Histopathological Investigation of the Effects of Methanol Extract of *Justicia Carnea* Leaves on the Pancreas, Liver and Kidney of Streptozotocin-Induced Diabetic Wistar Rats.

Samuel I. Ojeaburu* and Nathan Eimoga

Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

***Corresponding Author**

DOI: https://doi.org/10.51244/IJRSI.2024.1108042

Received: 17 July 2024; Revised: 30 July 2024; Accepted: 03 August 2024; Published: 05 September 2024

ABSTRACT

Introduction/Aim: Diabetes manifests as a condition of persistent hyperglycemia and poses considerable risks of complications in various organ systems. This study investigated the histopathological impact on the pancreas, liver, and kidney of streptozotocin-induced diabetic Wistar rats and the role of methanol leave extract of *Justicia carnea* in the amelioration of the damages inflicted on the referenced organs. **Materials and Methods:** Thirty-six (36) male Wistar rats weighing 180 to 200 g (mean weight = 190 ± 10 g) were divided into six (6) groups of six (6) rats each. Group 1 served as the normal control and received only water and grower's pellet throughout, group 2 was induced with diabetes but untreated, group 3 animals were induced with diabetes and treated with 50 mg/kg bw of metformin), groups 4, 5 and 6 were induced with diabetes and treated with 100, 200 and 500 mg/kg bw of methanol extract of *Justicia carnea* leaves respectively. Diabetes mellitus was induced in the rats by intraperitoneal injection of 50 mg/kg bw of streptozotocin (STZ). After 21 days of the treatment, the animals were sacrificed and the pancreas, liver and kidney were harvested and processed for histopathological screening. **Results:** The results obtained showed severe vascular ulceration, congestion and periportal infiltrates of inflammatory cells in the liver of the diabetic rats. However, treatment with *Justicia carnea* leave extract resulted in the restoration to near normal hepatocytes, portal vein and bile ducts. In the kidney of the diabetic rats, there was interstitial congestion, glomerular shrinkage and tubular necrosis which became normal after treatment. The Pancreas of diabetic rats showed evidence of degenerating islets, vascular congestion and stenosis but the conditions were ameliorated after treatment with the methanol extract of *Justicia carnea* leaves. **Conclusion:** *Justicia carnea* methanol leave extract ameliorated the histopathological lesions on the pancreas, liver and kidney of STZ-induced diabetic rats. Therefore, we are proposing the methanol extract of *Justicia carnea* leave as a potential candidate for antidiabetic drug or a candidate for adjuvant drug with other antidiabetic drugs.

Keywords: Diabetes, Pancreas, Liver, kidney, *Justicia carnea*, Histopathology.

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases that are characterized by chronic hyperglycemia resulting from direct insulin secretion, insulin action or both. Low level of the hormone insulin may result to various metabolic abnormalities in carbohydrate, lipids and proteins due to the significant role of the hormone [1]. The severity of the symptoms experienced by patients depends on the type and duration of the disease, although some patients are asymptomatic especially those with type 2 diabetes during the early

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

years of the disease. Some symptoms of the disease may include polyphagia, polyuria, polydipsia, weight loss, blurred vision and in severe cases, may lead to stupor, coma and even death if not treated [2,3]. It is classified into Type 1 Diabetes Mellitus (T1DM), an autoimmune condition resulting in the destruction of pancreatic β-cells, and Type 2 Diabetes Mellitus (T2DM), primarily associated with insulin resistance and impaired insulin secretion. T1DM typically manifests early in life, requiring lifelong insulin therapy. T2DM, more common in adults, is linked to lifestyle factors, genetics, and obesity. Complications of diabetes include cardiovascular diseases, kidney dysfunction, neuropathy and retinopathy [4]. Diabetes is becoming more common and has major consequences as an epidemic. The condition may lead to various organ system issues, such as kidney disease and blindness. The leading causes of morbidity and death in diabetics are peripheral artery disease, cardiovascular disease, and stroke, hence early detection and proper management and treatment is essential [5]. The kidneys play a crucial role in maintaining urine production and homeostasis through processes such as filtration, reabsorption, and secretion [3]. Diabetes mellitus is a prevalent cause of kidney failure, contributing to the development of chronic kidney disease even under controlled conditions. Elevated levels of blood glucose can result in nephron damage and impair blood filtration processes [6]. Diabetes, a metabolic disorder, affects various bodily systems, including the liver. Insulin resistance primarily drives hyperglycemia, disrupting lipid, carbohydrate, protein metabolism and predisposing individuals to non-alcoholic fatty liver disease (NAFLD) which may progress to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) [5]. The basic mechanism involves increased oxidative stress and dysregulated inflammatory responses, triggering transcriptional activation of pro-apoptotic genes and hepatocyte injury. Liver damage is one of the severe complications of diabetes, with insulin resistance aggravating oxidative stress and inflammation, thereby increasing liver pathology. Insulin resistance ultimately contributes to the development of chronic and potentially fatal liver conditions such as cirrhosis and end-stage liver failure [7,8]. Furthermore, excessive hepatic fat accumulation exacerbates insulin resistance and contributes to severe metabolic dysfunction. Prolonged diabetes distorts several functions of the heart like reducing blood flow and increasing the likelihood of myocardial infarction due to vascular occlusion by hyperglycemia. Myocardial infarction occurs when blood flow to a coronary artery is abruptly interrupted, resulting in myocardial damage. Some common symptoms of the effect of diabetes in the heart include severe chest pain radiating to the neck or left arm, as well as dyspnea. Diabetic individuals may experience asymptomatic myocardial infarction due to neuropathy-related symptom attenuation [9]. Streptozotocin (STZ) (2-deoxy -2- (3-methyl-3 nitrosourea) -1-0-glucopyranose is a naturally occurring alkylating antineoplastic agent that is particularly toxic to the insulin producing β -cell of the pancreas in mammals. it is a mixture of α- and β-Stereoisomers with molecular weight of 265 g/mol [10]. STZ is taken into the pancreatic β – cells of mammals by glucose transporter -2. When STZ is injected into an adult rat, it causes type 1 diabetes with severely elevated blood glucose level [11]. The therapeutic use of plants for maintaining good health has been extensively documented. Historically, plants and their derivatives have formed the basis of many pharmaceuticals that are employed in treating various ailments [12,13]. It is estimated that about 80% of the world's population relies on plant-based alternative medicines for healthcare [14]. Medicinal plants, which contain bioactive compounds that are used for therapeutic purposes or as precursors for vital drugs, become recognized as such when their biological activity is either ethnobotanically reported or scientifically established [15]. Africa has more than 500 plant species with known medicinal value. Many of these plants are being evaluated for their phytochemical compositions, medicinal and therapeutic properties such as antioxidant, anti-ulcer, anti-cancer, anti-diabetic, anti-hypertensive, hepatoprotective and nephroprotective effects amongst others. The World Health Organization recognizes the importance of conducting scientific research on herbal medicine, as these natural products have the potential to make a significant contribution to healthcare [16]. *Justicia carnea* is a flowering plant, widely distributed in various parts of Africa. In Nigeria, the shrubs of *Justicia carnea* are grown around homesteads and are easy to propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil [17]. In the Eastern parts of Nigeria, it is referred to as "Ogwu Obara" where a decoction of the plant is used as blood tonic administered to anemic patients, pregnant women as well as women undergoing menstruation [18]. In addition to its traditional use as a hematinic, it is also used locally in the treatment and management

