ORIGINAL RESEARCH REPORT

Effect of Vasicine on Kidney Histomorphology in Streptozotocin-Induced Diabetic *Wistar* Rats

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ABSTRACT

Background: *Justicia adhatoda* is a perennial shrub with various potential quinazoline alkaloids, including vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, and vasicinol. **Objective**: The study aimed to investigate the effect of vasicine, a quinazoline alkaloid, on diabetes-associated nephropathy caused by streptozotocin in rats. **Material and Method**: Vasicine was extracted from the leaves of *Justicia adhatoda*. Four groups of thirty male Wistar rats were used: the negative control group (received plain water orally), the positive control group (received intraperitoneal administration of Streptozotocin at 55 mg/kg body weight dissolved in citrate buffer, pH 4.5), and treatment groups III and IV, which received glibenclamide (5 mg/kg body weight dissolved in 0.5% DMSO) and vasicine (0.9 mg/kg body weight dissolved in 0.5% DMSO), respectively. Blood glucose was monitored weekly. Serum analysis for creatinine and blood urea nitrogen was performed. On day 28, the kidneys were removed and prepared for routine histopathological observation using H&E staining. **Result**: The histopathological changes in the kidneys were consistent with the biochemical values. Vasicine treatment helped restore renal histoarchitecture with a few areas of vacuolation in the cortex and medulla, and reduced serum creatinine and blood urea nitrogen levels significantly (p=0.01 and p=0.180, respectively). **Conclusion**: Vasicine extracted from *Justicia adhatoda* may help protect against diabetic nephropathy.

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Highlights

1. *Justicia adhatoda* leaves can be used as herbal medicine to treat diabetes and its associated conditions.
2. Vasicine in Justicia leaves helps protect kidney health in individuals with diabetes.

BACKGROUND

Diabetic nephropathy (DN), commonly referred to as diabetic kidney disease, is a chronic condition that impairs the kidneys' ability to function properly in individuals with type I and type II diabetes. The etiology of diabetic kidney disease (DN) is primarily excessive blood sugar, which damages the kidney's blood vessels and causes dysfunction (Rao, et al., 2019). Traditionally, abnormal homeostasis, including hemodynamic abnormalities, metabolic disorders, and hormone synthesis, such as angiotensin II, contributes to the development of diabetic kidney disease (DN). DN progresses through 5 stages, with microalbuminuria being the first disorder to occur, followed by macroalbuminuria and a decline in kidney function (Samsu, 2021).

Just like human diabetic nephropathy, which causes changes in the glomeruli and tubulointerstitial lesions, a standard animal model of the disease should also exhibit rising albuminuria and a decline in renal function (Kitada, et al., 2016).

Justicia adhatoda L., of the family Acanthaceae, is known to treat various disorders. Commonly referred to as vasaka, this evergreen perennial shrub is found throughout the tropical regions of Southeast Asia, including India (Dhankhar, et al., 2011). The plant contains several important substances, including phytosterols, glycosides, polyphenolics, and alkaloids. The leaves contain two main alkaloids: vasicinone and vasicine. According to Sharma, et al., (2016), these alkaloids possess antiallergic and antiasthmatic properties. In addition to vasicine and vasicinone, the leaves also contain vasicoline, vasicolinone, vasicinol, adhatodine, adhatonine, and anisotine (Yadav & Yadav, 2018).

For centuries, *Justicia adhatoda* has been used to treat conditions such as asthma and chronic bronchitis, as well as to stimulate uterine contractions during childbirth. In addition to these primary uses, the plant has been found to have insecticidal, anti-ulcer (Singh & Tiwari, 2016), and wound-healing properties (Khandelwal, et al., 2024). However, D'souza, et al., (2021) observed that ethanolic extracts of *Justicia adhatoda* leaves had a significant effect on diabetic rat kidneys.

In the present study, it was hypothesized that vasicine, derived from *Justicia adhatoda* leaves, could be a potential drug candidate for the treatment of diabetic nephropathy. The aim of this study was to investigate the role of vasicine, a quinazoline alkaloid obtained from *Justicia adhatoda* leaves, in diabetic rat kidneys, since ethanolic extracts of the plant have been shown to significantly improve kidney function.

