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Research Paper

A Potential Treatment Strategy for the Treatment of Diabetic Kidney Disease in Streptozotocin-induced Diabetic Rats: *Leptadenia Hastata* Extract

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ABSTRACT

Background: Kidney disorders are serious outcomes of diabetes, resulting in renal ailments.

This study seeks to provide an alternative treatment for the disorder through the use of medicinal plants that offer renal protection and ameliorate the deleterious effect of diabetes.

Methods: Thirty rats were divided into six groups of five and hyperglycemia was induced by intraperitoneal injection of 50 mg/kg streptozotocin. The rats with a fasting blood glucose level exceeding 250 mg/dl were selected. The *L. hastata* leaf extract was administered orally and a daily insulin injection was given intramuscularly into the left thigh for 28 days. Rats were then sacrificed and the urine was collected for urinalysis. The kidneys were harvested and examined histologically. The micrographs obtained were subjected to morphometric analyses to evaluate several parameters.

Results: The extract-treated groups showed preservation of the cytoarchitecture of the renal tubules and glomeruli as compared to the diabetic control group, which showed distortion of the glomeruli and atrophied renal tubules. The rats that received the extract showed a significantly increased glomerular perimeters and areas ($P<0.01$). The cellularity was significantly increased ($P<0.001$); however, the glomerular basement membranes were significantly thicker in these groups compared to the controls ($P<0.001$). Both the extract-treated and insulin-treated rats showed reduced urine glucose concentrations. Urobilirubinogen, protein, and ketone levels were elevated in the diabetic controls compared to the extract-treated rats.

Conclusion: Administration of the *L. hastata* extract led to renal protection by preserving the cytoarchitecture of the glomeruli and renal tubules, restoring the kidneys' function.

Keywords: Diabetes mellitus, Glomeruli, Leptadenia hastata, Histology, Kidney, Streptozotocin, Urinalysis

Introduction

Diabetes Mellitus (DM) is a metabolic disorder caused by pancreatic dysfunction in insulin secretion and normal cellular response to glucose, lipid, and protein metabolism [1]. The incidence and prevalence of diabetes have steadily increased worldwide over the past two decades. In 2019, approximately 463 million people were projected to have diabetes, with China being at the top of the list, followed by India and USA [2]. According to the International Diabetes Atlas (2019), the number is pro-

jected to increase to 578 million in 2030 and to 700 million in 2045 [3]. Diabetes is a growing epidemic and the most common cause of Chronic Kidney Disease (CKD), and renal shutdown in patients with this metabolic disorder. Kidneys play an important role in normal physiology, including plasma filtration of metabolic waste products, plasma volume regulation, hormone secretions, acid-base homeostasis, and vitamin D metabolism [3, 4].

Diabetic kidney disease, or diabetic nephropathy is a major chronic condition experienced by some individuals with diabetes [5]. This occurs when the disease damages the small blood vessels supplying the kidneys. When this occurs, the kidneys are unable to filter blood adequately, causing the body to retain more water and salt. This in turn leads to the accumulation of unfiltered waste biproducts in the body [6]. Globally, the 12th cause of death in humans has been attributed to CKD. This disorder is also the 17th cause of disability in people with diabetes worldwide [7]. These individuals are more disposed to Cardiovascular Diseases (CVD) than ordinary people [8]. Around 30% of patients with diabetes develop diabetic nephropathy and CKD, making them the top complications of diabetes [7, 9, 10].

Pathologically, the first deviations seen in the histology of the kidneys are thickened glomerular basement membranes and accumulation of matrix materials in the mesangium of the glomeruli. Subsequently, nodular deposits are observed, and glomerulosclerosis worsens as heavy proteinuria develops, glomeruli progressively destroyed, and the renal function continue to deteriorate. Diabetic nephropathy is commonly treated with medications that lower blood pressure, hence, protecting the kidneys. These drugs may reverse kidney structural damages and are prescribed as soon the evidence of protein in the urine, i.e., proteinuria is detected [6, 8].

Furthermore, the socioeconomic burden imposed by diabetic neuropathy includes patients' hospitalizations, cardiovascular disease, damaged renal blood vessels and eventually death. These highlight the importance of identifying at-risk individuals and managing them aggressively [10, 11]. However, successful management of diabetic kidney disease relies on changes in the patient's lifestyle including the incorporation of exercise and changes in diet combined with using oral hypoglycemic agents and/or insulin injections. This is done to lower blood pressure and maintain normal blood glucose levels, both of which are necessary for slowing down the progression of the resultant nephropathy [11, 12]. Insulin is a hormone that is administered to control blood glucose levels. Managing the blood glucose levels in individuals with diabetes in low income and developing countries is a daunting task. The main reasons are limited health care facilities and availability of medications to most diabetic patients. Therefore, an alternative solution to the rising concern would be to consider the use of herbal medications, which are both affordable and available to help in the treatment and/or management of diabetic kidney disease and its complications. Streptozotocin is commonly used in research to induce pancreatic cell injury for creating rat models of diabetes type I, which leads to renal injury that resembles the diabetic nephropathy in humans [12].