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

of different ailments such as diarrhea, arthritis, liver disease, diabetes, inflammation, gastrointestinal disorders and rheumatism [19,20]. The plant has also been reported to possesses antidiabetic, antioxidant, antiviral, hypocholestrolemic, and cardioprotective properties [21,22,23]. Although *Justicia carnea* extracts have been demonstrated to have antidiabetic potential [24,25,26], there is no comparative study on the impact of *Justicia carnea* methanol leave extract on the histomorphology of the pancreas, liver and kidneys of STZ-induced diabetic rats. This present work therefore investigated the histopathological effects of the methanol leave extracts of *Justicia carnea* on the pancreas, liver and kidney of STZ-induced diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade and were products of either British Drug House (BDH) (England), or Sigma Aldrich Ltd. (USA)

Collection and Identification of Plant

Fresh leaves of *Justicia carnea* were harvested from an open forest in Ovbiogie community, Ovia North - East Local Government Area, Benin City, Nigeria and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A voucher specimen (UBH-J386) was deposited at the Departmental Herbarium for future reference.

Preparation of Plant Sample

The fresh samples of *Justicia carnea* leaves were washed and air-dried. The dried leaves were pulverized using a mechanical grinder. The extraction of the pulverized plant material was done by maceration over a 72 hours period [27]. A portion (500 g) of the powdered leaves was soaked in 5000 ml methanol. The resultant methanol extract was filtered with a muslin cloth. The methanol solvent was removed by condensation using a rotary evaporator at 60°C and the moisture was removed by freeze drying using a lyophilizer to finally obtain the *Justicia carnea* methanol extract which was stored in the laboratory refrigerator at – 4°C until required for use.

Experimental Design

A total number of thirty-six (36) male Wistar rats (8 weeks old) weighing between 180g – 200g (mean weight = 190±10) were used in this study. The animals were obtained from the animal house of the Department of Biochemistry, University of Benin, Benin city, Edo state, Nigeria. The animals were acclimatized for two weeks under healthy and hygienic condition. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to pelletized growers mash and clean drinking water. All the experiments were carried out in accordance with the National Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23) revised 1996. The Wistar rats were randomized into 6 groups of 6 animals each. Group 1 was the control group which received only food and water throughout the duration of the experiment. Groups 2 to 6 animals were earlier induced with diabetes following intraperitoneal injection of streptozotocin (50 mg/kg bw). Group 2 was the diabetic untreated group, group 3 was the diabetic group treated with Metformin (25 mg/kg bw), while groups 4, 5 and 6 were diabetic groups treated with 100 mg/kg bw, 200 mg/kg bw and 500 mg/kg bw respectively of methanol extract of *Justicia carnea* leaves. The treatments lasted for 21 days after which the rats were sacrificed after an overnight fast.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

Acute Toxicity Text

An oral acute toxicity study was conducted on the methanol extract of *Justicia carnea* leaves using the Lorke method [28]. A total of eighteen (18) rats were used in this two-phase study. In phase 1, the rats were randomized into 3 groups of 3 animals each. Each group received a designated oral gavage dose of the methanol extracts (10, 100, 1000 mg/kg body weight) respectively. The animals were initially observed for signs of toxicity 60 minutes after administration and were continuously monitored for 24 hours. The absence of mortality in phase I necessitated a second phase. In phase 2, three rabbits were allocated to separate groups, with each group receiving a single, high-dose oral gavage of the methanol extracts (1500, 2500, and 5000 mg/kg body weight) respectively. The animals were observed for signs of toxicity within 24 hours, with extended monitoring for an additional 48 hours to assess for delayed mortality.

Induction of Diabetes and Administration of Extracts

Streptozotocin (STZ) (50 mg/kg bw) was administered intraperitoneally to induce diabetes. Fasting blood glucose levels were checked after three days. Rats with fasting blood glucose >200 mg/dL were considered diabetic. Fasting blood glucose (FBG) levels were determined with the aid of ACCU-CHEK Advantage II Active glucometer and strips. The test strip was inserted into the glucometer; blood sample was collected from the tail of the rat by tail tipping using a surgical blade. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded in mg/dl. Prior to the induction of diabetes, the animals were subjected to an overnight fast. The next morning, the fasting blood glucose levels of the experimental animals were measured and recorded. The blood glucose level was subsequently recorded on weekly basis for 21 days. The methanol extract of *Justicia carnea* was orally administered to the rats daily for 21 days of the experiment and the weight of the rats were also recorded on a weekly basis.

Histological study

At the end of the 21 days of treatment, the animals were sacrificed and the organs of interest (Pancreas, liver and kidney) were harvested for histological studies. The organs were rinsed in normal saline, soaked in 10% formalin for 12 hours and prepared according to the paraffin wax embedding technique by staining with hematoxylin and eosin for microscopic evaluation. Histopathological examination was carried out under light microscope. In each H&E section, exactly 25 circular tubules were measured in two axes drawn perpendicular to each other with the aid of an image analyzer (Image Proplus, version 3.0).

Statistical Analysis

Count data are expressed as mean ± standard error of mean. The statistical analysis was performed using SPSS (version 20). The various treatment groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Acute Toxicity

The animals did not show any toxicity sign and symptom at the highest dose of 5000 mg/kg bw. The methanol extract of *Justicia carnea* did not produce any mortality in the rats (Table 1 and Table 2).

Furthermore, the animals did not display any drug-related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Therefore, the extract was deemed to be safe at dose of 5000 mg/kg, and the median lethal dose (LD50) was considered to be > 5000 mg/kg.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

Table 1: Phase 1 Acute toxicity Test

Dose (mg/kg bw of methanol extract of *J. carnea*)	Mortality
10	0/3
100	0/3
1000	0/3

Table 2: Phase 2 Acute toxicity Test

Dose (mg/kg bw of methanol extract of *J. carnea*)	Mortality
1500	0/3
2500	0/3
5000	0/3

Effect of *J. carnea* on Glucose Concentration of Streptozotocin-Induced Diabetic Rats

Graded doses of methanol extract of *J. carnea* leaves significantly reduced the blood glucose concentration of streptozotocin-induced diabetic rats ($p < 0.05$) (Table 3).