OBJECTIVE

The study aimed to investigate the effect of vasicine, a quinazoline alkaloid from *Justicia adhatoda* leaves, on treating diabetic nephropathy induced with streptozotocin in rats.

MATERIAL AND METHOD

This study was an analytical observational study using control and treatment groups, conducted from October 2023 to June 2024 at the Pharmacovigilance Laboratory for Animal Feed and Food Safety and the Histology Laboratory at the Department of Veterinary Anatomy and Histology, Madras Veterinary College, Chennai, India.

Preparation of plant extract: Fresh *Justicia adhatoda* leaves were collected from the botanical garden of the Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), TANUVAS. Vasicine was extracted and stored at 4°C for further use (Ravali, et al., 2024).

Chemicals: Streptozotocin (Catalogue no. A10868) was obtained from Zanosar to induce diabetes. Glibenclamide (Catalogue no. 15009) from Cayman Chem was used as a standard drug. Biochemical analysis was performed using an automatic serum analyzer.

Experimental animals: Thirty-two male Wistar albino rats, aged 8–10 weeks, were procured from the Laboratory Animal Unit, TANUVAS, Chennai, India. The animals were housed under standard laboratory conditions with a room temperature of 22±5°C, 55% humidity, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and ad libitum water (Yu & Shang, 2014).

Experimental design: The 30 male Wistar rats were allocated into four groups. Group I served as the negative control group (i.e., the animals received plain water orally). Group II was the positive control

group. The rats in Group II were induced with Streptozotocin (55 mg/kg body weight), dissolved in citrate buffer (pH 4.5), and administered intraperitoneally (Furman, 2015). On the third day, the fasting blood glucose of the induced animals was tested using a commercial glucometer. Diabetic animals were defined as having blood glucose levels greater than 250 mg/dL. Groups II and IV were treatment groups. Group III rats were treated with glibenclamide (5 mg/kg body weight), dissolved in 0.5% DMSO (Garcia, et al., 2014). Group IV rats were treated with vasicine (0.9 mg/kg body weight), dissolved in 0.5% DMSO. Blood glucose levels were monitored weekly. Serum biochemical analysis for kidney function tests was performed using serum samples collected before the induction of diabetes, on the 14th day during treatment, and at sacrifice on the 28th day (Dash, et al., 2010).

Organ collection: The rats were euthanized by a humane method (ketamine-xylazine injection method). For histological analysis, kidney samples were obtained and preserved in 10% neutral buffered formalin (Al Drees, et al., 2017) and chilled acetone (4°C) for immunohistochemical analysis (Matsuyama, et al., 2018). The fixed tissues were processed, and 5 µm sections were made for routine histological and immunohistochemical staining techniques. Histopathological observations were made using a Leica microscope (CH9345 Heerbrugg) under different magnifications.

Statistical analysis: Duncan's multiple range test was used for statistical comparison following one-way ANOVA. The experimental data were presented as mean ± SE (Kim, 2014). P-values less than 0.05 were considered statistically significant. The software used for analysis was SPSS for Windows, version 15 (SPSS Inc., Chicago, IL, USA).

RESULT

Biochemical analysis

In Wistar albino rats, the estimated normal ranges for blood urea nitrogen (BUN), serum creatinine, and total protein were 11-25 mg/dL, 0.2-0.7 mg/dL, and 6.2-7.3 g/dL, respectively. BUN levels did not vary significantly between the groups before the induction of diabetes or on the 14th day during treatment. However, significant variation was observed at sacrifice and also within the groups throughout the study (Table 1).

Table 1. Blood Urea Nitrogen level (mg/dl) in animals of different groups during treatment (n=6, Mean ± SE).