A number of plants have shown the property to not only restore the kidney function in people with diabetes but also inhibit the renal toxicities of several drugs against the kidneys [13]. *Leptadenia hastata* (*L. hastata*) is a member of plant Family, Asclepiadaceae. This is an edible non-domesticated plant collected in the wild throughout Africa [14]. Ethno-botanical and vocal communications obtained from traditional practitioners in northern Nigeria and during this study revealed that it was used locally for the treatment of people with diabetes. Many effects of this herb have been reported previously by other studies [14-17]. Earlier, two of these studies [17-20] have evaluated the plant's glucose-lowering properties. Although *L. hastata* has proven efficient in the management of diabetes by reducing the serum glucose in patients, there is ample research literature, supporting its positive effect in the treatment of diabetic complications including the nephropathy.

Aims of the study: This study primarily sought to examine the histological alterations in the renal glomeruli and tubules found in diabetic kidneys. Secondarily, we assessed the therapeutic effects of the extract of *L. hastata* leaves in reversing the histopathological changes in the affected rat kidneys.

Materials and Methods

Animal husbandry: In the current study, the experiments were carried out on 30 Wistar albino rats of both sexes. These rats weighed between 135 and 190 grams and were three months old at the onset of the study. They were kept in the animal housing of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri, Nigeria, for two weeks before being used for the experiments. They had access to normal food pellets and water *ad libitum*.

Extraction of plant material: The plant *L. hastata* leaves were identified and validated by a plant taxonomist from the Department of Botany and processed for the present study, using the maceration technique as described by a previous study [20].

Animal grouping: The rats were randomly divided into six groups of five based on the categories presented in Table 1, and were administered the assigned treatment protocol as indicated later.

Determination of dosage: The dosage administered to the animals in each group was as determined following an acute toxicity carried out in a pilot study prior to the current study [19].

Induction of diabetes with streptozotocin: Hyperglycemia was induced using streptozotocin and confirmed in Wistar rats following the protocols previously described by two studies [21, 22]. One week after the streptozotocin administration, the rats with fasting blood glucose levels exceeding 250 mg/dl (13 mmol/dl) were selected for the study as indicated by previous studies [16-18]. Urinalysis tests were performed to confirm diabetes and renal retinopathy in all rat groups based on an established method [23]. The presence of proteins in the urine (proteinuria) confirmed the kidney damages in the diabetic rats. The *L. hastata* leaf extract was orally administered to the rats and insulin injections were made intramuscularly in the thigh region of the animals in Group 6.

Sacrifice and specimen collection: On the 29th day of the study, all rats were sacrificed by first inducing a dazed state using ketamine hydrochloride (Rotexmedica, Trittau, Germany). The kidneys were then harvested and were weighed and preserved in 10% formalin. These tissues were subjected to routine histological processes prior to staining with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS).

Urinalysis: Combostik-11 urinalysis strips (DFI Co., Iran) was procured from the University of Maiduguri Teaching Hospital. During the animal sacrifice, the bladders were neatly dissected out and the residual urine was carefully collected and tested for the presence of several parameters, using the Combostik-11 strips.

Histological evaluation of the kidneys: The kidneys were excised and cut to pieces for quick perfusion of the fixative. The tissue processing for dehydration with ethyl alcohol, clearing with xylene, and infiltration with paraffin followed the established protocols. The embedded tissue samples were then prepared in 3×4 mm blocks enclosed in a tissue cassette and properly labeled. These were placed in an automatic tissue processor (Leica TP1020, Austria) for making 5µ thick sections followed by staining with H&E and PAS.

Histomorphometric analyses: The images from the histological sections were obtained using an Amscope light microscope (MBJX-ISCOPE, Los Angeles, USA), fitted with a digital camera (M500, X64, version 3.7) and using 10X and 40X lenses. The images were analyzed using Amscope ImageJ software. A standardized ocular micrometer was also used to approximate areas of interest in the histological slides for morphometric analyses based on established methods [24-26]. The morphometric analyses were performed, using the computerized image analysis system, ImageJ 1.53a (Wayne Rasband, National Institutes of Health, USA). A micrometer was used to calibrate ImageJ using the same objective and pixel resolution as that for the micrographs being examined. In each rat, 50 glomeruli and convoluted tubules, both distal and proximal ones, were examined. The parameters and measurements were adopted and reported based on the previously established methods [24, 26].

Table 1. Animal groupings and treatment protocols

Group	Treatment Protocol
A	Control normal rats, administered with the corresponding volume of citrate buffer and olive oil.
B	Non-diabetic rats, administered with 200 mg/kg extract of Leptadenia hastata.
C	Streptozotocin-induced diabetic rats (50 mg/kg) administered olive oil for 28 days.
D	Streptozotocin-induced diabetic rats (50 mg/kg), treated with n-hexane extract of *Leptadenia hastata* (100 mg/kg) orally, dissolved in olive oil for 28 days.
E	Experimentally Streptozotocin-induced diabetic rats (50 mg/kg) were treated with n-hexane extract of *Leptadenia hastata* (200 mg/kg) orally, dissolved in olive oil for 28 days.
F	Experimentally Streptozotocin-induced diabetic rats (50 mg/kg) were treated with an intramuscular injection of insulin (6IU/kg) dissolved in olive oil for 28 days.

Data analyses: The data were statistically analyzed, using GraphPad InStat software, v. 3.10 (San Diego, CA, USA,) by one-way Analysis of Variance (ANOVA) and expressed as the Mean±Standard Error of the means, and percentages followed by Turkey's post hoc test for multiple comparisons. A $P<0.05$ was considered for the statistical significance.

