

# Renoprotective Effects of *Moringa oleifera* Leaf Extract in Streptozotocin-Induced Diabetic Male Rats

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**Abstract.** Diabetes mellitus is a major global health challenge, often leading to severe metabolic and systemic complications such as diabetic nephropathy. Hyperglycemia and oxidative stress contribute significantly to renal impairment, characterized by glomerular hypertrophy, basement membrane thickening, and elevated plasma creatinine. This study investigated the renoprotective potential of aqueous *Moringa oleifera* leaf extract in streptozotocin (STZ)-induced diabetic male Sprague Dawley rats. A total of 40 rats were divided into four groups: normal control, negative control (diabetic untreated), positive control (diabetic treated with 500 mg/kg metformin), and treatment group (diabetic treated with 400 mg/kg *Moringa oleifera* leaf extract). Treatments were administered orally for 14 and 21 days, respectively. Renal parameters assessed included kidney weight, glomerular diameter, plasma creatinine levels, and histological alterations. Diabetic control rats exhibited marked renal abnormalities with glomerular disruption, tubular inflammation, and significantly elevated plasma creatinine. Administration of *Moringa oleifera* leaf extract, however, reduced plasma creatinine levels significantly compared to diabetic controls, restored glomerular architecture, and attenuated kidney hypertrophy and glomerular enlargement after 21 days. *Moringa oleifera* leaf extract demonstrated renoprotective effects likely mediated by its antioxidant bioactive compounds. These findings support its potential role as a complementary therapeutic agent for managing diabetic nephropathy.

**Keywords:** antioxidant, diabetic nephropathy, *Moringa oleifera*, oxidative stress, renoprotection

## 1 Introduction

Diabetes mellitus is a chronic metabolic disorder associated with metabolic and systemic complications leading to irreversible organ damage [1]. Metabolic complications include diabetic ketoacidosis, hyperglycemia, polydipsia, polyuria and visual disturbances. The systemic complications include diabetic foot injury due to impaired blood flow to the lower

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extremities, which can lead to ulcer formation and bacterial infections, ultimately requiring amputation if untreated [2]. Other major complications include retinopathy [3], neuropathy [4], and nephropathy [5].

Diabetic nephropathy (DN), also referred to as diabetic kidney disease (DKD) or chronic kidney disease (CKD), is characterized by the presence of microalbuminuria and proteinuria, leading to end-stage renal disease (ESRD) [6]. The histological changes in diabetic kidneys include glomerular hypertrophy, mesangial cell proliferation, thickening of the basement membrane, and accumulation of extracellular matrix proteins [7]. Oxidative stress caused by excessive reactive oxygen species (ROS) disrupts renal structure and function [8], contributing to glomerulosclerosis and renal hypertrophy [9].

Medicinal plants rich in antioxidants may offer renoprotective benefits by reducing oxidative stress. *Moringa oleifera* is widely consumed in Asia and Africa, known for its rich antioxidant profile and therapeutic uses in addressing sexual dysfunction, hypertension, and diabetes [10]. Previous studies demonstrated that *Moringa* leaf extract reduced glucose, creatinine, and urea levels [11] in a diabetic model. This study investigates the renoprotective effects of aqueous *Moringa oleifera* leaf extract on renal structure and function in STZ-induced diabetic male rats.

## **2 Methodology**

### **2.1 Animal preparation**

All experimental procedures in this study were approved and conducted according to the ethical guidelines given by the Animal Ethics Committee, Faculty of Medicine, Universiti Kebangsaan Malaysia (FST/2017/MAHANEM/29-MARCH/833-MARCH-2017-FEB. 2019). 40 male Sprague Dawley rats were divided into two main groups: Group A (14 days, N=20) and Group B (21 days, N=20). Each group was further subdivided into four subgroups (n=5): normal control (distilled water), negative control (STZ-induced diabetic, untreated), positive control (STZ-induced, diabetic treated with 500 mg/kg metformin), and treatment group (STZ-induced diabetic, treated with 400 mg/kg *Moringa oleifera* leaf aqueous extract). Diabetes was induced by intravenous injection of STZ (50 mg/kg body weight) via the tail vein. Rats with fasting blood glucose levels more than 13 mmol/L after one week were included. Treatments were administered orally via feeding gavage.

