, 1976). $C_{cr} = (C_u/C_s) \times V$, where C_u is the concentration of creatinine in urine, C_s is the concentration of creatinine in serum, and V is the urine flow rate in millilitres per minute.

Serum and urine albumin concentrations

Serum albumin concentration was measured in a Multistat III Plus machine with reagents from Fisher Scientific (St. Louis, MO). Urine samples were collected in test tubes and stored at –40°C for analysis. The concentration of urinary albumin was determined using standard diagnostic kits, an enzyme-linked immunosorbent assay (ELISA) (Nephrat Exocell Inc., Philadelphia, PA, USA).

Assessment of urine/serum and tissue nitrite concentration

Urine samples were deproteinised by equal volumes of 0.3 M NaOH and 5% zinc sulphate and centrifuged at 6400 × g for 20 min. The supernatants were added in duplicates to 96-well plates and reacted with Greiss Reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water). Also, serum and tissue nitrite were estimated using Griess Reagent and served as an indicator of NO production; 500 μL of Greiss Reagent was added to suitably diluted 100 μL of serum and supernatant of the tissue as described above and corrected by protein amount. Nitrite levels were assessed by measurement of absorbance at 540 nm using a microplate reader (Model 550; Bio-Rad laboratories, Hercules, CA) [6]. Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as μmol/mL in serum and urine and as μmol/mg protein in homogenate, respectively.

Statistical analysis

Results are presented as means ± EM). Analysis of variance (ANOVA) with *post hoc* multiple comparison test and Wilcoxon signed-rank test were used in statistical analysis of the data. A p values less than 0.05 were considered significant.

RESULTS

General characteristics of the animals

The untreated diabetic group of rats exhibited a significant ($p < 0.05$) body weight loss during the 4-week study period. FEE treatment prevented diabetes-induced weight loss and facilitated the growth of animals, albeit at a moderately slower rate than seen in the 'control' normal animals (Table 1). Administration of STZ also resulted in a significant ($p < 0.05$) increase in kidney weight when compared with the normal 'control' and FEE-treated groups of rats. The FEE treated group of rats showed insignificant changes in kidney and body weights (Table 1).

Urine/serum and tissue nitrite levels

STZ treatment significantly increased ($p < 0.05$) urine, serum, and tissue nitrite levels in STZ-treated rats. *F. exasperata* treatment significantly decreased ($p < 0.05$) the elevated nitrite levels in urine, serum, and tissues of STZ + FEE-treated rats. However, FEE *per se* has no significant effect ($p > 0.05$) on urine, serum, and tissue nitrite levels of group D rats (Fig. 1).

Serum creatinine, blood urea nitrogen, and albumin levels

STZ treatment significantly increased ($p < 0.05$) serum creatinine, BUN, and urine albumin levels as compared with the 'control' group of rats. However, serum albumin levels were significantly lower ($p < 0.05$) in STZ-treated rats (Table 2). Although treatment of the diabetic rats with FEE significantly prevented a rise in serum creatinine, BUN, and urine albumin, the

Table 2. Assessment of renal functions in the experimental animal groups treated with STZ and FEE

Parameter	Control	STZ	STZ + FEE	FEE
Serum creatinine [mg/dL]	0.66 ± 1.0	2.57 ± 2.1*	0.85 ± 1.2	0.65 ± 1.8**
Urine creatinine [mg/dL]	58.7 ± 1.7	97.1 ± 1.6*	65.3 ± 1.1	59.2 ± 1.4**
Serum urea [mg/dL]	22.9 ± 1.2	92.3 ± 2.1*	26.1 ± 1.7	19.9 ± 3.1**
Urine urea [mg/dL]1	89.6 ± 3.0	109.1 ± 2.0*	95.2 ± 5.0	90.4 ± 5.0**
Urine albumin [mg/dL]	12.5 ± 1.2	29.7 ± 1.5*	16.4 ± 1.2	12.2 ± 1.4**
Serum albumin [mg/dL]	25.5 ± 3.7	13.9 ± 1.9*	26.9 ± 1.2	27.2 ± 1.2**
Creatinine clearance [mL/min]	1.12 ± 0.3	0.56 ± 0.6*	1.09 ± 0.3	1.14 ± 0.3**
Urea clearance [mL/min]	1.42 ± 0.1	0.41 ± 0.4*	1.31 ± 0.1	1.41 ± 0.5**