The following morphometric parameters were measured in all groups:

• **Glomerular basement membrane thickness (μm^2):** this was determined as a mean of the distance obtained by manual tracing of lines, using the freehand tool along both sides of the basement membranes.

• Glomerular area (μm^2) was obtained by the area occupied by the glomerular tuft in pixels converted to μm^2.

• The area of the proximal and distal renal tubules (μm^2) was determined as the number of pixels in the traced area converted to μm^2.

• The nuclear-cytoplasmic ratio was calculated as the area of the nucleus of a cell of the proximal/distal convoluted tubules and the area of the cytoplasm of the cell.

• Cellularity was determined as the number of cells in the μm^2 of area.

• Feret's diameter was calculated as the measured distance between theoretical analogous lines restricting the object perpendicular to that direction calculated as the ratio of the object perimeter (P) and pi (π), i.e., F = P/.

• The perimeter was determined as the length of the outline of each object.

• The circularity of each object was determined by the following formula: $4\ \pi$*area/perimeter^2.

Results

Histological analyses: The analysis of the micrographs from the renal tissue showed extensive morphological damages in the form of tubular cell degeneration and glomerular congestion in the untreated diabetic rats (Group C; Figure 1c). Also, the Bowman's spaces were dilated in this group, the glomerular membranes were thick, and tubular lumens widened. In the groups treated with the extract (Groups D and E), the renal tubules were preserved and the tubular cells' cytoarchitecture appeared intact (Figures 1d and 1e). In the diabetic animals treated with insulin (Group F), the renal tubular cells were also reduced and the tubules were widened (Figure 1f).

Figure 2 represents the photomicrographs of the renal tissue stained with PAS at higher magnification. The staining highlighted the basement membranes underlying the glomerular capillary loops and renal tubular cells giving a clearer view of the apical brush border, a feature of the proximal convoluted tubules (Figures 2a, 2B, 2D & 2E). Group C had a thickened layer of glomerular basement membranes dilated tubules, and marked damages to proximal convoluted tubules observed as evident by the absence of the apical brush border, and dilated appearance. Group D revealed almost complete absence of the histopathological alterations as observed in other groups.

Histomorphological analyses: The data from the histomorphological analyses of the kidney tissues from the six groups are represented in Table 2. The glomerular areas had decreased significantly ($P<0.01$) in Group C as evident by the condensed glomeruli seen in the micrographs. The rats in Group D that received the extract at 100 mg/kg showed a significant increase in the perimeters compared to those noted in other groups. Group E rats that received the extract at 200 mg/kg had reduced perimeters and glomerular areas. The cellularity in Groups D and E had significantly increased ($P<0.001$), compared to those in Groups C and F that showed higher number of glomerular cells. The glomerular basement membranes were significantly thicker in Groups B, C, and D, compared to those in Groups A, E, and F ($P<0.001$). The distal tubular areas and the nuclear-cytoplasmic ratio for the epithelial cells of the proximal and distal tubules showed no statistically significant differences between the controls and experimental groups. Also, there was no statistically significant difference among all groups with respect to the areas of the proximal and distal convoluted tubules.

Urinalysis: The results of the urinalysis for all rat groups are presented in Table 3. The findings indicated an elevated glucose level in the urine of the rats in Group C (300 mg/dl), compared to those in Groups A and B, whose urine glucose level was normal. The rat groups, treated with the extract or insulin (Groups D, E, and F), showed reduced glucose levels in the urine. The urobilirubinogen and ketone levels were also elevated in Group C rats compared to those treated with the extract. Also, the mean protein concentration in the urine samples from Groups C and F was 300mg/dl. However, the urine protein levels were significantly reduced in Groups D and E. No blood smears were detected in the urine samples with

Table 2. Results of the morphometric analysis of the rats' kidneys

Variables	Mean±SEM					
	A	B	C	D	E	F
Glomerular Area (μm²)	2376.28±179.1[b]	3183.09±210.9[b]	1976.17±233.0[a]	2838.69±302.9[a,b]	2300.01±141.4[b]	2945.40±315.5[b]
Glomerular Perimeter (μm)	1207.22±163.8 [b]	1016.65±144.3[b]	1065.0±154.4[b]	2022.30±284.4[a]	898.19±179.1[b]	786.6±116.3[b]
Feret's Diameter (μm)	89.49±4.5[b]	85.19±3.3[b]	84.40±5.6[b]	110.02±9.3[c]	86.73±6.9[b]	87.45±3.9[b]
Circularity (Glomerulus)	0.40±0.02[b]	0.79±0.04[b]	0.90±0.02[b]	0.50±0.08[c]	0.036±0.8[b]	0.80±0.05[b]
Cellularity (cell/μm²)	0.7200±0.6[a]	0.88±0.8[b,c]	0.58±0.3[b]	0.83±0.7[b,c]	0.79±0.5[b]	0.88±0.6[b,c]
GBM Thickness (μm)	0.1067±0.1[a]	0.249±0.02[b]	0.189±0.01[b]	0.196±0.01[b]	0.126±0.01[a,c]	0.130±0.01[b,c]
PCT area(μm)	1962.40±208.3	1411.60±245.1	1643.70±262.9	1974.49±262.9	1665.33±229.7	1930.90±189.9
PCT Perimeter (μm)	185.40±15.8	145.95±12.5	178.14±19.1	184.10±17.7	156.60±9.9	170.85±9.1
Nucleus Area PCT [N] (μm)	24.27±1.6[b]	20.69±1.6[b,c]	16.01±1.6[a]	22.12±0.7[c]	25.88±.2.2[b]	24.26±1.5[b,c]
Area of PCT [C] (μm)	113.94±10.8[b]	102.61±9.2[b]	100.79±9.8	127.12±8.7ab	117.84±5.6[a]	161.60±6.4[c]
N/C of PCT	0.213±0.07	0.201±0.06	0.158±0.08	0.174±0.09	0.219±0.06	0.150±0.01
Circularity (PCT)	0.717±0.6	0.92±0.04	0.81±0.6	0.732±0.2	0.853±0.3	0.831±0.3
Area of DCT (μm)	1282.64±112.1	2049.15±282.2	1721.87±260.4	2249.79±239.4	1946.90±257.7	1833.6±113.2
Perimeter of DCT (μm)	148.67±10.5	180.75±14.75	175.08±16.5	200.99±14.74	185.56±14.3	177.7±6.9
Nuclear Area of DCT [N] (μm)	15.89±1.1	14.23±1.1	15.62±1.0	20.62±2.4	18.80±2.3	17.58±1.6
Area of DCT Cell [C] (μm)	93.30±4.4	66.30±5.9	84.50±3.1	72.70±2.8	96.50±1.9	107.0±5.1
N/C of DCT	0.3±0.01	0.214±0.2	0.3±0.01	0.4±0.23	0.34±0.19	0.2±0.1
Circularity (DCT)	0.729±0.4	0.788±0.4	0.706±0.6	0.700±0.7	0.710±0.7	0.90±0.72