Groups	Before induction of diabetes	During treatment 14th day	At sacrifice 28th day	p-values
DMSO control	12.92 ± 0.87^{aA}	13.03 ± 1.19^{aA}	11.57 ± 1.20^{aA}	0.590
Diabetic control	19.28 ± 1.60^{bA}	22.28 ± 2.28^{abA}	21.13 ± 1.32^{aA}	0.499
Diabetes-induced glibenclamide-treated	22.78 ± 1.32^{bA}	41.34 ± 8.26^{cA}	23.17 ± 6.40^{aA}	0.078
Diabetes-induced vasicine-treated	19.41 ± 0.65^{bB}	30.53 ± 3.11^{bcA}	22.34 ± 3.64^{aAB}	0.034
Non-diabetic vasicine-treated	20.94 ± 2.16^{bA}	25.64 ± 2.80^{abA}	21.18 ± 2.79^{aA}	0.379
p-values	0.001	0.002	0.180	

Legends: Means with similar superscripts (uppercase letters) in rows and superscripts (lowercase letters) in columns do not differ significantly.

Serum creatinine levels were within the normal range in all groups before the induction of diabetes. At sacrifice, the levels in the diabetic control group were higher, while the levels in the other groups remained within the normal range (Table 2).

Table 2. Serum creatinine (mg/dl) level in animals of different groups during treatment (n=6, Mean ± SE).

Groups	Before induction of diabetes	During treatment 14th day	At sacrifice 28th day	p-values
DMSO control	0.46 ± 0.02^{aA}	0.48 ± 0.02^{bA}	0.46 ± 0.04^{bA}	0.892
Diabetic control	0.53 ± 0.05^{aB}	0.79 ± 0.09^{bcAB}	0.94 ± 0.18^{aA}	0.098
Diabetes-induced glibenclamide-treated	0.48 ± 0.53^{aB}	1.33 ± 0.06^{aA}	0.32 ± 0.06^{bB}	0.000
Diabetes-induced vasicine-treated	0.53 ± 0.06^{aB}	1.13 ± 0.24^{acA}	0.43 ± 0.09^{bB}	0.012
Non-diabetic vasicine-treated	0.36 ± 0.06^{aB}	1.11 ± 0.13^{acA}	0.38 ± 0.01^{bB}	0.000
p-values	0.178	0.001	0.001	

Legends:Means with similar superscripts (uppercase letters) in rows and superscripts (lowercase letters) in columns do not differ significantly.

The serum total protein levels in all groups were within normal range before the induction of diabetes and at sacrifice. The levels were slightly higher on the 14th day of treatment, with significant variation observed between the groups. The total protein level in the diabetic group was significantly higher (Table 3).

Table 3. Serum Total protein (g/dl) in animals of different groups during treatment (n=6, Mean ± SE).

Groups	Before induction of diabetes	During treatment 14th day	At sacrifice 28th day	p-values
DMSO control	5.88 ± 0.46^{aA}	5.78 ± 0.75^{bA}	5.82 ± 0.70^{abA}	0.994
Diabetic control	6.70 ± 0.31^{aB}	8.23 ± 0.42^{aA}	7.78 ± 0.58^{aAB}	0.078
Diabetes-induced glibenclamide-treated	7.25 ± 1.70^{aA}	7.40 ± 0.25^{aA}	5.25 ± 1.06^{bA}	0.109
Diabetes-induced vasicine-treated	6.80 ± 0.21^{aAB}	7.70 ± 0.48^{aA}	6.32 ± 0.14^{abB}	0.022
Non-diabetic vasicine-treated	6.40 ± 0.75^{aB}	7.75 ± 0.40^{aA}	6.68 ± 0.08^{abB}	0.014
p-values	0.264	0.017	0.087	

Legends: Means with similar superscripts (uppercase letters) in rows and superscripts (lowercase letters) in columns do not differ significantly.

