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Original Article

Effects of *Neocarya macrophylla* Chloroform Fraction on Glycemic Control and Tissue Integrity in Experimental Type 2 Diabetes

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ABSTRACT

Background: Type 2 diabetes mellitus is associated with persistent hyperglycemia, dyslipidemia, hepatic dysfunction, renal impairment, and progressive tissue injury. Despite the availability of conventional antidiabetic drugs, limitations relating to cost, accessibility, and adverse effects continue to drive interest in medicinal plants with potential therapeutic value. *Neocarya macrophylla* (*Nm*) has been traditionally used in the management of diabetes; however, its pharmacological efficacy remains insufficiently validated. This study investigated the antidiabetic and organ-protective effects of the chloroform fraction of *Nm* leaves in high-fat diet/streptozotocin–induced type 2 diabetic Wistar rats.

Methods: Twenty-four male Wistar rats were randomly assigned into four groups (n = 6): normal control, diabetic control, *Nm* chloroform fraction-treated (180 mg/kg), and metformin-treated (200 mg/kg). Type 2 diabetes was induced using a high-fat diet followed by a single intraperitoneal injection of streptozotocin (40 mg/kg). Treatments were administered orally for 4 weeks. Fasting blood glucose (FBG), body mass index, liver function indices, lipid profile, renal biomarkers, hematological parameters, and histopathological alterations in hepatic, renal, and pancreatic tissues were evaluated.

Results: Administration of the chloroform fraction significantly reduced FBG levels by −60% (210 mg/dL to 85 mg/dL) compared with diabetic controls ($P < 0.001$). Treatment with the chloroform fraction of *Nm* significantly reduced FBG compared with the diabetic control ($P = 0.0055$), improved Homeostatic Model Assessment of Insulin Resistance ($P = 0.0032$) and Homeostatic Model Assessment of β-cell Function ($P = 0.0003$), reduced aspartate aminotransferase ($P < 0.001$), alanine aminotransferase ($P < 0.001$), urea and creatinine ($P < 0.05$), improved lipid profile ($P = 0.001–0.004$), and restored pancreatic, hepatic, and renal histoarchitecture ($P < 0.001$).

Conclusions: The observed antidiabetic activity of the chloroform fraction of *Nm* may be attributed, at least in part, to bioactive phytoconstituents previously reported in *Nm*, including flavonoids (quercetin, catechin, epicatechin, and catechin glycosides), phenolic compounds, triterpenoids, steroids such as stigmasterol, and alkaloids. These compounds have been associated with anti-hyperglycemic, antioxidant, anti-inflammatory, and insulin-sensitizing activities in experimental models of diabetes and may contribute to the glucose-lowering effects observed in the present study. This calls for further mechanistic and toxicological investigations.

Key words: *Neocarya macrophylla* (Nm), chloroform fraction, antidiabetic activity, high-fat diet, streptozotocin, diabetes

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia due to defects in insulin secretion, action, or both. [1,2] This condition leads to significant hematological changes, including alterations in the morphology, size, and function of red blood cells (RBCs), white blood cells (WBCs), and platelets, which are directly related to endothelial dysfunction and inflammation. [3,4]

According to the International Diabetes Federation, approximately 537 million adults were living with diabetes globally in 2021, and this number is projected to increase to 783 million by 2045. Nigeria remains among the countries experiencing a growing burden of diabetes and its associated complications. [5]

Addressing diabetes involves multiple challenges, such as insufficient funding for non-communicable diseases, lack of population-specific studies and guidelines, medication scarcity, and healthcare disparities. [6] Given these challenges, there is a significant interest in traditional medicine. Over 400 plant species are reported to have antidiabetic properties, though not all have been scientifically validated. [7–9]

In Nigeria, many plants with potential antidiabetic effects have been documented. [10–13] One such plant is *Neocarya macrophylla* (*Nm*), known for its potent ethnomedicinal properties. It contains various bioactive compounds like carbohydrates, alkaloids, flavonoids, anthraquinone, saponin, tannin, glycosides, steroids, and triterpenes, which contribute to its pharmacological activities. [14,15]

Despite its traditional use and promising phytochemical profile, there is limited scientific documentation on the antidiabetic effects of *Nm*. Traditional medicine practitioners have long advocated its use, but further research is needed to establish its safety and efficacy as an antidiabetic agent. [16]

Previous phytochemical investigations of *Nm* have demonstrated the presence of flavonoids, alkaloids, tannins, saponins, phenolic compounds, steroids, and triterpenoids, many of which possess established antidiabetic and antioxidant activities. [15] Furthermore, preliminary investigations indicated that the chloroform fraction contains relatively higher concentrations of medium-polarity bioactive compounds and a better minimum effective dose, thereby justifying its selection for the present study. [17]

Although *Nm* is traditionally used in the management of DM, limited scientific evidence exists regarding its antidiabetic efficacy and organ-protective effects in experimental models of type 2 diabetes; therefore, this study investigated the effects of its chloroform leaf fraction on biochemical, hematological, and histopathological alterations in high-fat diet/streptozotocin (HFD/STZ)–induced diabetic rats.