Means carrying the same letter (superscript) within the same row are non-significantly different from each other's (P>0.05). Mean values carrying different letters (superscripts) within the same row are significantly or highly significantly different from each other's (significantly at P≤0.05 and highly significantly at P≤0.01). Group A: normal control, Group B: non-diabetic control; Group C: diabetic control; Group D: Diabetic rats treated with 100mg/kg hexane extract of *L. hastata*, Group E: diabetic rats treated with 200mg/kg hexane extract of *L. hastata*, Group F: diabetic rats treated with insulin (6 IU), PCT: Proximal Convoluted Tubule, DCT: Distal Convoluted Tubule, GBM: Glomerular Basement Membrane

Table 3. The results of urinalyses for all rat groups

Group	BLD	BIL	UROBIL (mg/dl)	KET (mg/dl)	GLC (mg/dl)	PRT (mg/dl)	NIT	LEU (WBC/μl)	pH	S.G./ DENS	ASC. A (mg/dl)
A	-VE	+	1.0	-VE	-VE	100	-VE	-VE	6	1.015	++40
B	-VE	++	1.0	-VE	-VE	100	-VE	-VE	6	1.010	+20
C	-VE	+	2.0	++40	300	300	+VE	+25	7	1.020	-VE
D	-VE	+	1.0	15	100	30	+VE	-VE	6	1.025	+20
E	-VE	+	1.0	-VE	100	-VE	-VE	-VE	6	1.015	++40
F	-VE	++	1.0	++40	50	300	+VE	+25	7	1.020	++40

BLD: Blood, BIL: Bilirubin, UROBIL: Urobilirubinogen, KET: Ketones, GLC: Glucose, PRT: Protein, NIT: Nitrogen, LEU: Leucocytes, pH: the acidity scale, S.G/DENS: Specific gravity/Density, ASC.A: Ascorbic acid. Group A: normal control, Group B: non-diabetic control, Group C: diabetic control, Group D: Diabetic rats treated with 100mg/kg hexane extract of Leptadenia hastata, Group E: diabetic rats treated with 200mg/kg hexane extract of *Leptadenia hastata*, Group F: diabetic rats treated with insulin (6 IU)

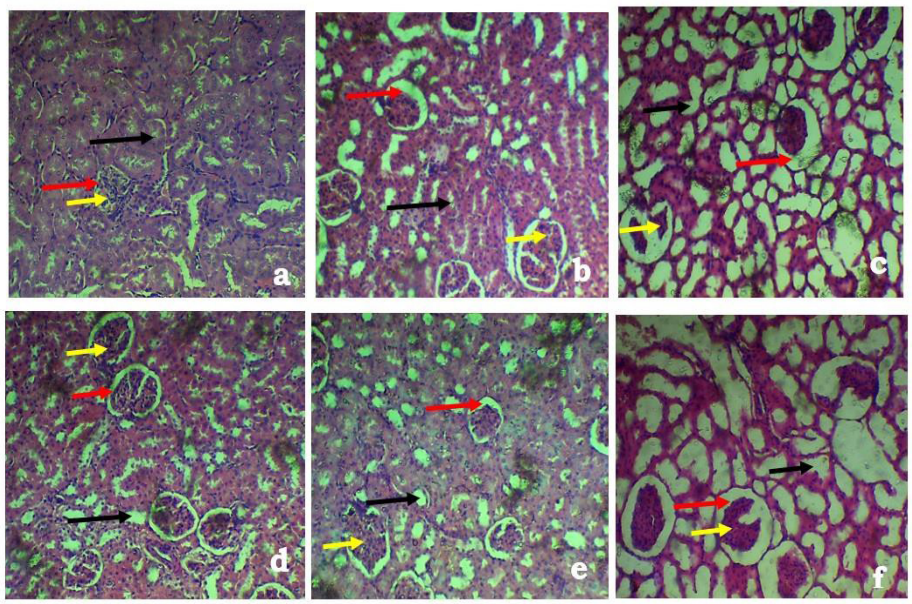

Figure 1. Photomicrographs representing the rat kidneys in all groups after the oral administration of n-hexane leaf extract of *Leptadenia hastata*. Bowman's space (red arrow) is widened in groups c and f, renal tubule (black arrow) is lined with cuboidal tubular cells which are preserved in the extract treated groups. The glomerulus (Yellow arrow) is compact and shrunken in the diabetic and insulin treated groups. Groups A (a), B (b), C (c), D (d), E (e) and F (f) H & E X 100.