Table 3: Blood Glucose Concentration (mg/dL) of Diabetic Rats

Group			**Week**		
	Initial	**1**	**2**	**3**	**4**
Group 1 (Control)	76.00±4.15	_	103.67±2.53^{b}	92.33±2.37^{b}	65.5±3.42^{b}
Group 2 (Diabetic Control)	94.67±14.45	305.00±27.40^{a}	475.00±41.02^{a}	368.00±54.08^{a}	369.33±28.01^{ab}
Group 3 (Metformin)	62.20±3.88	310.80±56.81^{a}	286.40±55.00^{ab}	204.60±51.17^{ab}	168.00±76.00^{ab}
Group 4 (100 mg/kg)	58.80±4.11	283.20±50.71^{a}	223.00±58.15^{ab}	222.00±59.05^{ab}	171.20±67.12^{ab}
Group 5 (200 mg /kg)	56.80±1.65	370.20±49.42^{a}	321.75±53.10^{ab}	295±112.155^{ab}	222.33±62.22^{ab}
Group 6 (500 mg/kg)	59.17±6.32	297.67±43.31^{a}	240.20±70.83^{ab}	190.00±55.08^{ab}	53.00±12.00^{b}

Values are expressed as Mean ± SEM (n=3). Values with superscript "b" are significantly different from the normal control while values with superscript "b" are significantly different from the diabetic control (p<0-05). Values with superscripts 'a and b' are significantly different from both normal and diabetic controls (p<0.05) but not significantly different when compared to the Metformin treated groups.

Effect of Graded Doses of Methanol Extract of *J. Carnea* Leaves on Rat Body Weight

Table 4 shows the changes in the body weights of diabetic rats treated with methanol extract of *J.carnea* leaves. There were increases in the body weights of the Wistar rats across the groups. Group 4 and 5 had a significantly lower weight gain when compared to the normal control and diabetic control.

Table 4: Changes in the Body Weight of Diabetic Rats Treated with *J.carnea* Extract

Groups	**Initial Body Weight (g)**	**Final Body Weight (g)**	**Change in Body Weight (g)**
Group 1 (Control)	109.53±3.13	192.80±14.34	83.28±14.49
Group 2 (Diabetic Control)	133.75±14.41	212.11±17.24	78.36±9.15
Group 3 (Metformin)	118.33±5.23	178.58±11.19	66.20±9.74
Group 4 (100mg of Extract)	118.362±12.64	176.67±18.24	58.31±9.52^{a}
Group 5 (200mg of Extract)	112.04±4.43	153.99±5.54	41.95±1.45^{a}
Group 6 (500mg of Extract)	125.81±6.59	211.08±15.88	85.27±9.29

Values are expressed as Mean ± SEM (n=3). Values with superscript ^{a} are significantly different ($p < 0.05$) from the control group.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

Effect of Graded Doses of Methanol Extract of *J. Carnea* Leaves on the Weight of the Rats' Pancreas, Liver and Kidney

The final weights of the kidney, pancreas, and liver is presented in table 5. There was an abnormal increase in the weight of the liver in group 2 (the diabetic untreated group) when compared to other groups. When the normal control was compared to the diabetic control, the weight of the kidney and pancreas did not differ significantly ($p>0.05$).

Table 5: Changes in the Weights of the Pancreas, Liver and Kidney

Groups	PANCREAS	LIVER	KIDNEY
Group 1 (Control)	0.68±0.10	4.57 ± 0.18^{b}	1.14±0.09
Group 2 (Diabetic Control)	0.63±0.06	8.04 ± 0.58^{a}	1.43±0.18
Group 3 (Metformin)	$0.37\pm0.06^{a,b}$	4.44 ± 0.28^{b}	$0.77\pm0.11^{a,b}$
Group 4 (100 mg/kg)	$0.36\pm0.03^{a,b}$	$3.53\pm0.34^{a,b}$	$0.66\pm0.03^{a,b}$
Group 5 (200 mg /kg)	$0.25\pm0.00^{a,b}$	3.78 ± 0.09^{b}	$0.67\pm0.04^{a,b}$
Group 6 (500 mg/kg)	$0.33\pm0.03^{a,b}$	4.18 ± 0.23^{b}	$0.76\pm0.05^{a,b}$

Values are expressed as Mean ± SEM (n=3). Values with different superscripts are significantly different from the controls ($p<0.05$).

Effects of methanol extract of *Justicia carnea* leaves on the Pancreas, Liver and Kidney Ultrastructure in STZ-induced Diabetic Rats.

The histopathological analyses of the Pancreas, Liver and Kidney of diabetic rats treated with methanol extracts of the leaves *of J. carnea* are presented in Figures 1,2 and 3 respectively.

Fig. 1a – 2f represent the photomicrographs of the Pancreas

Fig. 2a – 2f represent the photomicrographs of the Liver

Fig. 3a – 3f represent the photomicrographs of the Kidney

Fig. 1: Photomicrographs of the Pancreas

Fig. 1a Pancreas of group 1 rats (Normal control) showing normal Exocrine Acini (EA), Islets of Langerhans (IL), Pancreatic ducts (PD): H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

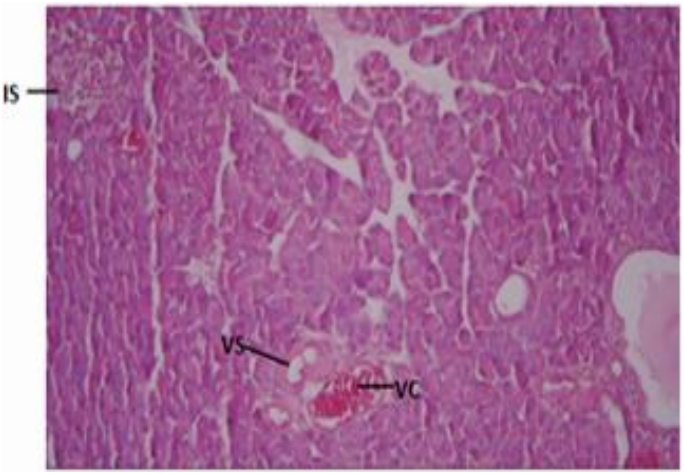

Fig. 1b. Pancreas of diabetic untreated rats (Group 2) showing degenerating islets (IS), vascular congestion (VC) and stenosis (VS): H&E x 400

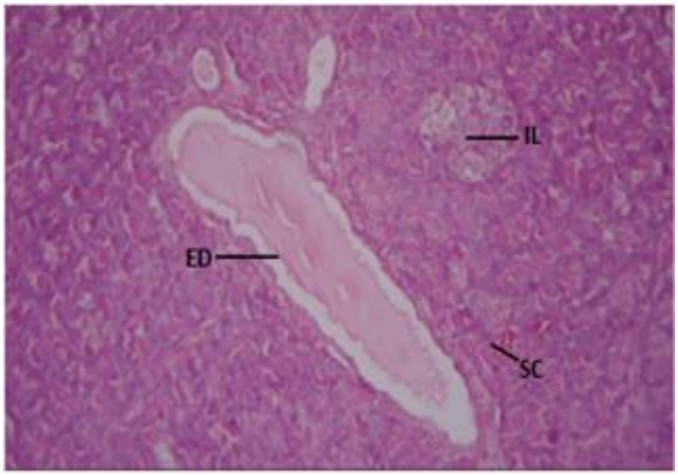

Fig. 1c. Pancreas of metformin treated diabetic rat (Group 3) showing normal islet of Langerhans (IL), dilated exocrine duct (ED) with active stromal congestion (SC): H&E x 400