MATERIALS AND METHODS

Experimental Materials

The study utilized male Wistar rats, oral gavage cannula, dissecting instruments, sample bottles, conical flasks, office pins, standard rodent feed, ethanol, methanol, formaldehyde, formalin, xylene, burettes, cotton wool, glucometer (Accu-Check), measuring cylinders, distilled water, HFD ingredients, stopwatch, anesthetizing chamber, precision weighing balance, phosphate-buffered saline, STZ (Sigma), metformin tablets (500 mg; Actavis, Chennai, India), and 1-mL syringes equipped with 23- and 25-gauge needles. Centrifugation was performed using an Eppendorf 5424R centrifuge (Germany).

Plant Collection and Preparation

Leaves of *Nm* were collected from Jega Local Government Area, Kebbi State, Nigeria, on June 18, 2022. Taxonomic authentication was conducted at the Herbarium Unit, Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, by Mallam Musa Magaji, and a voucher specimen was deposited under PCG/UDUS/CHRY/0001.

Leaves were washed, shade-dried, ground into fine powder, labelled, and stored at room temperature. A total of 1961 g of powdered leaves underwent maceration in 90% methanol for 6 days. Solvent evaporation produced 288 g of crude methanol leaf extract (MEL). A portion of 200 g MEL was suspended in distilled water, filtered, and successively partitioned with *n*-hexane (1 L), chloroform (2.5 L), and *n*-butanol (2.5 L) to yield respective fractions along with a residual aqueous fraction. The fractionation process yielded 2.8 g of chloroform fraction corresponding to a 1.4% yield. The chloroform fraction was selected for experimental evaluation based on the results obtained from the minimum effective dose determination and other preliminary studies. [17] The LD_{50} value of 565 mg/kg was adopted from a previous similar study. [9]

Experimental Animals

Seventy male Wistar rats (4 weeks old; 80–120 g) were obtained from the Animal Breeding Unit, Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. Animals were housed in standard polypropylene cages under controlled environmental conditions (22–25°C, relative humidity 50%–60%, and a 12 h light/12 h dark cycle) with free access to standard feed and water. Following a two-week acclimatization period, 36 rats were selected and fed an HFD ad libitum for four weeks to induce insulin resistance.

Treatments were administered orally once daily between 08:00 and 10:00 h throughout the study period to minimize circadian variations in metabolic responses. Feed and water were provided ad libitum except during scheduled fasting

periods before STZ administration and fasting blood glucose (FBG) determination.

A sample size of six rats per group was selected based on previous studies employing the HFD/STZ model and in accordance with the reduction principle of the ARRIVE 2.0 guidelines. [18] This sample size has been widely used in comparable antidiabetic studies and was considered adequate to detect biologically relevant treatment effects. [19,20]

Preparation of Chloroform Fraction Solution

The chloroform fraction of *Nm* was concentrated using a rotary evaporator at 40°C and stored at 4°C until use. Then the fraction was dissolved in 0.1% dimethyl sulfoxide and distilled water to prepare a stock solution of 24 mg/mL, ensuring solubility and stability for oral administration.

HFD Preparation

A 60% HFD was formulated following Gheibi et al. [21] Standard pellet feed (10 kg; 59.4% carbohydrate, 20% protein, 4.8% fat, 4.8% crude fiber, 7.6% acid detergent fiber, 16.4% neutral detergent fiber) was mixed with pig oil and groundnut in a 3:2 ratio (3.6 kg pig oil, 2.4 kg groundnut) to achieve a diet with 60% fat and 540 kcal.

Induction of HFD/STZ-Induced Type 2 DM

Following acclimatization, rats were fed an HFD for four weeks to induce insulin resistance. Thereafter, a single intraperitoneal injection of STZ (40 mg/kg body weight) was administered to induce partial pancreatic β-cell dysfunction. This HFD/STZ model closely mimics the pathophysiological characteristics of Type 2 DM, including insulin resistance, moderate β-cell impairment, hyperglycemia, and altered glucose homeostasis. [22]

To prevent acute hypoglycemia following STZ administration, rats received 5% glucose solution for 24 h. FBG levels were measured on days 3 and 10 post-induction using a glucometer. Animals with FBG concentrations ≥180 mg/dL on both occasions were considered diabetic and included in the study. Of the 40 animals subjected to induction, 35 developed diabetes successfully, corresponding to an induction success rate of 87.5%, while five animals that failed to meet the glycemic criterion were excluded before randomization.

Baseline body mass index (BMI) measurements were obtained after the 4-week HFD period and before treatment initiation. The elevated BMI observed in diabetic animals relative to the normal control group confirmed successful induction of obesity and insulin resistance.

Body weight and naso-anal length were measured weekly using a digital weighing balance and a calibrated measuring tape. BMI was calculated using the formula:

$$BMI = \text{weight (g)}/\text{length}^2\ (\text{cm}^2)$$

Experimental Design

Following confirmation of diabetes, animals were randomized into four groups (n = 6 per group) using a computer-generated randomization procedure. Investigators responsible for biochemical analyses and histopathological evaluations were blinded to treatment allocation throughout data collection and analysis.