### **2.2 Kidney analysis**

The right kidney was weighed, washed in saline, and fixed in 10% neutral buffered formalin (NBF). Tissue processing, paraffin embedding, and sectioning were performed, followed by hematoxylin-eosin (H&E) staining. Histological analysis focused on glomerular diameter ( $\mu\text{m}$ ) and structural changes.

### **2.3 Plasma creatinine analysis**

Blood samples were collected via cardiac puncture. Plasma creatinine levels were measured using a Rat Creatinine (Cr) ELISA kit (FineTest, China) according to the manufacturer's protocol.

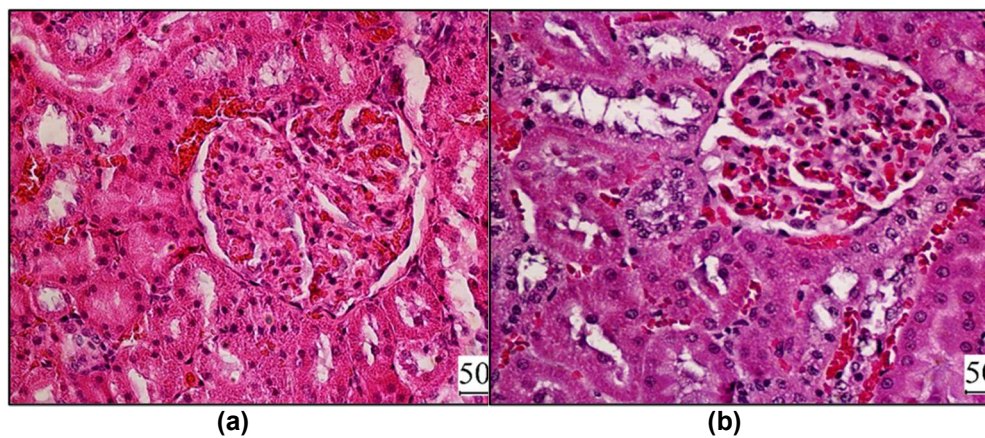
### **2.4 Statistical analysis**

Data were tested for normality and analyzed using one-way ANOVA in IBM SPSS Statistics. Post hoc comparisons were performed using Tukey's test. Results were presented as mean  $\pm$  SEM, and statistical significance was set at  $p < 0.05$ .