Histopathology

The DMSO control group rats exhibited normal kidney architecture, with intact tubules in both the cortex and medulla (Figure 1a). Significant degenerative changes were observed in the glomeruli of the diabetic control group rats. Prominent changes included glomerular shrinkage, tubular degeneration, and congestion, leading to increased urinary space (Figure 1b). Thickening of the mesangial matrix and multifocal mild to moderate vacuolar degeneration were noted. Perivascular fibrosis was predominantly observed in the cortex, as indicated by Mason's trichrome stain (Figure 1c). Pyknotic nuclei were present in the lining epithelium of the tubules in the medullary region. Hyalinization was characteristically found in the interstitium of the cortex.

In the kidneys of the diabetes-induced glibenclamide-treated group rats, the glomerular and cortical capillaries were congested (Figure 1d). Amyloid-like eosinophilic deposits were observed in the interstitium of the cortex, although these were considerably less than those seen in the diabetic control group (Figure 1e), and they were positive for Periodic Acid-Schiff (PAS) stain. Vacuolar degeneration was observed in the epithelium of the collecting tubules. Most of the renal corpuscles maintained normal histoarchitecture. In the kidneys of the diabetes-induced vasicine-treated group, the renal corpuscles and tubules of the nephron maintained normal histoarchitecture in most sections. However, there was multifocal mild vacuolar degeneration of the tubular epithelial cells in both the cortex and medulla (Figure 1f). Capillary congestion and hyaline deposits were less frequently observed.

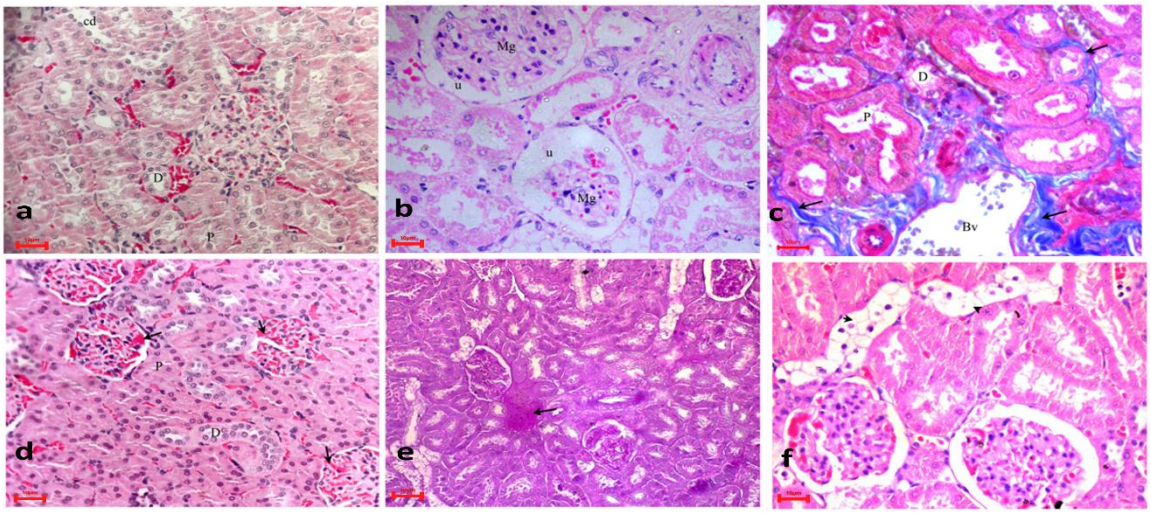

Figure 1. Photomicrograph of rat kidney depicting (a) regular histoarchitecture of proximal (P), distal (D) and collecting ducts (CD) in cortex of group 1 H & E X 400, (b) atrophied and hypertrophied mesangium (Mg), increased urinary space (u) in group II H & E X 400, (c) perivascular (Bv) and peritubular fibrosis (black arrows indicated) in group II Masson's trichrome X 400, (d) congested capillaries (black arrows indicated) in group III X H & E 400, (e) PAS positive hyaline deposition (black arrow indicated) in group III PAS X 100 and (f) tubular vacuolations in group IV H & E X 400.

Immunohistochemistry

The expression of GLUT 2 receptors in the proximal convoluted tubules was observed in the DMSO control group. The expression was higher in the diabetic control group. In the diabetes-induced glibenclamide and vasicine-treated groups, the expression of GLUT 2 receptors was comparatively lower than in the diabetic control group (Figure 2).