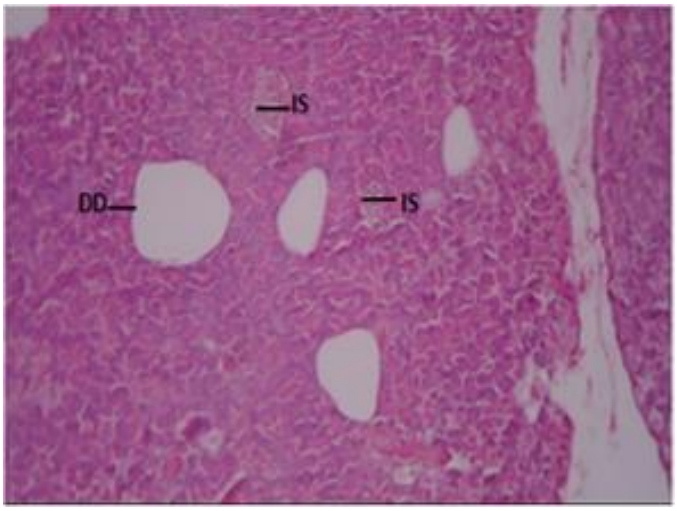

Fig. 1d. Pancreas of diabetic rats treated with 100mg/kg bw extract (Group 4) showing regenerating islets (IS) and dilated, ducts (DD): H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

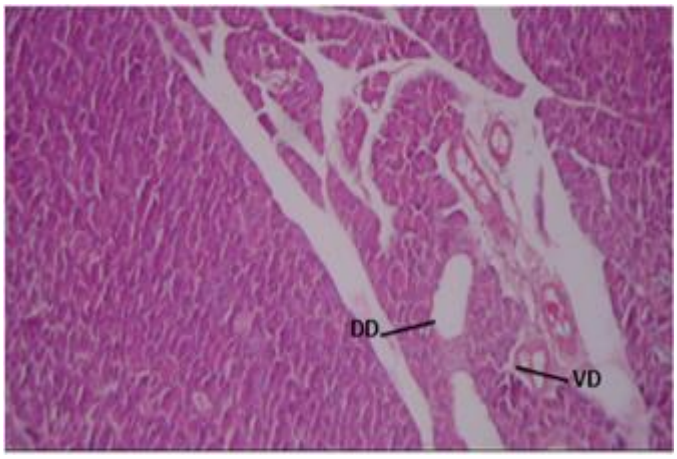

Fig. 1d. Pancreas of diabetic rats treated with 200mg/kg bw extract (Group 5) showing paucity of islets, dilated ducts (DD), and vascular obstruction (VD): H&E x 400

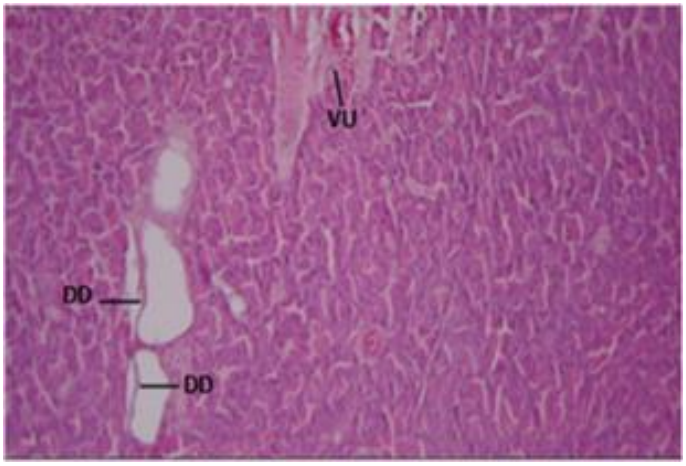

Fig.1e. Pancreas of diabetic rats treated with 500mg/kg bw extract (Group 6) showing: paucity of islets, dilated ducts (DD), and vascular ulceration (VU): H&E x 400

Figure. 2: Photomicrographs of the Liver

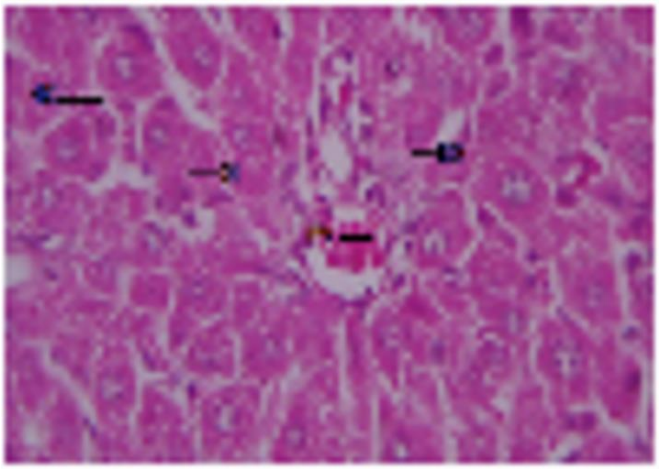

Fig. 2a. Liver of group 1 rats (Normal control) showing normal architecture: hepatocytes. (HC), sinusoids (SI), bile duct (BD), portal vein (PV): H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

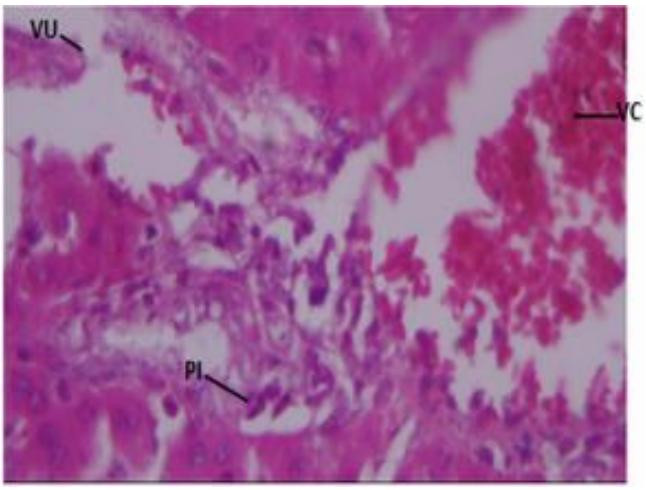

Fig. 2b. Liver of diabetic untreated rats (Group 2) showing: severe vascular ulceration (VU), congestion (VC), periportal infiltrates of inflammatory cells (PI): H&E x 400

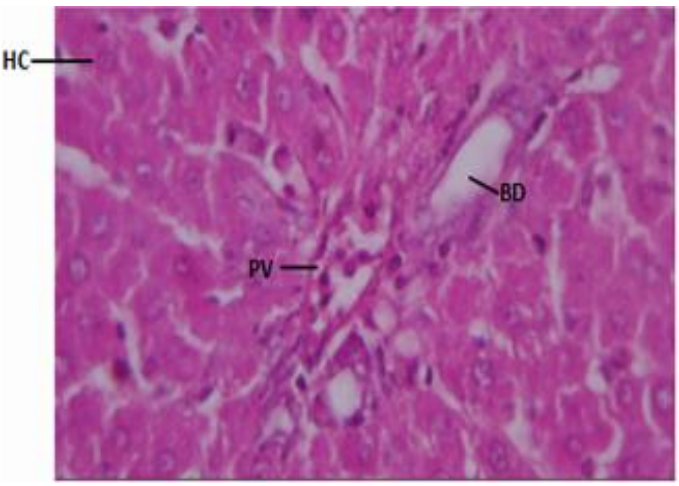

Fig. 2c. Liver of metformin treated diabetic rat (Group 3) showing normal hepatocytes (HC), portal vein (PV), and bile ducts (BD) H&E x 400