Values are expressed as means ± SEM of 8–10 rats; *statistically significant at $p < 0.05$ compared to control; **statistically significant at $p < 0.05$ compared to STZ-treated

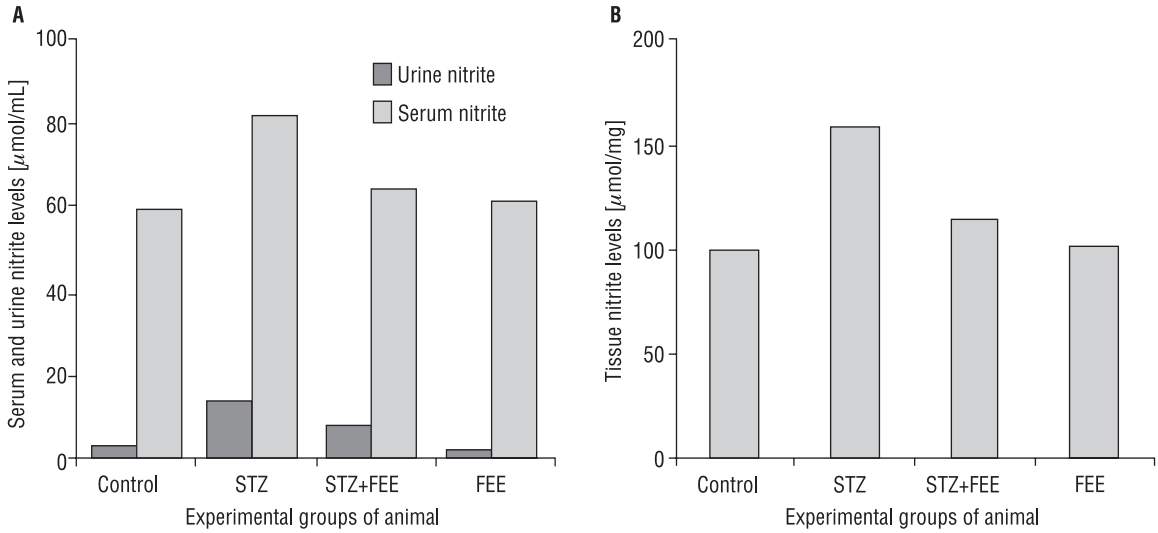

Figure 1. Changes in serum, urine (**A**), and tissue (**B**) nitrite concentrations in rats with renal injury after streptozotocin (STZ) and *F. exasperate* leaf aqueous extract (FEE) treatments. Results are expressed as means of 8–10 observations.

extract at the same time increased serum albumin in the STZ + FEE group of rats (Table 2). Creatinine and urea clearance, which were markedly decreased in STZ--treated rats, were significantly increased by FEE treatment (Table 2), but the extract did not restore creatinine and urea clearance values to the level seen in the normal, 'control' animals. FEE treatment *per se*, however, had no significant effect ($p > 0.05$) on these biochemical variables in group D rats (Table 2).

Renal and carotid blood flow

Induction of STZ diabetes produced a significant increase ($p < 0.05$) in renal and carotid blood flow throughout our experimental period (Fig. 2). Control and FEE-treated (group D) rats exhibited normal renal and carotid blood flow. A remarkable improvement was seen in group C rats treated with FEE (Fig. 2).

Urine/serum and tissue nitrite levels

Administration of STZ significantly increased ($p < 0.05$) urine, serum, and tissue nitrite levels in STZ--treated rats. *F. exasperata* leaf aqueous extract treatment significantly decreased ($p < 0.05$) the elevated nitrite levels in urine, serum, and tissues of STZ + + FEE-treated rats. However, FEE *per se* had no significant effect ($p > 0.05$) on urine, serum, and tissue nitrite levels of group D rats (Table 2, Fig. 1).