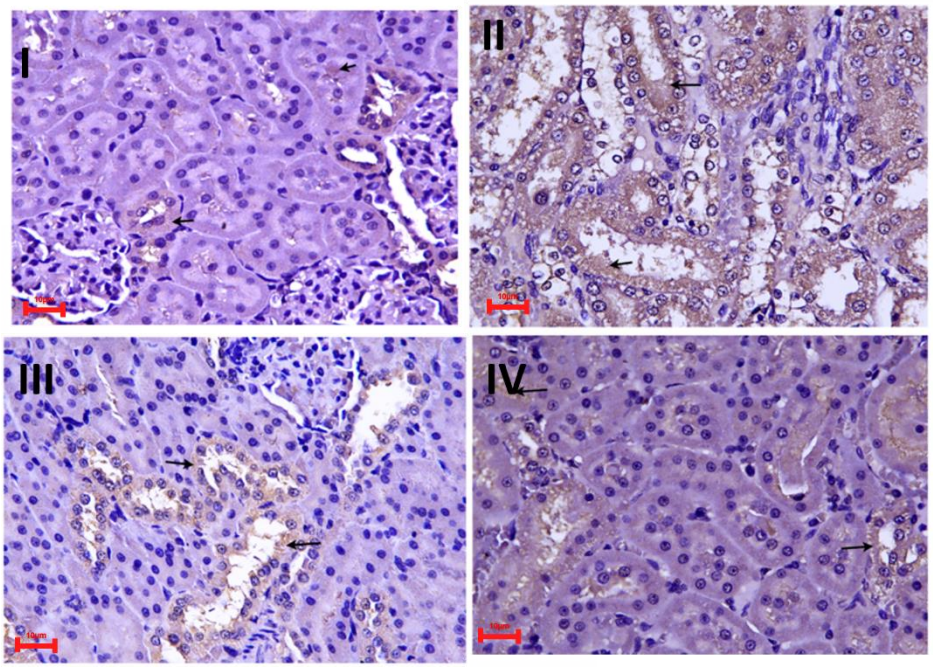

Figure 2. Photomicrograph of GLUT 2 receptor expression in proximal convoluted tubules (black arrows indicated) of rat kidneys in the negative control group (I) and treatment groups (II,III and IV).

DISCUSSION

The histopathology of human diabetic nephropathy shows that the mesangial matrix enlarges, the glomeruli become enlarged, and the glomerular basement membrane thickens. In addition to glomerular lesions, tubulointerstitial lesions are a major factor contributing to the decline in kidney function in individuals with diabetic nephropathy (Kitada, et al., 2016).

In the present study, BUN and serum creatinine levels increased after the onset of diabetes. An increase in creatinine levels not only indicates a reduction in renal function but also serves as a marker for identifying the harmful effects of compounds on the kidneys in rats (Al-Ghaithi, et al., 2004). Creatinine, identified as the end product of creatine phosphate breakdown in muscle, serves as an indicator of impaired glomerular filtration. Elevated serum creatinine levels reflected the severity of kidney disease. In the diabetic control group, the rise in creatinine levels was indicative of renal damage, including the destruction of renal tubules and nephrons. In the diabetes-induced glibenclamide and vasicine-treated groups, serum BUN and creatinine levels decreased at sacrifice. This suggested an improvement in kidney function and a reduction in the rate of muscle degradation (Elkader, et al., 2018).

Changes in serum creatinine and BUN levels were likely caused by a negative nitrogen balance, which also led to increased tissue proteolysis and decreased protein synthesis. This resulted in elevated levels of creatinine and serum urea, suggesting that diabetic animals have impaired renal function (Jensen, et al., 1986; Mir, et al., 2008).

The albumin levels in all the groups remained within the normal range throughout the study. Histological changes in the glomerular filtration barrier could lead to proteinuria. Additionally, hyperglycemia causes the detachment of podocytes from this membrane. In this case, normoalbuminuria progresses to microalbuminuria, which eventually leads to macroalbuminuria (Pourghasem, et al., 2015).