a similar urine pH in all groups, except for Groups C and F, in which the pH was 7.

Discussion

The present study was undertaken to determine the effect of the extract of *L. hastata* leaves on the histomorphology of the kidneys of streptozotocin-induced diabetic rats. These animals have been reported to reliably provide evidence of histologic and functional alterations in their kidneys due to the streptozotocin toxicity [6]. It is well known that the kidney glomeruli's principal function is to produce an ultrafiltrate of the plasma, that contains water, sodium ions, and urea for further filtra-

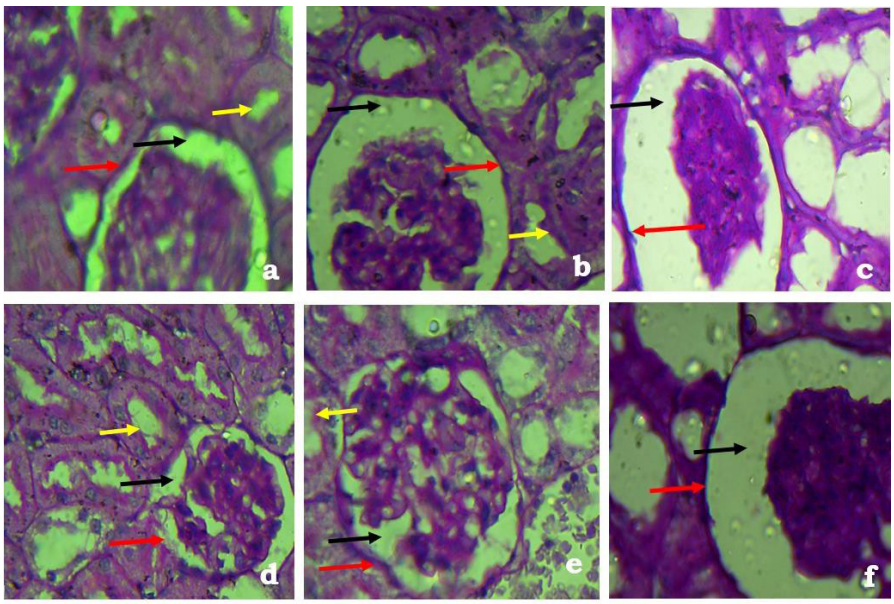

Figure 2. Photomicrographs representing the of kidney of rats in all groups after the administration of

N-hexane leaf extract of *L. hastata*. Bowman's space (black arrow), Bowman's capsule (red arrow) Groups A (a), B (b), C(c), D (d), E (e) and F (f) PAS X400. The cells in the extract treated groups (b, d & e) showed preserved renal tubular cells. Bowman's space in c and f are widened compared to extract-treated groups.

tion in the renal tubules, thus playing an important role in the maintenance of fluid and electrolyte homeostasis. The renal tubules also participate in the reabsorption of the plasma filtrates. Disorders of the kidneys are serious consequences of diabetes, which ultimately result in end-stage renal diseases. In recent studies [25, 26], histopathological examination of the rats' kidneys administered streptozotocin have shown lesions, such as glomerulosclerosis and membranous thickening, arteriolar hyalinization, and tubular cell necrosis.

In the current study, the histological and histomorphometric evaluations of the rats in the untreated diabetic group showed atrophy and condensations of the glomeruli. These alterations included a significant decrease in density, cellularity, and shrinkage of the glomerular and tubular cell injuries. In addition, there was diminished cellularity due to a significant reduction in the cells within the glomerular tufts together with Bowman's spaces widening. These abnormalities could lead to long-term kidney impairment, dysfunction, and failure which are the major complications of diabetes. The rats' kidneys treated with insulin also showed widened Bowman capsule spaces, compact glomerular tufts, and shrinkage in the proximal and distal convoluted cells. Also, similar pathological alterations were observed in the untreated diabetic rats compared to those groups treated with the extract. The findings demonstrated that the extract had a protective effect on the histologic structures of the renal tubular cells compared to those treated with insulin alone.

A study in 2016 has quantified the morphometric changes in the basement membranes of glomerular cells and the protective effect of quercetin versus the nephrotoxicity of cisplatin, a chemotherapeutic agent [26]. This study discovered that the quercetin extract protected against the development of cystic luminal dilatation, renal immune cell inflammations, and proximal tubules degeneration induced by cisplatin. Quercetin also reduced histopathological changes in the glomeruli and the basement membranes of the glomeruli [26].