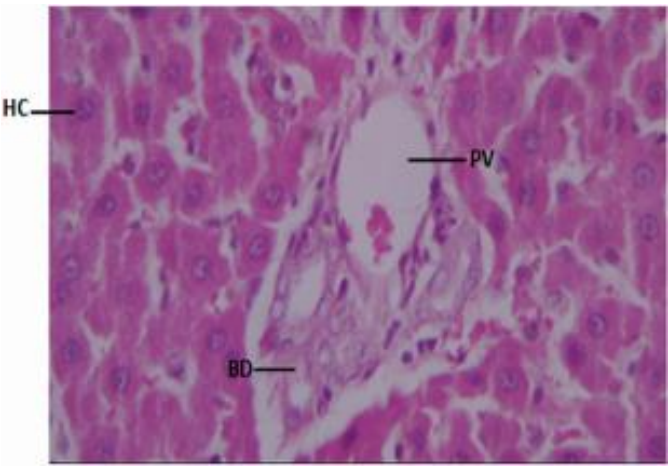

Fig. 2d. Liver of diabetic rats treated with 100mg/kg bw extract (Group 4) showing: normal architecture of hepatocytes (HC), portal vein (PV), and bile ducts (BD): H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

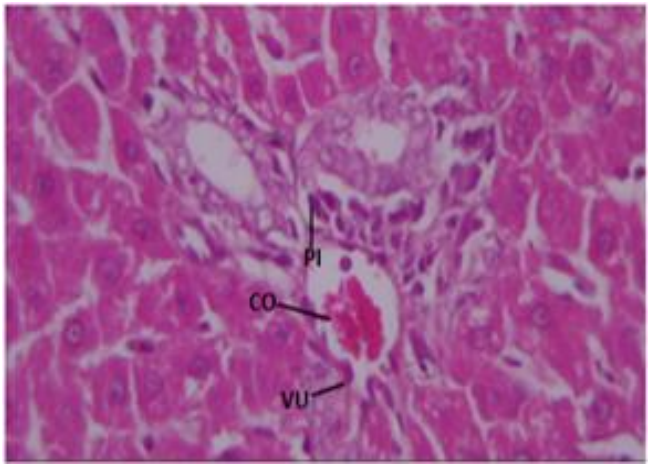

Fig. 2e. Liver of diabetic rats treated with 200mg/kg bw extract (Group 5) showing: periportal infiltrates of inflammatory cells (PI), focal portal vascular ulceration (VU), congestion (CO): H&E x 400

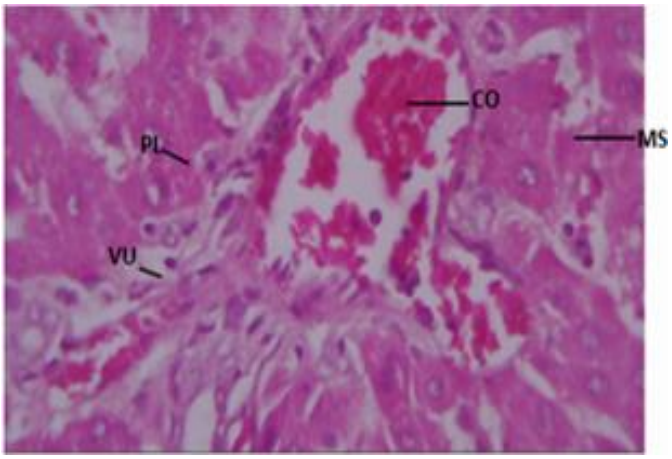

Fig. 2f. Liver of diabetic rats treated with 500mg/kg bw extract (Group 6) showing: mild periportal mobilization of lymphocytes, vascular ulceration, congestion, microvesicular steatosis: H&E x 400

Figure. 3: Photomicrographs of the kidney

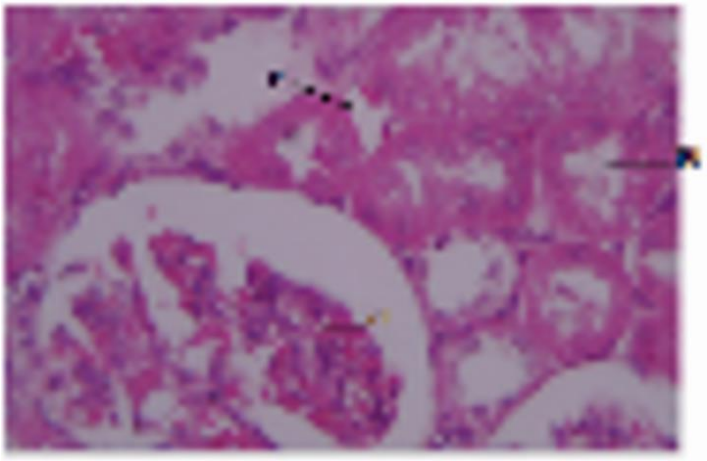

Fig. 3a. Kidney of group 1 rats (Normal control) showing normal architecture: interstitial space (IS), tubules (TU), glomeruli (GL): H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

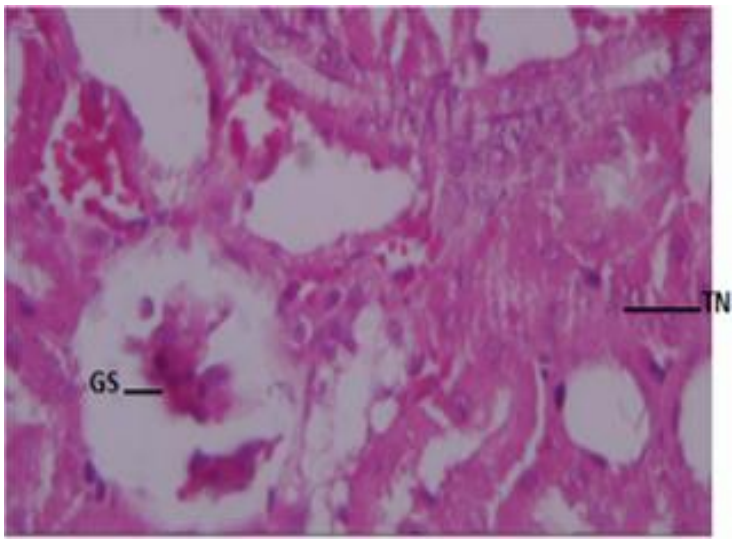

Fig. 3b Kidney of untreated diabetic rats (Group 2) showing: interstitial congestion, glomerular shrinkage (GS), focal (tubular necrosis (TN): H&E x 400