Plasma and kidney malondialdehyde results

The untreated diabetic animals exhibited a marked increase ($p < 0.05$) in both plasma MDA levels and kidney MDA contents when compared with 'control' and FEE-treated rats (Fig. 3). This observation probably suggests an increased ROS-mediated lipid peroxidation in the diabetic animals. FEE

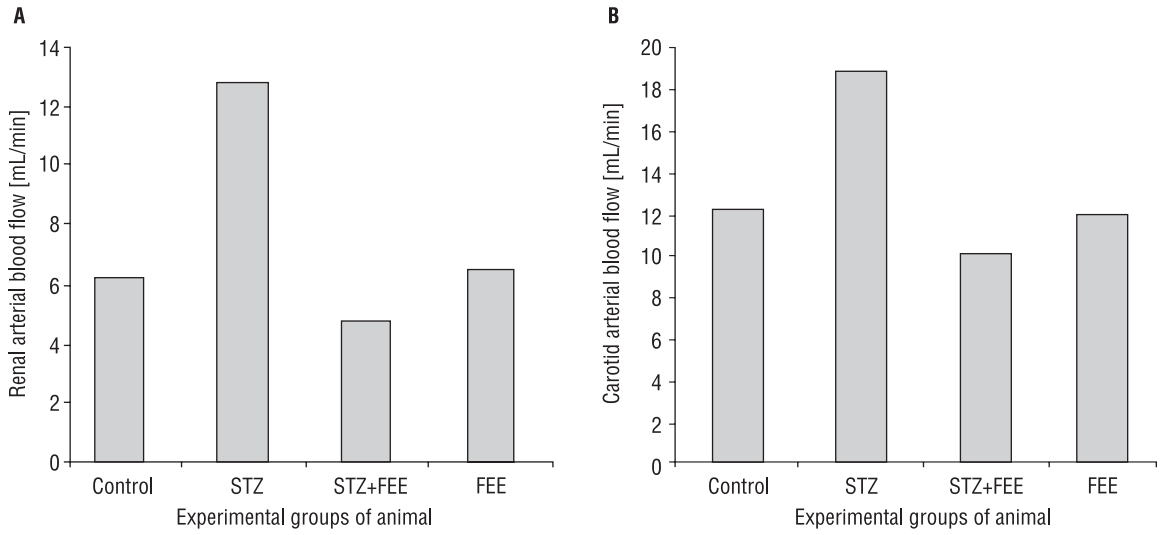

Figure 2. Changes in (**A**) renal arterial blood flow and (**B**) carotid arterial blood flow (n = 6).