Histomorphometric analysis of the kidney: Morphometric analysis of the kidney tissue revealed statistically significant differences in the renal tubules dimension, glomerular size and the basement membrane thickness, and the glomerular cellularity, comparing cisplatin versus quercetin [26]. Another study [27] has examined the histological changes in the kidneys in the alloxan model of diabetic rats. This research reported lipofushin pigments in the proximal convoluted tubules, reduced renal barrier capacity, cellular hypertrophy, and proliferation and vacuolization of the renal cells. These were not observed in the current study. The decrease that was reported in the glomerular basement membranes' thickness in the diabetic but untreated rats, was also consistent with the findings of our study.

Histological findings: The glomerular basement membranes of the animals in the group treated with the extract was significantly thicker than those in the control group, suggesting that the extract might have caused significant structural changes in the basement membrane. The thickening of glomerular basement membranes is thought to be early changes in both types I and II diabetic nephropathy, that progresses with the disease duration. The thickening is a consequence of extracellular matrix accumulation with deposits of normal extracellular matrix components, such as fibronectin, laminin, and collagen types IV and VI [28]. The thickenings might have resulted from the accumulation of these substances in response to the extract administration in the treated groups as observed in the micrographs. Also, there was an increased glomerular cellularity in the groups treated with the extract as compared to the diabetic rats. This suggests that the extract may have had a protective effect on the cellular components of the kidneys. This finding is in agreement with the findings in earlier studies [25, 26] where the use of medicinal plants preserved the glomerular and tubular cells, and improved the renal function.

Another study [29] has reported thickening in Bowman capsules, which was linked to the deposition of fibrous tissue following chronic inflammatory condition. It has been proposed that the mesangial tissue in the glomeruli may be permeated with inflammatory cells, leading ultimately to glomerulosclerosis [26, 27]. In the current study, the rats in groups administered with the extract demonstrated a distinct reformative capacity of the renal tubules and glomeruli, compared to either the untreated or insulin-treated groups. The findings suggest that the extract may have renal protective effect by preserving the cytoarchitecture of the glomeruli and tubules, and thus restoring renal function.

Effect of *L. hastata* on urinary parameters: The urinalysis revealed decreased ketone, urobilirubinogen, and protein concentrations in the diabetic rats treated with the extract, without ketoacidosis, suggesting improved renal inflammation. The reductions in proteinuria and ketone bodies in urine following the extract administration have been shown to be beneficial in the prevention or reducing the progression of chronic kidney disease in diabetes [25, 26].

Mechanism of action of *L. hastata* extract on the kidneys: Herbal plants, such as *Curcuma longa*, *Panax quinquefolium*, *Vitis vinifera*, and glycosides from *Stelechocarpus cauliflorus* have been shown to prevent renal lesions [30, 31]. The exact mechanism of how *L. hastata* extract ameliorates diabetic complications is still unclear. However, previous findings have suggested that traditional and herbal remedies impart their antidiabetic potentials through diverse cellular and molecular pathways. Major mechanisms include improving insulin secretion, α-glucosidase enzyme inhibitory activity, regeneration of pancreatic *β-cells*, reducing insulin resistance, anti-inflammatory effects, and attenuating the oxidative stress [30, 31]. Further, suppressing glucose output from the liver and enhancing glucose uptake by cells are believed to be the major contributors to the antidiabetic effect of natural remedies. These effects are mediated via stimulating glycolysis, glucose oxidation, glycogenesis, reducing glycogen breakdown, and promoting gluconeogenesis [20, 30]. A more recent study [32] has demonstrated that herbal medicines have proven effects in balancing metabolic conditions by reducing inflammatory responses, fibrosis, improving renal cells innate safety, and promoting microRNA metabolism. The dynamic constituents recognized in antidiabetic plants include polysaccharides, flavonoids, xanthones, and peptides [30, 31].

Investigation of the *L. hastata* extract in a prior study identified several phytochemicals, including triterpenoids which have been beneficial in the treatment of patients with diabetes [33]. A previous study [34] has also shown that triterpenoid derivatives have promising effects for the management of diabetic nephropathy. They offer an array of pharmacological properties, including anti-cancer, anti-inflammatory, anti-diabetic, and renal protective effect. The mechanism involved by phytochemicals to lower blood sugar includes glucose phosphorylation and tyrosine phosphatase inhibition [17]. Further, two former studies [33] have highlighted the pivotal role that inflammation plays in diabetic nephropathy and the effect of ursolic acid. It is believed that a triterpenoid compound exists in the kidneys of diabetic rats with the ability to protect against diabetic nephropathy by suppressing oxidative stress and inflammation. Further, the glucose-lowering property of the extract plays a pivotal role in the glycemic control, thereby reducing the risks of microalbuminuria and related complications that follows diabetic nephropathy.

Conclusion

In the present study, the *L. hastata* extract was found to preserve glomerular and tubular cells by maintaining the cellular integrity as compared to those observed in the diabetic control groups. The extract also reduced glucose, protein, and ketone levels in the urine, suggesting that it improved the tubular reabsorption in the treated kidneys. However, significant thickening occurred in the glomerular basement membranes in the treated rats, which is attributed to the extracellular accumulation of typical matrix components of the cell membranes of the rats that were treated with the *L. hastata* extract.

Limitations of the study: The present study considered only the gross and histological features in diabetic and non-diabetic kidneys in response to the n-hexane extract of *L. hastata*.