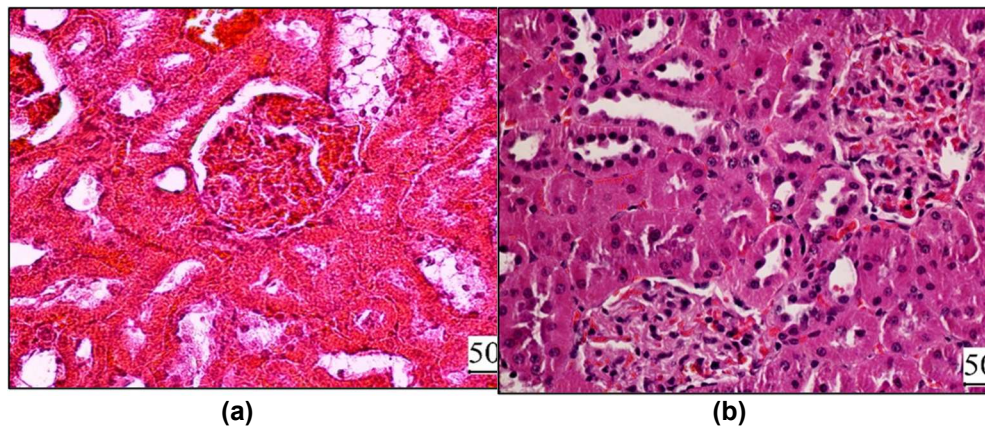
### 3 Results

#### 3.1 Kidney histology

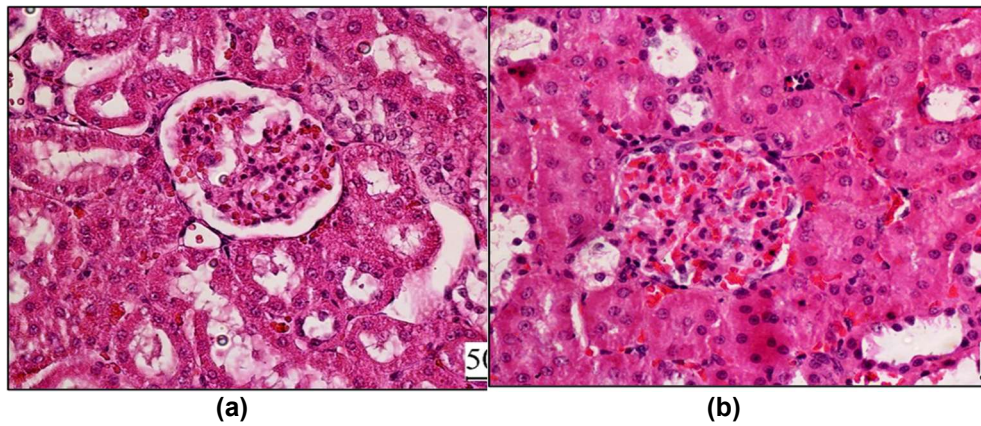
Negative control diabetic rats exhibited a distorted glomerular structure, tubular inflammation, thickened basement membranes, and hypertrophy of proximal tubular cells. In contrast, *Moringa oleifera*-treated rats showed improved glomerular structure with reduced thickening of the basement membrane and restoration of proximal tubules. Normal control rats showed intact kidney histology. Figures 1-4 show cross-sections of rat kidneys stained with Hematoxylin and Eosin (H&E) for all experimental groups: normal control, negative control, positive control, and *Moringa oleifera* treatment group.



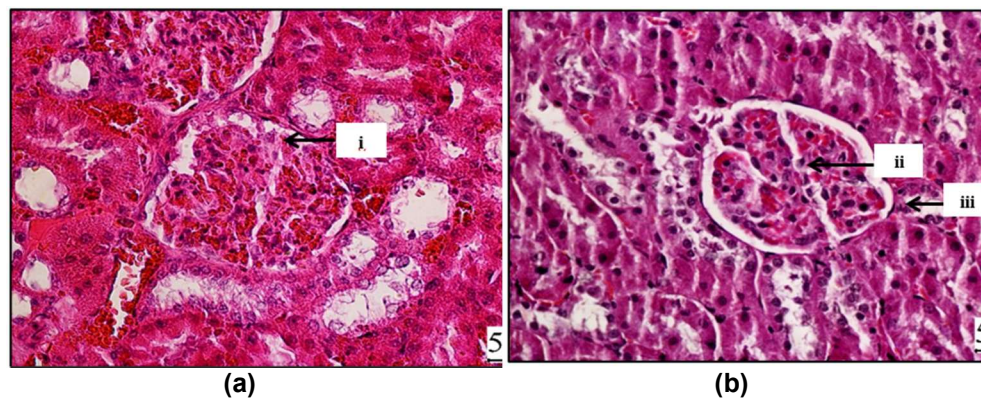
**Fig 1.** Cross-section of kidney stained with Hematoxylin and Eosin (H&E) showing the normal control group after (a) 14 days and (b) 21 days of treatment at  $\times 400$ .



**Fig 2.** Cross-section of kidney stained with Hematoxylin and Eosin (H&E) showing the negative control group after (a) 14 days and (b) 21 days of treatment at  $\times 400$  magnification.

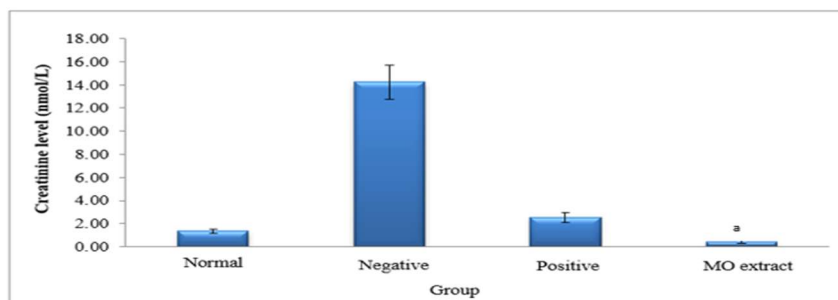


**Fig 3.** Cross-section of kidney stained with Hematoxylin and Eosin (H&E) showing the positive control group after (a) 14 days and (b) 21 days of treatment at  $\times 400$  magnification.