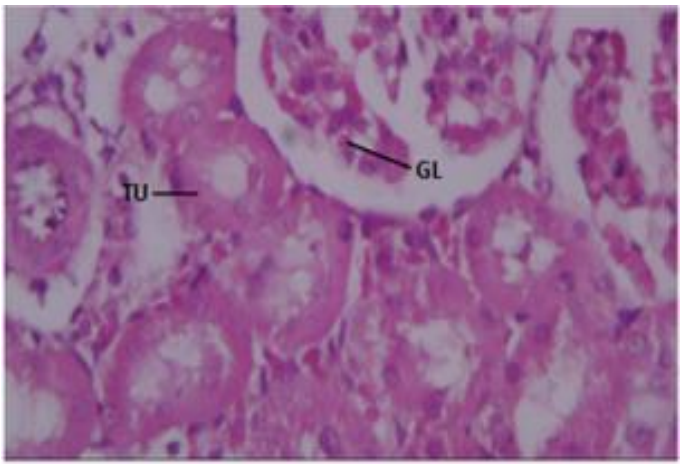

Fig. 3c Kidney of metformin treated diabetic rat (Group 3) showing normal tubules (TU) and normal glomeruli (GL), (H&E x 400).

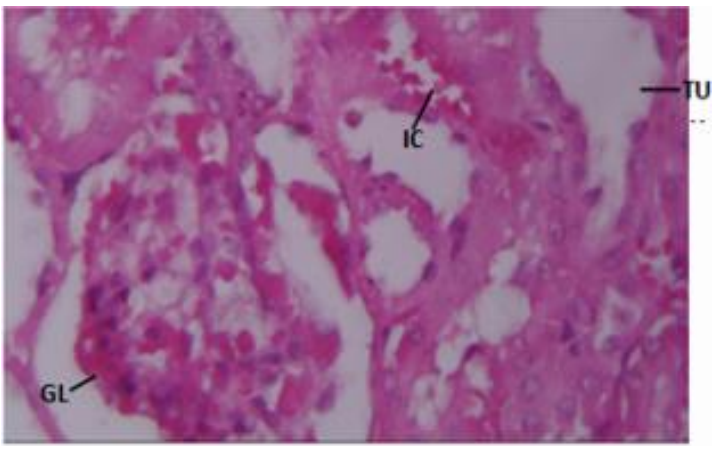

Fig. 3d. Kidney of diabetic rats treated with 100mg/kg bw extract (Group 4) showing: active interstitial congestion (IC), tubules, glomeruli (GL), all normal: H&E x 400

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

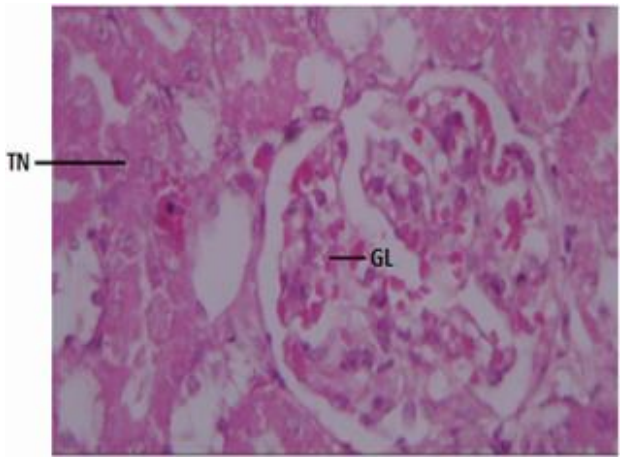

Fig. 3e. Kidney of diabetic rats treated with 200mg/kg bw extract (Group 5) showing: focal tubular necrosis (TN), and normal glomerulus (GL): H&E x 400

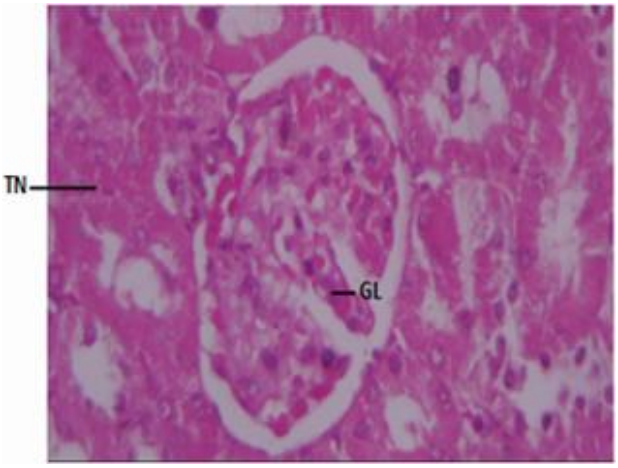

Fig. 3f. Kidney of diabetic rats treated with 500mg/kg bw extract (Group 6) showing: mild focal tubular necrosis (TN), and normal glomerulus (GL): H&E x 400

DISCUSSION

Diabetes mellitus is a group of metabolic diseases that is becoming more common worldwide [29] and characterized by multiorgan failures, peripheral neuropathy, retinopathy, nephropathy, hyperlipidemia, and a variety of cardiovascular disorders [3,5]. *Justicia carnea* extracts have been demonstrated to have antidiabetic potential [24,25,26]. The photomicrograph of the pancreas of group 1 rats (normal control) showed intact and healthy islets of Langerhans, pancreatic duct and exocrine acini (Fig.1a) while the photomicrograph of untreated diabetic group represented in Fig. 1b showed some features of complications of diabetes in the blood vessel such as vascular obstruction, ulceration and disappearance of the islets of Langerhans. When the diabetic rats were treated with metformin there was regeneration of the islets of Langerhans (Fig. 1c) similar to those of the normal control (Fig. 1a). The 100 mg/kg bw of the extract was also effective in the regeneration of the islets of Langerhans that was destroyed by diabetes hence ameliorating the effect of diabetes as shown in Fig. 1d. The rate of regeneration of the islet of Langerhans in groups 5 and 6 rats (Fig. 1e and Fig.1f) respectively, was less than that of the 100 mg/kg bw (Fig.1d), hence very few islets of Langerhans were present as shown in the photomicrographs (Fig. 1e and Fig. 1f respectively). Some vascular complications such as dilation of the blood vessel and vascular obstruction were also noticed in the pancreas of groups 5 and 6 (Fig.1e and Fig.1f) respectively. It has been reported in a