Recommendations for further studies: It is recommended that oxidative stress and biochemical parameters be further investigated in response to the administration of the *L. hastata* extract. Electron microscopic images of the glomerular membranes should be considered to obtain detailed views of these renal structures. Nanosized contrast agents may be used to observe the cellular sings of renal inflammation through Magnetic Resonance Imaging (MRI).

Ethical Considerations

Compliance with ethical guidelines

This study was conducted following the University of Maiduguri Research (UNIMAID) and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The study protocol was approved by the Ethics Committee of the Department of Human Anatomy, the University of Maiduguri Research (UNIMAID) (Code: UM/HA/PGP 18.19-08802).

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Authors' contributions

Conceptualization: All Authors; Acquisition and design: Martha Orendu Oche Attah and Tamunotonye Watson Jacks; Methodology: All Authors; Administrative support/Supervision: Tamunotonye Watson Jacks and Sani Hyedima Garba; Provision of study materials: Martha Orendu Oche Attah and Tamunotonye Watson Jacks;

Data collection and assembly: All Authors; Data analysis and interpretation: All Authors; Funding Acquisition: Martha Orendu Oche Attah and Tamunotonye Watson Jacks; Writing, Review and Editing: All Authors; Final approval of manuscript: All Authors.

Conflict of interest

The authors declare no conflict of interests.

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References

[1] Pourghasem M, Shafi H, Babazadeh Z. Histological changes of kidney in diabetic nephropathy. Caspian J Intern Med. 2015; 6(3):120-7. [PMID]

[2] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157:107843. [PMID]

[3] International Diabetes Foundation (IDF). IDF Diabetes Atlas Ninth Edition. Brussels: International Diabetes Federation; 2019. https://diabetesatlas.org/atlas/ninth-edition/

[4] Sundaram SS, Suresh K, Sundaram SP. Traditional knowledge of medicinal plants used to treat kidney related diseases in selected areas of Madurai district, Tamil Nadu, India. J Med Plants Stud. 2019; 7(4):250-3. https://www.plantsjournal.com/archives/?year=2019&vol=7&issue=4&part=D&ArticleId=1030

[5] Wang M, Wang Z, Zhou J, Sun W, Wang Y, Han M, et al. Effects of traditional Chinese herbal medicine in patients with diabetic kidney disease: Study protocol for a randomized controlled trial. Trials. 2018; 19(1):389. [DOI:10.1186/s13063-018-2749-6] [PMID] [PMCID]

[6] Shafi S, Tabassum N, Ahmad F. Diabetic nephropathy and herbal medicines. International Journal of Phytopharmacology. 2012; 3(1):10-7. www.sciepub.com/reference/278623

[7] Das S, Vasudeva N, Sharma S. Kidney disorders and management through herbs: A Review. J Phytopharm. 2019; 8(1):21-7. http://www.phytopharmajournal.com/Vol8_Issue1_06.pdf

[8] Ritz E, Rychlik I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. Am J Kidney Dis. 1999; 34(5):795-808. [DOI:10.1016/S0272-6386(99)70035-1]

[9] U.S. Renal Data System (2009). USRDS 2012 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda. https://www.usrds.org/annual-data-report/

[10] Iqbal S, Alam A. Renal disease in diabetes mellitus: Recent studies and potential therapies. J Diabetes Metab. 2013; S9:006. [DOI:10.4172/2155-6156.S9-006]

[11] Zajjari Y, Aatif T, Hassani K, Benbria S, El Kabbaj D. Renal histology in diabetic patients. Saudi J Med Med Sci. 2019; 7(1):22-7. [DOI:10.4103/sjmms.sjmms_76_18] [PMID] [PMCID]

[12] Tesch GH, Allen TJ. Rodent model of streptozotocin-induced diabetic nephropathy. Nephropathy (Carlton). 2007; 12(3):261-6. [DOI:10.1111/j.1440-1797.2007.00796.x] [PMID]

[13] Mardani S, Nasri H, Rafieian-Kopaei M, Hajian S. Herbal medicine and diabetic kidney disease. J Nephropharmacol. 2013; 2(1):1-2. http://eprints.skums.ac.ir/2839/1/20.pdf

[14] Bello A, Aliero AA, Saidu Y, Muhammad S. Phytochemical screening, polyphenolic content and alpha-glucosidase inhibitory potential of leptadenia hastata (Pers.) Decne. Niger J Basic Appl Sci. 2011; 19(2):181-6. https://www.ajol.info/index.php/njbas/article/view/73830

[15] Aliero AA, Wara SH. Validating the medicinal potential of leptadenia hastata. Afr J Pharm Pharmacol. 2009; 3(6):335-8. https://academicjournals.org/journal/AJPP/article-full-text-pdf/54B03CB35154

[16] Sanda KA, Sandabe UK, Auwal MS, Bulama I, Bashir TM, Sanda FA, et al. Hypoglycemic and antidiabetic profile of the aqueous root extracts of leptadenia hastata in albino rats. Pak J Biol Sci. 2013; 16(4):190-4. [DOI:10.3923/pjbs.2013.190.194] [PMID]

[17] Umaru IJ, Badruddin FA, Umaru HA. Phytochemical, antifungal and antibacterial potential ofLeptadenia hastata stem-bark extract. MOJ Toxicol. 2018; 4(4):263-8. https://medcraveonline.com/medcrave.org/index.php/MOJT/article/view/20316