The histological changes observed in the diabetic kidney could be attributed to the effect of STZ, as kidney cells also express GLUT2 glucose transporters. In the diabetic kidney, hyaline deposits were present. The exudative lesions consisted of plasma protein deposits beneath the endothelium, which tested positive for periodic acid-Schiff (PAS) stain (Alicic, et al., 2017). Eosinophilic deposition is an early sign of renal fibrosis, occurring when the extracellular matrix (ECM) becomes glycated, disrupting the balance between ECM protein synthesis and degradation (Sugimoto, et al., 2007).

The kidneys of diabetic individuals treated with glibenclamide exhibited normal histoarchitecture, although some hyaline deposits were observed in the cortical tubules (Adeva-Andany, et al., 2023). Alotaibi, et al., (2019) also reported that glibenclamide treatment maintained kidney structure in diabetic animals, although eosinophilic secretions were present in the tubules. Yassin, et al. (2004) suggested that the recovery of glibenclamide-treated kidneys occurred at a slower rate. The presence of regenerative cells in the areas of the uriniferous tubules indicated ongoing recovery.

In the vasicine-treated group, the kidneys showed normal histology, with a few areas of vacuolation in the cortex and medulla. Tubular vacuolization may be a cellular response to stress, potentially leading to cell damage. This is also associated with subnuclear lipid vacuolization or glycogen deposition. In cases of significant hyperglycemia, a deposition known as Armanni-Ebstein cells can occur (Pourghasem, et al., 2015).

D'souza, et al., (2021) also reported minimal tubular dilatation and degeneration, with reduced Bowman's space in diabetic rats treated with ethanolic *Adhatoda zeylanica* extract. The antioxidant properties of the *Adhatoda zeylanica* plant could be responsible for reversing the undesirable kidney changes associated with hyperglycemia-induced oxidative stress.

Primarily located in the proximal convoluted tubules of the kidney, the number of GLUT2 transporters increases in the diabetic state (Vrhovac, et al., 2015; Ghezzi, et al., 2018). In conditions such as hyperglycemia, increased glomerular filtration rate, and chronic kidney disease, the GLUT2 receptors translocate from the basolateral membrane to the brush border membrane of proximal tubules (Ahmad, et al., 2022).

The results highlight the potential benefits of vasicine treatments, not only in controlling blood glucose but also in protecting kidney structure and function. This approach may also be applicable to human health, using vasicine for diabetic patients at risk of diabetic nephropathy.

Strength and limitations

The strength of the study includes its understanding of the effects of vasicine on kidney's health in diabetic conditions. However, a limitation is the short duration of treatment (28 days). Since the treatment period was relatively brief, it may not capture the long-term effects of vasicine on kidney function and structure. Chronic effects may require a longer observation period. Additionally, urinalysis for glomerular function proteins, particularly transferrin and type IV collagen, could provide more insightful results regarding kidney damage status, even with normal albuminuria levels. This aspect was lacking in the current study.

CONCLUSION

This study investigated how vasicine affected kidney health in streptozotocin-induced diabetic Wistar rats from both histomorphological and biochemical perspectives. The findings demonstrated that vasicine treatment preserved kidney architecture, with a notable reduction in glomerular and tubular damage compared to the diabetic control group. Biochemical markers such as serum creatinine and BUN indicated improved renal function, suggesting that vasicine may counteract the deleterious effects of diabetes on the kidneys. Additionally, changes in GLUT2 receptor expression highlight potential mechanisms through which vasicine exerts its protective effects in diabetic nephropathy.

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Conflict of Interest

No conflict of interest among the authors.

Ethic Consideration

Institutional Animal Ethical Committee (IAEC), Madras Veterinary College approved the experiment (App. No. IAEC/SA/08 on 05-05-2022).

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Author Contribution

RKSS contributed to the conception and design of the manuscript. US contributed to the corrections and final approval. TAK contributed to critical revision, and final approval. SP contributed to statistical analysis.

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