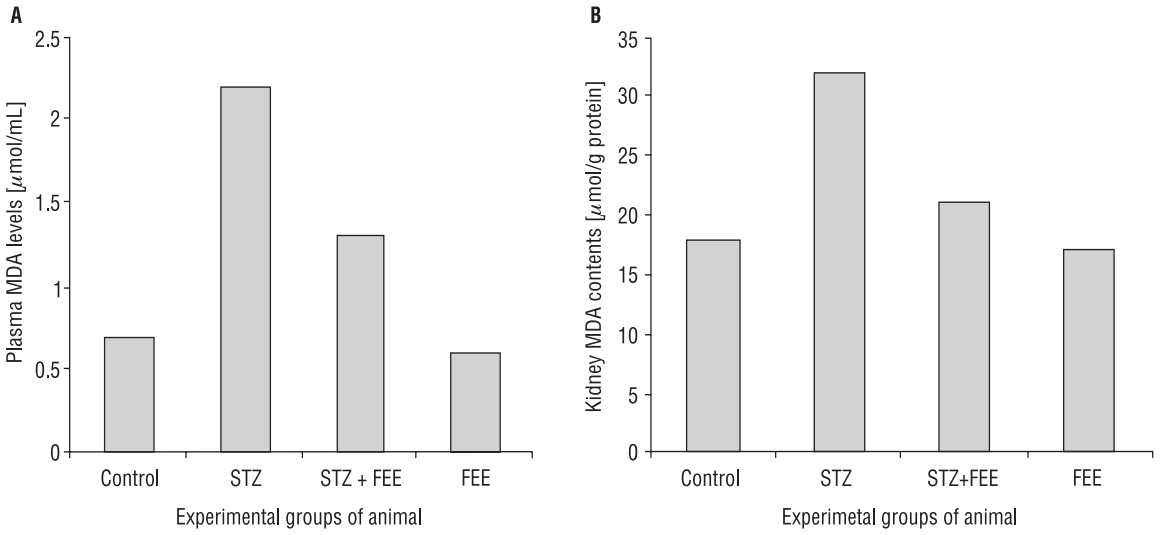

Figure 3. Changes in (**A**) plasma malondialdehyde (MDA) levels and (**B**) kidney malondialdehyde (MDA) contents in rats with renal injury after STZ and FEE treatments. $P < 0.05$ versus 'control' and FEE-treated rat groups.

treatment resulted in a significant but incomplete reduction ($p < 0.05$) in plasma and kidney MDA concentrations in diabetic animals (Fig. 3).

Histopathological findings

STZ administration alone caused prominent histopathological damage in the kidney compared with 'control' rats. Kidney sections from 'control' and FEE-treated groups of rats showed normal glomeruli and tubulo-interstitial cells (Fig. 4A, 4D). In contrast, the kidneys of STZ-treated rats showed marked deleterious histological changes. The principal findings within the STZ-treated kidney sections were glomerular and tubular degenerations, varying from thickening of periglomerular arteriolar walls and glomerular basement, interstitial inflammation, and tubular cell swelling. STZ exposure also aggravated loss of peritubular capillaries (Fig. 4B). The FEE-treated group of rats preserved normal morphology of the kidney and showed normal architecture of the kidney (Fig. 4C).

DISCUSSION

In this study we examined the effects of *F. exasperata* leaf aqueous extract on the renal function of STZ-treated rats. The most impressive finding, however, is related to the histological findings. Indeed,