[18] Attah MOO, Jacks TW, Garba SH, Balogun JB. Hypoglycemic and anti-diabetic profile of n-hexane extract of leptadenia hastata leaves on streptozotocin-induced diabetes in albino rats. Sumerianz J Med Healthc. 2019, 2(4):42-6. https://www.sumerianz.com/pdf-files/sjmh2(4)42-46.pdf

[19] Attah M, Jacks T, Garba SH, Dibal NI, Ojo P. Evaluation of acute oral toxicity induced by n-hexane extract of Leptadenia hastata Leaves in wistar rats. Int J Veterina Sci Animal Husband. 2019; 4(1):40-4. https://www.veterinarypaper.com/archives/2019/4/1/A/4-1-5

[20] Azwanida NN. A review on the extraction methods used in medicinal plant: Principles, strengths and limitations. Med Aromat Plants. 2015; 4(3):196. https://www.longdom.org/open-access/a-review-on-the-extraction-methods-use-in-medicinal-plants-principle-strength-and-limitation-2167-0412-1000196.pdf

[21] Etuk EU. Animals models for studying diabetes mellitus. Agric Biol JN Am. 2010; 1(2):130-4. https://scihub.org/ABJNA/PDF/2010/2/1-2-130-134.pdf

[22] Attah M, Jacks W, Garba H, Dibal I. Pancreatic morphology and morphometric analysis of streptozotocin-induced diabetes in albino rats treated with n-hexane extract of leptadenia hastata leaves. J Med Histol. 2018; 2(2):173-80. [DOI:10.21608/jmh.2019.7631.1049]

[23] Benmehdi H, Azzi R, Djaziri R, Lahfa F, Benariba N, Tabti B. Effect of saponosides crude extract isolated from Citrullus Colocynthis (L.) seeds on blood glucose level in normal and streptozotocin induced diabetic rats. J Med Plants Res. 2011; 5(31):6864-8. [DOI:10.5897/JMPR11.1369]

[24] World Health Organization. Basic laboratory methods in medical parasitology. Geneva: World Health Organization; 1997. http://apps.who.int/iris/bitstream/handle/10665/40793/9241544104_(part1).pdf;jsessionid=2BFDFC32F63A0D57E032D92F69E8310A?sequence=1

[25] Komolafe OA, Ofusori DA, Adewole OS, Ajayi SA, Ijomone OM, Fakunle JB. Effects of four herbal plants on kidney histomorphology in STZ-induced diabetic wistar rats. J Cytol Histol. 2013; 5(2):1-5. https://www.hilarispublisher.com/open-access/effects-of-four-herbal-plants-on-kidney-histomorphology-in-stzinduced-diabetic-wistar-rats-2157-7099.1000210.pdf

[26] Ilic S, Stojiljkovic N, Veljkovic S, Veljkovic M, Randjelovic P, Sokolovic D, et al. Morphometric study of structural kidney damages caused by cisplatin in rats. Effects of quercetin. Acta Microscopica. 2016; 25(3):121-30. https://acta-microscopica.org/acta/article/view/129

[27] Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classificatin of diabetic nephropathy. J Am Soc Nephrol. 2010; 21(4):556- 63. [DOI:10.1681/ASN.2010010010] [PMID]

[28] Ali BH, Inuwa I, Al Za'abi M, Al Bahlani S, Al Issaei H, Ramkumar A, et al. Renal and myocardial histopathology and morphometry in rats with adenine - induced chronic renal failure: Influence of gum acacia. Cell Physiol Biochem. 2014; 34(3):818-28. [DOI:10.1159/000363045] [PMID]

[29] Farzaei MH, Rahimi R, Farzaei F, Abdollahi M. Traditional medicinal herbs for the management of diabetes and its complications: An evidence-based review. Int J Pharmacol. 2015; 11(7):874-87. [DOI:10.3923/ijp.2015.874.887]

[30] Lu Z, Zhong Y, Liu W, Xiang L, Deng Y. The efficacy and mechanism of Chinese herbal medicine on diabetic kidney disease.J Diabetes Res. 2019; 2019:2697672. [DOI:10.1155/2019/2697672] [PMID] [PMCID]

[31] Attah MOO, Jacks TW, Garba SH, Mshelia HE. Physicochemical and phytochemical screening of n-hexane extract of leptadenia hastata leaves: A proposed herbal remedy in the treatment of diabetes mellitus. Int J Res - Granthaalayah. 2019; 7(2):45- 57. [DOI:10.29121/granthaalayah.v7.i2.2019.992]

[32] Mkhwanazi BN, Van Heerden FR, Mavondo GA, Mabandla MV, Musabayane CT. Triterpene derivative improves the renal function of streptozotocin-induced diabetic rats: A follow-up study on maslinic acid. Ren Fail. 2019; 41(1):547-54 [DOI:10.1080/0886022X.2019.1623818] [PMID] [PMCID]

[33] Zafar M, Naqvi SN, Ahmed M, Kaimkhani ZA. Altered kidney morphology and enzymes in streptozotocin induced diabetic rats. Int J Morphol. 2009; 27(3):783-90. [DOI:10.4067/S0717-95022009000300024]

[34] Xu HL, Wang XT, Cheng Y, Zhao JG, Zhou YJ, Yang JJ, et al. Ursolic acid improves diabetic nephropathy via suppression of oxidative stress and inflammation in streptozotocin-induced rats. Biomed Pharmacother. 2018; 105:915-21. [DOI:10.1016/j.biopha.2018.06.055] [PMID]