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

previous work [30] that there was considerable regeneration of the islet of Langerhans in the pancreas following administration of ethanolic extract of *Alchornea cordifolia* leaf in alloxan-induced diabetic Wistar rats. The plant extract of *J. carnea* is probably able to induce the proliferation of quiescent (inactive) cells to replace lost ones. The photomicrograph of the liver of group 1 rats (Fig.2a) shows normal hepatic histo-architecture. The liver parenchyma shows normal portal hepatic features with the central vein, sinusoids radiating from the central vein and the hepatocytes appearing normal. The hepatocytes are densely packed with normal cellular structure. However, there were structural alterations in the liver parenchyma of group 2 rats. These alterations which included desquamated, dilated, and congested central vein, apoptosis of hepatocytes with aggregation of macrophages, are in line with previous report [31]. The hepatocytes (HC), sinusoids (SI), bile duct (BD) and portal vein (PV) on the portal zone were all normal. The diabetic untreated group showed that there was dilatation of the blood vessels leading to thinning of the wall causing vascular ulceration (Fig.2b). This ulceration of the blood vessel is a complication of atherosclerosis induced by diabetes [32]. This condition is a diabetic complication known as vasculopathy. The photomicrograph also indicates inflammation around the portal vein, this condition is known as portal hepatitis (inflammation of the portal zone of the liver).The liver of the diabetic group treated with Metformin (Fig.2c) showed a significant reversal of the vasculopathy induced by diabetes, the alteration and other complications as a result of diabetes were also ameliorated .This pattern of restoration of the liver's ultrastructure was also observed for group 4 animals which were diabetic rats treated with 100 mg/kg bw of the methanol extract of *Justicia carnea* (Fig.2d). The liver of the diabetic rats treated with 200 mg/kg bw of the extract, also showed a reversal of some structural complications induced by diabetes (Fig.2e) but some inflammatory cells were still present in the liver with congestion and mild vascular ulceration. This observation is consistent with previous findings [33]. In this present study, it was observed that the photomicrograph of diabetic rats treated with 100 mg/kg bw of the extract (Fig.2e) was more similar to the normal control when compared to the photomicrograph of diabetic rats treated with 200 mg/kg bw (Fig.2f). The restoration to normal of the hepatocytes (diabetic rats treated with 500 mg/kg bw) was less pronounced compared to group 4 rats treated with 200 mg/kg bw of the extract due to the level of congestion and vascular ulceration in the liver. The doses have varying effects on the impact of diabetes on the liver and in comparing the effect of the various doses of the extract to the normal control and the drug (Metformin), the 100 mg/kg bw of the methanol extract of *Justicia carnea* was more effective in the amelioration of the effect of STZ – induced diabetes in the liver than 200 and 500 mg/kg bw of the extract. However, it has been reported [25] that both low (200 mg/kg) and high (800 mg/kg) doses of *Justicia carnea* extract was effective in ameliorating the effect of diabetes on the liver. Diabetic kidney disease is the leading cause of end-stage kidney disease [34]. It is considered a microvascular complication and occurs in both diabetes mellitus type 1 and diabetes mellitus type 2. Thirty to 40 percent of patients with diabetes mellitus develop diabetic nephropathy [35]. The exact cause of diabetic nephropathy remains unknown, but insulin resistance, genetics, hyperglycemia, and an autoimmune process may be the causes [35,36]. The photomicrograph of the kidney of the normal control group showed normal architecture of interstitial spaces, tubules and glomeruli (Fig.3a). The untreated group, showed congestion of the interstitial space, shrinkage of the glomeruli and tubular necrosis (Fig.3b) which are some of the features of diabetic nephropathy caused by diabetes [35,37]. When the diabetic rats were treated with Metformin, the photomicrograph of their kidneys (Fig.3c) were similar to that of the normal control which is an indication of the effectiveness of metformin in reversing the effect of diabetes on the kidney. The photomicrograph of diabetic rats treated with 100 mg/kg bw of the extract (Fig.3d) showed normal glomeruli, tubules and the interstitial spaces, were normal in comparison with the normal control, suggesting the effectiveness of the dose in the amelioration of the effect of diabetes on the kidney just like the standard drug, metformin. The photomicrographs of the kidneys of diabetic rats treated with 200 and 500 mg/kg bw of the extract respectively (Fig.3e and fig.3f), showed no shrinkage of the glomeruli compared to the diabetic control (Fig.3b) but there was focal necrosis. From the histomorphology of the kidneys of diabetic rats treated with 100, 200 and 500 mg/kg bw of the extract, it is clear that the various doses of the extract were effective in the amelioration of the diabetes-induced nephrotoxicity. This is also corroborating the findings as previously reported that there was restoration of

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VIII August 2024

the normal general structure of the kidney and the normal appearance of glomeruli and tubules after treatment of diabetic rats with *Justicia carnea* extract [25].

CONCLUSION

This study demonstrated the significant therapeutic potential of the methanol extract of *Justicia carnea* leaves in the management of diabetes mellitus and its complications in the pancreas, liver and kidney. Histopathological analysis revealed that the methanol extract of *Justicia carnea* showed varying degree of amelioration of the various organs' lesions at different doses. This study revealed that the 100 mg/kg body weight dose of the extract was particularly very effective in ameliorating the diabetes-induced damage across all examined organs. These findings underscore the medicinal properties of *Justicia carnea* and support its potential use in the management of diabetes mellitus. Therefore, we are proposing the methanol extract of *Justicia carnea* leave as a potential candidate for antidiabetic drug or a candidate for adjuvant drug with other antidiabetic drugs. Further research and clinical trials are warranted to fully explore its potential, establish standardized dosing regimens for therapeutic applications and determine the mechanism of action of the leave extract.

FUNDING

This research project did not receive any funds from any agency. It was self-funding

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest related to the publication of this paper

ACKNOWLEDGEMENTS

The authors appreciate the Natural Product Research and Disease Control Laboratory (NPRDC), Department of Biochemistry, University of Benin for providing the facilities needed to carry out this study. The authors also thank the following persons: Mr Shuaibu Braimah, Mr Kingsley Akpeh and Mr Emmanuael Nwemuche for their technical support.

REFERENCES

1. American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus Diabetes Care. 37 (1):81-90
2. Craig, M. E., Hattersley, A. and Donaghue, K. C. (2009). Definition, epidemiology and classification of diabetes in children and adolescents. Pediatr diabetes. 10(12): 3-12
3. Fayez, K. A., Abdullah, A. A., Ibrahim, A. A., Fahad, A. A., Mohammed, H. A., Mohammed, G.Y., Alfaifi, F. R., Abdulaziz K. and Hatem A. A. (2023). Diabetes Mellitus. International Journal of Engineering Applied Sciences and Technology. 8(3): 43-52.
4. American Diabetes Association. (2021). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes. Diabetes Care, 44(Supplement 1), S15-S33
5. Smith, J. D. and Jane, K. D. (2020). "The Impact of Diabetes on Cardiovascular Health." Journal of Diabetes Research. 14(2): 123-134.
6. Reed, J., Bain, S.and Kanamarlapudi, V. (2021). A Review of Current Trends with Type 2 Diabetes: Epidemiology, Aetiology, Pathogenesis, Treatment and Future Perspective, Metabolic Syndrome and Obesity: Targets and Therapy 14: 3567–3602. https://doi.org/10.2147/DMSO.S319895
7. Levinthal, G. N and Tavill, A.S. (1999). Liver disease and diabetes mellitus. Clin Diabetes.17:73
8. Mohamed,J., Nafizah,A.H., Zariyantey, A.H. and Budin,S.B. (2016). Mechanisms of Diabetes-