Group 1: Normal control (standard diet + distilled water)

Group 2: Diabetic control (HFD/STZ + distilled water)

Group 3: HFD/STZ rats treated with chloroform fraction of *Nm* (180 mg/kg)

Group 4: HFD/STZ rats treated with metformin (200 mg/kg)

Treatments were administered orally once daily for 4 consecutive weeks. The dose of 180 mg/kg was selected based on previous studies demonstrating significant antidiabetic activity and acceptable safety margins. [17] This dose represents approximately one-third of the reported LD50 value (565 mg/kg), placing it within a safe therapeutic range.

Biochemical Analysis

At the end of the treatment period, rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intramuscularly and euthanized by cardiac puncture. Approximately 4 to 5 mL of blood was collected from each animal into EDTA and plain tubes and transported on ice to the Clinical Biochemistry Laboratory, Usmanu Danfodiyo University, Sokoto, for analysis.

Blood collected in plain tubes was centrifuged at 3000 rpm for 10 minutes using an Eppendorf 5424R centrifuge (Germany), and the resulting serum was used for biochemical analyses. Serum concentrations of urea, creatinine, electrolytes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, total protein, albumin, globulin, and total cholesterol (TCh) were determined using a fully automated clinical chemistry analyzer (TRX 7010, Biorex Mannheim, Germany).

Quantification of Insulin Sensitivity, Resistance, and β-Cell Function

Insulin resistance and β-cell function were assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and the Homeostatic Model of β-cell Function (HOMA-β), respectively, calculated from FBG and fasting serum insulin concentrations, as described by Matthews et al. [23]

At the end of the experimental period, rats were fasted overnight for 12 to 16 hours with free access to water to ensure stable basal metabolic conditions and reduce postprandial variation in glucose and insulin levels. Following the fasting period, animals were anesthetized lightly, and blood samples were collected via cardiac puncture into plain sample tubes.

The blood samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 to 15 minutes to obtain clear serum. The serum was used for fasting insulin determination using a standard immunoassay method, while FBG was measured at the point of collection using a calibrated glucometer (or enzymatic colorimetric method, if applicable).

FBG values (mg/dL) were converted to mmol/L using the conversion factor: 1 mmol/L = 18 mg/dL before calculating HOMA indices.

The indices were calculated using the following standard formulas:

HOMA-IR = (fasting insulin [μU/mL] × fasting glucose [mmol/L])/22.5

HOMA-β = (20 × fasting insulin [μU/mL])/(fasting glucose [mmol/L − 3.5])

Insulin sensitivity (IS) was calculated as:

$$IS = \frac{1}{\text{Log (fasting insulin} \left(\frac{U}{L}\right) \times \log \text{(fasting glucose (mg/dL))}}$$

All measurements were performed in duplicate, and mean values were used for statistical analysis.

Renal Function Test Parameters

Serum samples were also used to measure renal function parameters, namely potassium, sodium, chloride, urea, and creatinine. Additionally, the estimated glomerular filtration rate (eGFR) was calculated for each group using the following formula. [24]

$$eGFR = \frac{k \times (\text{weight (g)}0.914)}{\text{Creatinine (μmol/L)}}$$

k = 7.5

Semi-Quantitative Histopathological Assessment

The semi-quantitative histopathological scoring system was adapted from previously validated experimental pathology protocols commonly employed in diabetic animal studies. [25]

Histopathological alterations in the liver, kidney, and pancreas were assessed semi-quantitatively by a blinded histopathologist using a light microscope at ×100 and ×400 magnifications. Tissue lesions were graded according to the severity of pathological changes using a modified semi-quantitative scoring system adapted from previously established experimental pathology methods.

For the liver, parameters assessed included hepatocellular degeneration, sinusoidal congestion, inflammatory cell infiltration, and vacuolar degeneration. Renal tissues were evaluated for glomerular degeneration, tubular necrosis, inflammatory infiltration, and vascular congestion, while pancreatic tissues were assessed for islet cell degeneration, necrosis, inflammatory infiltration, and distortion of islet architecture.

Lesions were scored as follows:

0 = normal histological appearance

1 = mild lesion (<25% tissue involvement)

2 = moderate lesion (25%–50% tissue involvement)

3 = severe lesion (51%–75% tissue involvement)

4 = very severe lesion (>75% tissue involvement)

The mean histopathological score for each parameter was calculated for all experimental groups and expressed as mean ± standard error of mean (SEM).

Statistical Analysis

Data were analyzed using GraphPad Prism 9. All results are presented as mean ± SEM (n = 6). Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple pairwise comparisons. For variables measured repeatedly over time (e.g., BMI), repeated-measures one-way ANOVA was applied to assess treatment and time effects as well as their interaction. When overall ANOVA results were significant, Tukey's test was used to adjust for multiple comparisons and minimize Type I error. Statistical significance was set at $P < 0.05$.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria (Approval Number: UDUTH/HREC/2024/1371/V1). All procedures adhered to institutional and international guidelines for the care and use of laboratory animals.