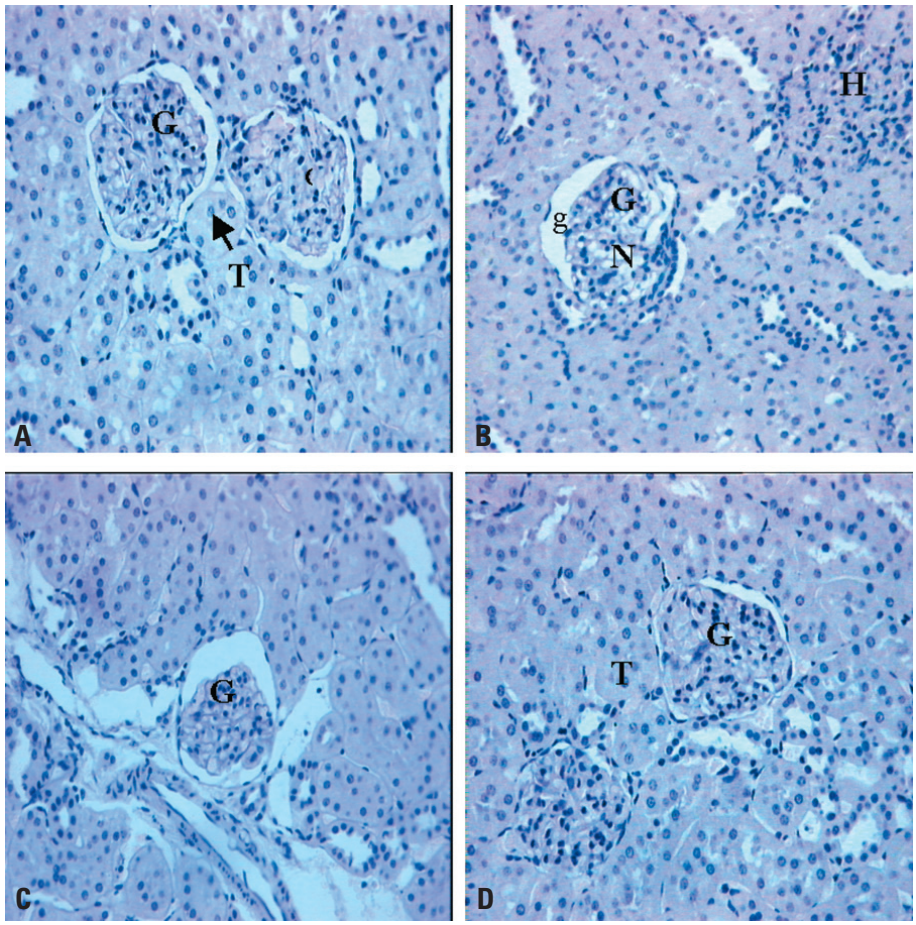

Figure 4. Photomicrographs of kidney sections stained with haematoxylin-eosin and Masson's trichrome under light microscope. **A.** 'Control' rats showing normal rat kidney with normal tubular brush-borders (T) and intact glomeruli (G). No evidence of congestion or inflammation was observed in the sinusoid; **B.** Streptozotocin-treated rats showing loss of tubular brush-borders, glomerular hypercellularity (H), and thickened glomerular basement membrane (g); **C.** STZ + FEE-treated rats showing attenuated hypercellularity with improved tubular and glomerular architecture; **D.** FEE-treated rats showing normal morphology of the kidney. X 200.

one of the major effects of STZ treatment was to cause a marked worsening of microvascular injury, as characterised by arteriolar thickening and peritubular capillary loss, which was associated with more tubular injury and greater interstitial inflammation. The development of preglomerular microvascular disease can be a mechanism for renal dysfunction progression since structural changes alter renal autoregulation and favour development of glomerular hypertension ("the Herrera hypothesis") [9].

Oxidative stress contributes to renal injury, and antioxidant supplementation mitigates renal disease in diabetic and non-diabetic rats [15]. The untreated diabetic animals in this study showed significant increases in plasma MDA concentration and marked elevation of kidney MDA content. These findings suggest ROS-mediated modifications of lipid, carbohydrate, and protein molecules, denoting the presence of oxidative stress, which is a known feature of DM [16]. Interestingly, tissue, serum, and urine nitrite were significantly higher in the untreated diabetic animals, probably due to the presence of oxidative stress that could have raised nitrite abundance via ROS-NO interaction. This phenomenon can be explained, in part at least, by the NO production coupled with elevation of glucose and free fatty acids, rendering them the dominant targets of ROS in the untreated diabetic rats. Accordingly, the by-products of ROS interaction with glucose and fatty acids (MDA) were increased in diabetic animals. This phenomenon, together with attenuation of tubuloglomerular feedback response (occasioned by enhanced proximal tubular sodium reabsorption) [21], contributes to the associated rise in renal blood flow and glomerular filtration rate.

FEE administration resulted in marked but incomplete reductions in plasma glucose and MDA concentrations and tissue MDA contents. This reduction was coupled with a significant rise in renal urea and creatinine clearance rate. The latter could be due to a constellation of up-regulation of NOS isoforms, residual oxidative stress associated with good glycaemic control, and reduction in competition by glucose and fatty acids for interaction with ROS. In contrast, a number of other studies have shown up-regulation of NO production and NOS expression in diabetic animals and cultured endothelial cells exposed to high glucose concentration [19].

Uncontrolled diabetes of 4 weeks duration in rats is associated with enhanced lipid, glucose, and protein oxidation, renal dysfunction, hypertension, down-regulation of NOS expression, and reduced nitrotyrosine abundance. It can be concluded that administration of FEE to STZ-treated diabetic rats decreased blood glucose levels. This hypoglycaemic effect of the extract may be due to depression of key gluconeogenic enzymes, an increase in the levels of glucose transporters, and/or stimulation of glucose uptake in skeletal muscle cells. Another obvious effect of this plant's extract is to preserve nephron and renal functions, resulting in a significant improvement in deranged renal function.

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