Induced Liver Damage: The role of oxidative stress and inflammation. Sultan Qaboos Med J.16(2): e132–e141. doi: 10.18295/squmj.2016.16.02.002

9. Sharan, B. (2019). Hyperglycaemia and Myocardial Infarction. British Journal of Medical Practitioners. 12(2): 1 – 4.
10. Eleazu, C. O., Eleazu, K. C., Chukwuma, S. and Essien, U. N. (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of diabetes & metabolic disorders. 12**:** 1-7.
11. Furman, B. L. (2021). Streptozotocin-induced diabetic models in mice and rats. Current Protocols. 1: e78. doi: 10.1002/cpz1.78
12. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. Pharmacogn Rev, 6(11), 1-5. https://doi.org/10.4103/0973-7847.95849
13. Ochekwu, E. B., Ekeke, C., Nwadiaro, P. O., & Christopher, S. (2015). Phytochemical evaluation of leaves of some medicinal plants from parts of Niger Delta.IOSR journal of Pharmacy and Biological Sciences.10(2):26-31
14. World Health Organization (WHO). Nutritional Policy on Traditional Medicine and Regulation of Herbal Medicine. Geneva 2005 Report of WHO Global Survey, http://wwwwho.int,/topics/traditional medicine/en
15. Ahirwar, R.K. (2023). An Overview of Medicinal Plants: Drugs of Tomorrow. In: Singh, D., Mishra, A.K., Srivastava, A.K. (eds) Stress-responsive Factors and Molecular Farming in Medicinal Plants. Springer, Singapore. https://doi.org/10.1007/978-981-99-4480-4_116.
16. Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O., Akanmu, M. A., & Ukponmwan, O. E. (2006). Sedative, antiepileptic and antipsychotic effects of Spondias mombin L. (Anacardiaceae) in mice and rats. J Ethnopharmacol, 103(2), 166-175. https://doi.org/10.1016/j.jep.2005.07.019
17. Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*. **2**(3): 365-372.
18. Onyeabo, C., Achi, N. K., Ekeleme-Egedigwe, C. A., Ebere, C. U. and Okoro, C. K. (2017). Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Sci. Pol. Technol. Aliment.* **16**(2): 217–230
19. Badami, S., Aneesh, R., Sankar, S., Sathishkumar, M. N., Suresh, B. and Rajan, S. (2003). Antifertility activity of Derris brevipes variety coriacea. *J. Ethnopharm.* **84:** 99–104.
20. Correa, G. M. (2012). Chemical constituents and biological activities of species of Justicia: A review. *Braz J Pharmacogn*. **22:** 220-238.
21. Medapa, S., Singh, G. R. J. and Ravikumar, V. (2011). The phytochemical and antioxidant screening of Justicia wynaadensis. *Afr. J. Plant Sci.* **5**(9): 489–492
22. Abo, K.A., Fred-Jaiyesimi, A.A., Jaiyesimi, A.E.A., (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology 115, 67–71.
23. Orjiakor,C.A., Uroko, R.I., Njoku, O. U., and Ezeanyinka, L.U.S. (2019). Nutritive properties of aqueous extract of Justicia carnea leaves andsits effects on haematological and some biochemical indices of anaemia induced male wistar albino rats. Biomedical Research 30(4):645-654
24. Ukpabi-Ugo, J.C., Ndukwe, P.A.C.and Iwuoha, A.G. (2019) Hepatoprotective Effect of Methanol Extract of Justicia carnea Leaves on Carbon Tetrachloride-Intoxicated Albino Rats. Biochem Anal Biochem 8:381. doi: 10.4172/2161-1009.1000381
25. Ukpabi-Ugo, J.C., Uhuo, E.N., Ugwu, O.C. and Chukwuma, E.W. (2023). Evaluation of Subacute Toxicity of Methanol Extract of Justicia carnea Leaves in Wistar Albino Rats. Animal Research International. 20(1): 4862 – 4875
26. Ojeaburu, S.I. and Olasehinde, O. (2024). Ameliorative Effect of Justicia carnea Methanol Leaf Extract against Nephrotoxicity in Streptozotocin-Induced Diabetic Wistar Rats. Sahel Journal of Life Sciences.2(2):141-148 https://doi.org/10.33003/sajols-2024-0202-19
27. Abu, O.D., Imafidon, K.E. and Iribhogbe, M.E. (2015). Biochemical effect of aqueous leaf Extract ofIcacina trichanta on urea, creatinine and kidney oxidative status in CCl4-induced Wistar rats.

Nigerian Journal of Life Sciences. 5 (1): 85 – 89
28. Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. Arch Toxicol. 54:275-287
29. Alhassan, A.J., Sule, M.S., Atiku, M.K., Wudil, A.M., Abubakar, H.and Mohammed, S.A. (2012). Effects of aqueous avocado pear (Persea americana) seed extract on alloxan induced diabetic rats. Gr. J. of Med. Sci.2(1);005-011
30. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, and Shaw JE. (2014). Global estimates of diabetes prevalence for (2013) and projections for 2035. Diabetes Res Clin Pract.103(2):137–149. doi: 10.1016/j.diabres.2013.11.002
31. Eliakim-Ikechukwu, C.F and Obri, A.I. (2009). Histological changes in the pancreas Following administration of ethanolic extract of *Alchornea cordifolia* leaf in alloxan-induced diabetic Wistar rats. Nigerian Journal of Physiological Sciences 24(2):153-155 DOI:10.4314/njps. v24i2.52927
32. Poznyak, A., Grechko, A.V., Poggio, P., Myasoedova, V.A., Alfieri, V.and Orekhov, A.N. (2020). The Diabetes Mellitus-Atherosclerosis Connection: The Role of Lipid and Glucose Metabolism and Chronic Inflammation. Int J Mol Sci. 21(5):1835. doi: 10.3390/ijms21051835
33. Wilson, J. I., Ojieh, A.E., Ossai, N.R., Nwogueze, B.C.and Olise-Enewe,M.(2021). Histomorphological, Hypoglycaemic and Hepatoprotective Effects of Acute Administration of Methanolic Extract of Persea americana Seed in Alloxan-induced Diabetic Wistar Rats. Plant Cell Biotechnology and Molecular Biology. 22(41&42):247-260
34. Rabkin, R. (2003). Diabetic Nephropathy. Clin Cornerstone. 5(2):1-11.
35. Umanath, K. and Lewis, J.B. (2018). Update on Diabetic Nephropathy: Core Curriculum 2018. Am J Kidney Dis. 71(6):884-895.
36. Koye, D.N., Magliano, D.J., Reid, C.M., Jepson, C., Feldman, H.I., Herman, W.H. and Shaw, J.E. (2018). Risk of Progression of Nonalbuminuric CKD to End-Stage Kidney Disease in People with Diabetes: The CRIC (Chronic Renal Insufficiency Cohort) Study.Am J Kidney Dis. 72(5):653-661.
37. Ahmadi, N and Amouzegar A. (2024). Diabetic Kidney Disease Without Albuminuria: A New Entity in Diabetic Nephropathy. Iran J Kidney Dis.1(1):1-8.