RESULTS

Effect of the Chloroform Fraction of *Nm* on BMI

The BMI was significantly lower in the normal control group compared with the chloroform-treated group ($P = 0.0001$) and the metformin-treated group ($P < 0.0001$). However, no significant difference was observed between the diabetic control and chloroform-treated groups ($P = 0.1231$), between the diabetic control and metformin groups ($P = 0.1468$), or between the chloroform and metformin groups ($P = 0.9999$), indicating that the treatments did not produce a statistically significant effect on BMI (**Table 1**).

Table 1: Effect of *Nm* chloroform fraction on body mass index (g/cm^2) in high-fat diet/streptozotocin–induced Wistar rats.

Experimental groups	Normal control	Diabetic control	*Nm* chloroform fraction (180 mg/kg)	Metformin (200 mg/kg)
Duration (weeks)	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
Week 0	0.49 ± 0.01	1.21 ± 0.03	1.22 ± 0.04	1.13 ± 0.04
Week 1	0.56 ± 0.01	1.24 ± 0.03	1.18 ± 0.05	1.15 ± 0.03
Week 2	0.53 ± 0.02	1.27 ± 0.03	1.17 ± 0.05	1.20 ± 0.06
Week 3	0.58 ± 0.01	1.28 ± 0.04	1.16 ± 0.05	1.22 ± 0.05
Week 4	0.60 ± 0.02	1.29 ± 0.04	1.18 ± 0.05	1.24 ± 0.06

SEM, standard error of mean; *Nm*, *Neocarya macrophylla*. Values are expressed as mean ± SEM (n = 6).

Effect of Treatment on Blood Glucose Levels

Administration of the chloroform fraction of *Nm* (180 mg/kg) significantly reduced FBG levels throughout the treatment period, with values decreasing from approximately 210 mg/dL at baseline to 85 mg/dL after 4 weeks. A significant treatment effect was observed on FBG levels ($F(3,16) = 17.2$, $P < 0.001$). FBG in the chloroform-treated group was significantly lower than in the normal control ($P = 0.0260$) and diabetic control groups ($P = 0.0055$). In contrast, untreated diabetic rats exhibited progressive hyperglycemia, with FBG increasing from approximately 200 mg/dL to 245 mg/dL over the same period (**Figure 1**).

Quantification of IS, Resistance, and Beta Cell Function in Type 2 Diabetic Wistar Rats

Repeated significant differences were observed in FBG among the experimental groups ($F(3,20) = 282.50$, $P < 0.001$), as shown in **Table 2**. The chloroform fraction significantly reduced FBG compared with the diabetic control group ($P < 0.0001$) and the metformin group ($P = 0.002$). In addition, significant differences were also observed in HOMA-IR ($F(3,20) = 77.82$, $P < 0.001$), with the chloroform fraction significantly lowering HOMA-IR relative to the diabetic control ($P = 0.0032$) and metformin groups ($P = 0.0062$). HOMA-β showed significant variation across groups ($F(3,20) = 24.56$, $P < 0.001$), with a significant increase in the chloroform-treated group compared with the diabetic control ($P = 0.0003$), but remaining lower than the normal control group ($P = 0.0159$).

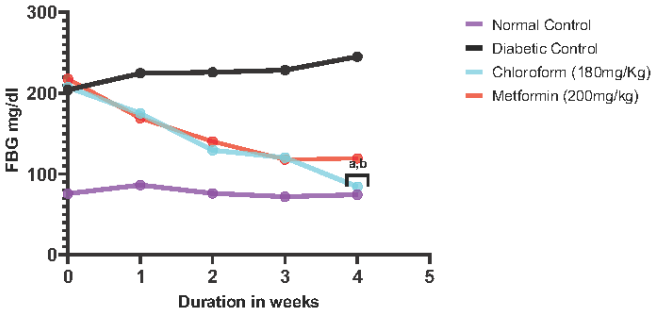

Figure 1: Effect of chloroform fraction of *Nm* on fasting blood glucose. Data were presented as the mean ± SEM, rats per group (n = 6). SEM, standard error of mean. 'a': $P = 0.0260$, chloroform-treated group vs normal control. 'b': $P = 0.0055$, chloroform-treated group vs diabetic control.

Effect of *Nm* Chloroform Fraction on Liver Function Parameters

The liver function test results are presented in **Table 3**. Albumin levels did not differ significantly among the experimental groups. However, total protein levels differed significantly ($F(3,20) = 13.05$, $P < 0.001$), with the chloroform-treated group showing higher values than both the diabetic control ($P < 0.001$) and metformin-treated groups ($P = 0.0018$). Significant differences were also observed for AST ($F(3,20) = 86.13$, $P < 0.001$), ALT ($F(3,20) = 34.74$, $P < 0.001$), and ALP ($F(3,20) = 80.39$, $P < 0.001$). The chloroform fraction significantly reduced AST compared with the normal control ($P = 0.0026$), diabetic control ($P < 0.001$), and metformin groups ($P < 0.001$). Also, ALT levels were significantly lower than in the diabetic control group ($P < 0.001$) but comparable to the metformin group ($P = 0.969$). Similarly, ALP levels were significantly reduced compared with the diabetic control ($P < 0.0001$) and metformin groups ($P < 0.001$), with no significant difference from the normal control.