**Fig 4.** Cross-section of kidney stained with Hematoxylin and Eosin (H&E) showing the *Moringa oleifera* leaf extract treatment group after (a) 14 days and (b) 21 days of treatment at  $\times 400$  magnification; (i) Bowman's capsule, (ii) glomerulus, and (iii) proximal convoluted tubule (PCT).

### 3.2 Plasma creatinine analysis



**Fig. 5.** The bar chart illustrates plasma creatinine levels in the control and *Moringa oleifera* leaf extract treatment groups after 21 days. A significant difference ( $p < 0.05$ ) was observed compared with the <sup>a</sup>negative control group.

After 21 days of treatment, plasma creatinine was significantly lower ( $p < 0.05$ ) in *Moringa oleifera*-treated rats ( $0.42 \pm 0.14$  nmol/mL) compared to diabetic controls ( $14.25 \pm 1.48$  nmol/mL). Metformin-treated rats also showed a reduction in creatinine levels ( $2.53 \pm 0.41$  nmol/mL), while normal controls recorded  $1.34 \pm 0.15$  nmol/mL (Figure 5).

### 3.3 Kidney weight and the diameter of the glomerular

At 14 days, no significant changes were observed. However, after 21 days, Moringa treatment significantly reduced kidney weight ( $1.26 \pm 0.10$  g) and glomerular diameter ( $106.30 \pm 5.26$   $\mu$ m) compared to the negative control group ( $1.74 \pm 0.10$  g;  $135.76 \pm 4.57$   $\mu$ m,  $p < 0.05$ ) (Table 1).

**Table 1.** Analysis of kidney weight and the diameter of glomerular after 14 and 21 days of treatment.

Group	Kidney weight (g)		Diameter of glomerular ( $\mu$ m)	
	14 days	21 days	14 days	21 days
Normal	$1.50 \pm 0.19$	$1.28 \pm 0.12$	$124.06 \pm 5.07$	$71.80 \pm 9.03$
Negative	$1.50 \pm 0.15$	$1.74 \pm 0.10$	$119.98 \pm 12.10$	$135.76 \pm 4.57$
Positive	$1.26 \pm 0.12$	$1.36 \pm 0.14$	$102.94 \pm 6.58$	$116.22 \pm 7.19$
MO extract	$1.54 \pm 0.21$	$1.26 \pm 0.10^a$	$131.13 \pm 10.37$	$106.30 \pm 5.26^a$

Significant at  $p < 0.05$  compared with the <sup>a</sup>negative control group.

## 4 Discussion

This study demonstrates that aqueous *Moringa oleifera* leaf extract confers significant renoprotective effects in STZ-induced diabetic rats. Diabetic nephropathy is characterized by glomerular hypertrophy, thickening of the glomerular basement membrane, and increased creatinine levels [12]. In agreement with previous reports, STZ-induced diabetic rats in this study showed marked renal damage. Treatment with *Moringa oleifera* significantly reduced plasma creatinine, kidney hypertrophy, and glomerular enlargement, suggesting improved renal function. These effects may be attributed to its high antioxidant content, which scavenges ROS and reduces oxidative stress [13]. Flavonoids and terpenoids in Moringa may also stimulate insulin secretion from pancreatic  $\beta$ -cells, contributing to improved glycemic control and reduced renal stress [14]. The findings are consistent with earlier studies that reported *Moringa oleifera* extracts reduce creatinine, urea, and proteinuria in diabetic rats [15].

## 5 Conclusion

Aqueous *Moringa oleifera* leaf extract exhibits potent renoprotective effects in STZ-induced diabetic male rats. Oral administration for 21 days significantly reduced plasma creatinine, improved renal histology, and attenuated kidney hypertrophy and glomerular enlargement. These results suggest that *Moringa oleifera* may serve as a complementary therapeutic agent in managing diabetic nephropathy.

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