Effect of *Nm* Chloroform Fraction on Lipid Profile

The lipid profile in diabetic rats treated with the chloroform fraction of *Nm* showed marked improvement compared

Table 2: Quantification of IS, resistance, and beta cell function in type 2 diabetic Wistar rats.

Groups	Normal control	Diabetic control	Metformin (200 mg/kg)	*Nm* chloroform (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
FBG (mg/dL)	74.44 ± 5.64	245.75 ± 5.72	119.37 ± 3.72	84.27 ± 3.43[b,c]
HOMA-IR	6.78 ± 0.22	9.22 ± 0.62	8.98 ± 1.02	5.72 ± 0.14[b,c]
HOMA-beta	10.41 ± 1.21	1.26 ± 0.15	5.29 ± 0.91	6.80 ± 0.15[a,b]
IS	0.15 ± 0.01	0.10 ± 0.04	0.11 ± 0.02	0.18 ± 0.02

FBG, fasting blood glucose; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-beta, pancreas activity; IS, insulin sensitivity; *Nm*, *Neocarya macrophylla*; SEM, standard error of mean. Data were presented as mean ± SEM, rats per group (n = 6). 'a', Chloroform-treated group vs normal control. 'b', Chloroform-treated group vs diabetic control. 'c', Chloroform-treated group vs metformin-treated group.

Table 3: Effect of *Nm* fraction of chloroform on liver function test.

Variable	Normal control	Diabetic control	Metformin (200 mg/kg)	*Nm* chloroform fraction (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
ALB (g/L)	28.5 ± 0.94	24.5 ± 0.31	28.1 ± 0.32	26.5 ± 1.32
Protein (g/L)	48 ± 0.62	39 ± 2.54	43.5 ± 0.94	55 ± 2.56[b,c]
AST (IU/L)	193.4 ± 2.73	263 ± 2.34	204.5 ± 7.8	168 ± 1.13[a,b,c]
ALT (IU/L)	88.5 ± 1.52	165 ± 4.24	109 ± 5.86	112.5 ± 8.23[a,b]
ALP (IU/L)	129 ± 1.25	178.5 ± 3.84	153 ± 2.72	128 ± 2.17[b,c]

ALB, albumin; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; *Nm*, *Neocarya macrophylla*; SEM, standard error of mean. Data were presented as mean ± SEM, rats per group (n = 6). 'a', Chloroform-treated group vs normal control; 'b', chloroform-treated group vs diabetic control; 'c', chloroform-treated group vs metformin-treated group.

with the diabetic control group, as presented in **Table 4**. TCh showed significant differences among the experimental groups (F(3,20) = 7.33, *P* = 0.015), with elevated levels in the diabetic control group compared with the normal control, and normalization following chloroform treatment. Triglyceride levels also differed significantly (F(3,20) = 42.70, *P* = 0.001), with the chloroform-treated group showing a significant reduction compared with both the diabetic control and metformin groups. High-density lipoprotein (HDL) levels varied significantly across groups (F(3,20) = 134.80, *P* = 0.004), with the chloroform fraction significantly increasing HDL compared with the diabetic control and metformin groups. Similarly, low-density lipoprotein (LDL) levels showed significant differences among groups (F(3,20) = 152.10, *P* = 0.001), with the chloroform-treated group exhibiting a significant reduction compared with the diabetic control group, indicating improved lipid metabolism.

Effect of Chloroform Fraction of *Nm* Leaf on Some Parameters of Renal Function Test in Type 2 Diabetic Wistar Rat

Renal function analysis showed no significant changes in potassium and sodium levels. Bicarbonate differed significantly (F (3,20) = 69.20, *P* < 0.001), with reductions in the chloroform group versus diabetic control (*P* < 0.001). Chloride also varied significantly (F (3,20) = 9.799, *P* = 0.0003), showing improvement versus diabetic control (*P* = 0.0054). Urea (F (3,20) = 11.69, *P* = 0.001) and creatinine (F (3,20) = 12.1, *P*<0.001) were significantly reduced by treatment compared with diabetic control.

Similarly, eGFR was markedly improved by the chloroform fraction versus diabetic and metformin groups (*P* < 0.0001), with overall significance confirmed (F (3,20) = 21.08, *P* < 0.001; **Table 5**; **Figure 2**).

Effect of *Nm* Chloroform Leaf Fraction on Complete Blood Count

The effect of *Nm* chloroform fraction on hematological parameters was presented in **Table 6**. The chloroform-treated group demonstrated significant improvements compared with the diabetic control. Packed cell volume (PCV) differed significantly among groups (F (3,20) = 25.93, *P* = 0.014), with higher values in the chloroform group versus diabetic control (*P* = 0.014) and metformin (*P* = 0.017). WBC count also showed a significant effect (F (3,20) = 28.64, *P* = 0.021), with increases in the chloroform group compared with diabetic

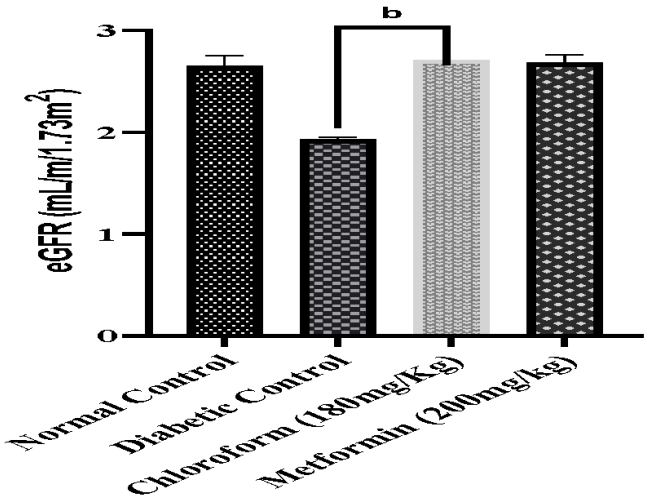

Figure 2: Estimated glomerular filtration rate (eGFR) among treated and untreated groups. SEM, standard error of mean. Data were presented as the mean ± SEM, rats per group (n = 6). 'b': chloroform vs diabetic control.

Table 4: Effect of *Nm* chloroform leaf fraction (180 mg/kg) on lipid profile.

Variables	Normal control	Diabetic control	Metformin (200 mg/kg)	*Nm* chloroform (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
TCh (mmol/L)	3.6 ± 0.13	4.3 ± 0.04	3.9 ± 0.13	3.7 ± 0. 13[b]
Tg (mmol/L)	1.1 ± 0.02	2 ± 0.09	1.13 ± 0.09	1.3 ± 0.0[b]
HDL (mmol/L)	1.8 ± 0.09	0.5 ± 0.02	1.4 ± 0.02	1.5 ± 0.02[a,b]
LDL (mmol/L)	1.2 ± 0.13	3.0 ± 0.02	1.2 ± 0.06	1.2 ± 0.02[b,c]

TCh, total cholesterol; Tg, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *Nm*, *Neocarya macrophylla*; SEM, standard error of mean. Data were presented as mean ± SEM of six rats per group. 'a': Chloroform-treated group vs normal control. 'b': Chloroform-treated group vs diabetic control. 'c': Chloroform-treated group vs metformin-treated group.

Table 5: Effect of chloroform fraction of Nm leaf (180 mg/kg) on renal function test parameters in type 2 diabetic Wistar rats.

Variables	Normal control	Diabetic control	Metformin (200 mg/kg)	Chloroform (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
K^+ (mmol/L)	3.1 ± 0.01	3.2 ± 0.02	3.0 ± 0.06	2.9 ± 0.27
Na^+ (mmol/L)	140.8 ± 0.18	145.5 ± 0.44	148.0 ± 2.17	144.8 ± 2.19
HCO_3^- (mmol/L)	10.5 ± 0.11	11.8 ± 0.03	11.2 ± 0.05	11.4 ± 0.04
Cl^- (mmol/L)	33.8 ± 0.06	36.5 ± 0.12	35.3 ± 0.44	34.5 ± 0.58
Ur (mmol/L)	2.0± 0.04	3.6 ± 0.04	2.4± 0.23	2.1 ± 0.36[b]
Cr (µmol/L)	62.5 ± 1.32	126.8 ± 1.30	67.0 ± 4.05	65.5 ± 3.44[b]

K^+, potassium; Na^+, sodium; HCO_3^-, bicarbonate; Cl^-, chloride; Ur, urea; Cr, creatinine; *Nm*, *Neocarya macrophylla*; SEM, standard error of mean. Data were presented as mean ± SEM, rats per group (n = 6). 'a': Chloroform-treated group vs normal control. 'b': Chloroform-treated group vs diabetic control, 'c': Chloroform-treated group vs metformin-treated group.

Table 6: Effect of *Nm* fraction of chloroform on hematological indices.

Variables	Normal control	Diabetic control	Metformin (200 mg/kg)	*Nm* chloroform (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
RBC (10^{12}/L)	5.4 ± 0.2	2.9 ± 0.1	4.6 ± 0.1	3.3 ± 1.4
Hb (g/dl)	9.85 ± 0.2	5.6 ± 0.6	8.7 ± 0.5	8.3± 1.5
PCV (%)	31 ± 1.4	16 ± 1.4	24 ± 0.5	20.7 ± 0.6
MCV (fL)	53.5 ± 0.7	52.5 ± 0.7	51 ± 1.4	51 ± 1.4
MCH (pg)	20.0 ± 1.4	18.0 ± 0.2	19.0 ± 0.9	18.2 ± 0.3
MCHC(g/L)	32.5 ± 0.7	34.0 ± 0.2	34.5 ± 0.7	33.5 ± 0.6
WBC (10^9/L)	6.1 ± 0.2	2 ± 0.1	7.8 ± 0.9	4.2 ± 0.1^{a,b,c}
Neutrophil (%)	36.9 ± 0.4	26.3 ± 2.7	41.5 ± 1.5	42.8 ± 2.2^{b}
Monocyte (%)	2.1 ± 0.4	5.5 ± 3.4	3.5 ± 1.9	3.8 ± 1.9
Lymphocyte (%)	55 ± 1.3	95.0 ± 2.6	66.0 ± 0.7	46.0 ± 0.8^{a,b,c}
Eosinophil (%)	ND	ND	1.5 ± 1.6	1.0 ± 0.3
Platelet (10^9/L)	25.0 ± 2.1	32.0 ± 0.3	39.5 ± 0.7	37.5 ± 0.4^{a,b}

RBC, red blood cells; WBC, white blood cell; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; ND, not detected; *Nm*, *Neocarya macrophylla*; SEM, standard error of mean. Different superscript letters in the same row indicate significant differences ($P < 0.05$) between groups. Same superscript letters indicate no significant difference; data were presented as mean ± standard error of the mean of six rats per group. 'a': Chloroform-treated group vs normal control. 'b': Chloroform-treated group vs diabetic control, 'c': Chloroform-treated group vs metformin-treated group.

control ($P = 0.021$) and metformin ($P = 0.029$). Neutrophil levels were significantly affected ($F(3,20) = 15.47$, $P = 0.026$), with higher values in the chloroform group versus diabetic control ($P = 0.026$) and metformin ($P = 0.027$). Lymphocyte levels showed a marked effect ($F(3,20) = 189.40$, $P < 0.001$), while platelet count was also significantly affected ($F(3,20) = 32.72$, $P < 0.001$), with higher values in the chloroform group compared with normal control ($P < 0.0001$) and diabetic control ($P = 0.0131$).

Effect of *Nm* Fraction of Chloroform on the Histology of Pancreas, Kidney, and Liver

Histological examination of renal sections revealed that the normal control group exhibited well-preserved kidney architecture with intact Malpighian corpuscles, normal glomeruli, and properly organized proximal and distal convoluted tubules (**Figure 3A–D**). In contrast, the diabetic control group showed extensive renal damage, characterized by focal destruction of renal corpuscles, loss of glomerular tufts, pyknotic nuclei, and epithelial desquamation.

Treatment with 180 mg/kg of the chloroform fraction of *Nm* markedly improved renal architecture, restoring glomerular tufts and tubular integrity. Similarly, the Metformin-treated group (200 mg/kg) demonstrated nearly normal renal histology. The pancreatic sections of the normal control group showed preserved islets of Langerhans and healthy acinar cells, indicating normal endocrine and exocrine morphology as in **Figure 4**. The diabetic control group exhibited degenerative changes in the islet cells, while the acinar cells remained relatively intact, consistent with diabetes-induced pancreatic injury. Treatment with 180 mg/kg of the chloroform fraction of *Nm* preserved both islet and acinar structures, though mild vascular congestion and arteriolar dilation were observed. The Metformin-treated group (200 mg/kg) also showed restoration of pancreatic architecture with focal islet necrosis and mild mononuclear infiltration.

Liver histology of the normal control group revealed typical hepatic architecture, including centrally located veins, radiating hepatocytes, and well-preserved sinusoids. The diabetic control group displayed marked hepatic abnormalities, with inflammatory cell infiltration within the portal triads and distorted hepatocyte arrangement. Administration of 180 mg/kg of the chloroform fraction of *Nm* improved liver structure, showing restoration of normal hepatocyte morphology and central vein integrity. Likewise, Metformin treatment (200 mg/kg) produced comparable hepatoprotective effects, with improved hepatic organization and only mild residual inflammatory infiltration (**Figure 5**).

Effect of *Nm* Fraction of Chloroform on the Histology of Pancreas, Kidney, and Liver

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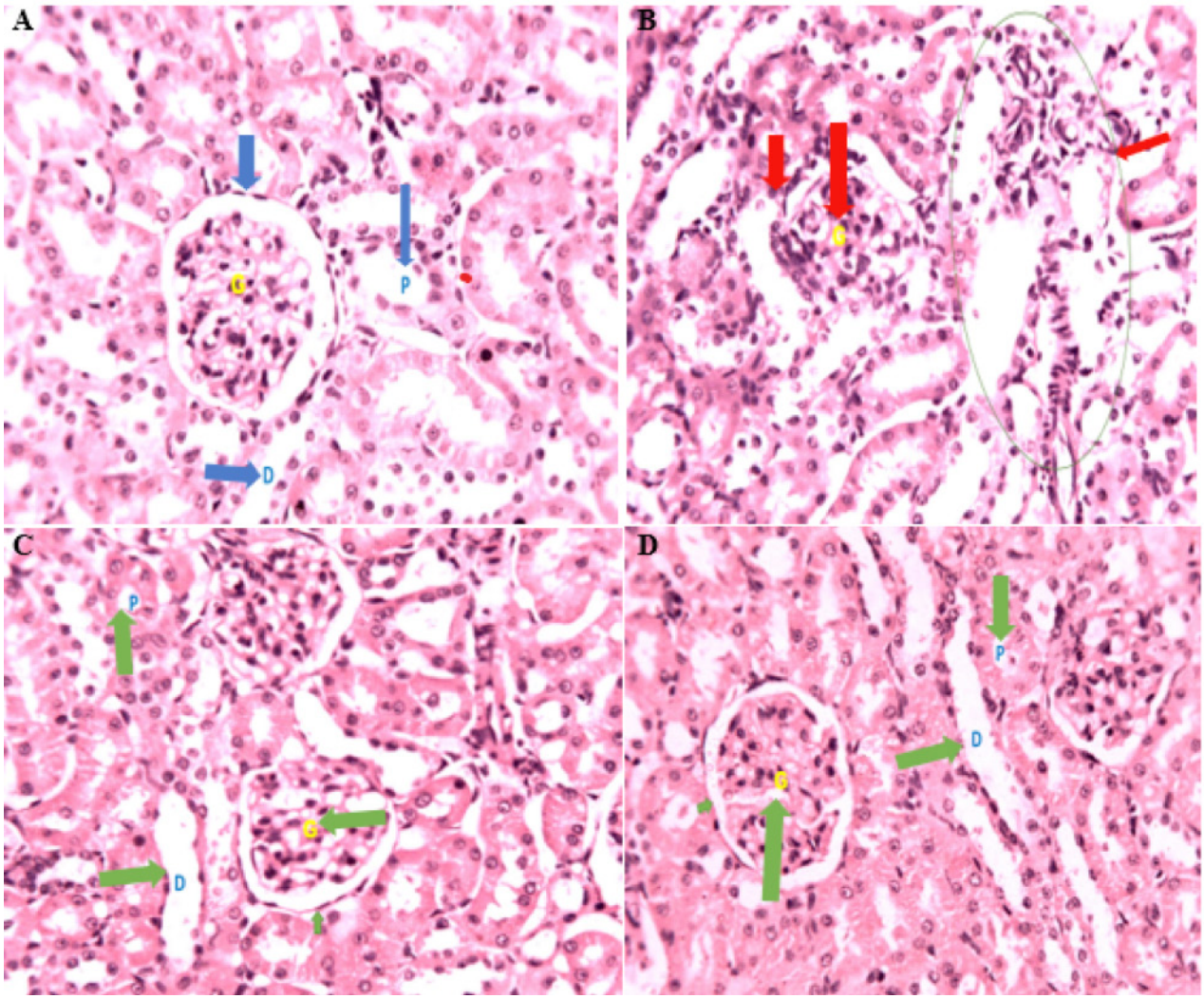

Figure 3: Photomicrographs of renal sections (H&E x200). A, Normal control group exhibited well-preserved renal architecture, with intact malpighian corpuscles, normal glomeruli, and properly organized proximal and distal convoluted tubules. B, The Diabetic control group showed extensive renal damage, including focal destruction of renal corpuscles, loss of glomerular tufts, pyknotic nuclei, and epithelial desquamation. C, Chloroform group180 mg/kg: Administration of 180 mg/kg of the chloroform fraction of *Nm* restored renal structure to a considerable extent, as evidenced by preserved glomerular tufts and intact proximal tubules. D, The Metformin-treated group (200 mg/kg) also demonstrated normal renal histoarchitecture, indicating effective renoprotective action comparable to that of the *Nm* leave fraction.

The Metformin-treated group (200 mg/kg) also showed restoration of pancreatic architecture with focal islet necrosis and mild mononuclear infiltration. Liver histology of the normal control group revealed typical hepatic architecture, including centrally located veins, radiating hepatocytes, and well-preserved sinusoids. The diabetic control group displayed marked hepatic abnormalities, with inflammatory cell infiltration within the portal triads and distorted hepatocyte arrangement. Administration of 180 mg/kg of the chloroform fraction of *Nm* improved liver structure, showing restoration of normal hepatocyte morphology and central vein integrity. Likewise, Metformin treatment (200 mg/kg) produced comparable hepatoprotective effects, with improved hepatic organization and only mild residual inflammatory infiltration (**Figure 5**).

Semi-Quantitative Histopathological Scores of Liver, Kidney, and Pancreatic Tissues

Histopathological evaluation (shown in **Table 7**) demonstrated significant tissue protection by the chloroform fraction of *Nm* in diabetic rats. Hepatic architecture was markedly preserved, with significant reductions in hepatocellular degeneration, sinusoidal congestion, inflammatory infiltration, and vacuolar changes ($F(3,20) = 36.29–485.40$, $P < 0.001$), indicating strong hepatoprotective activity compared with the diabetic control group. Renal histoarchitecture was similarly improved, with significant attenuation of glomerular degeneration, tubular necrosis, interstitial inflammation, and vascular congestion ($F(3,20) = 154.60–307.60$, $P < 0.001$), with additional improvements observed relative to the metformin group in

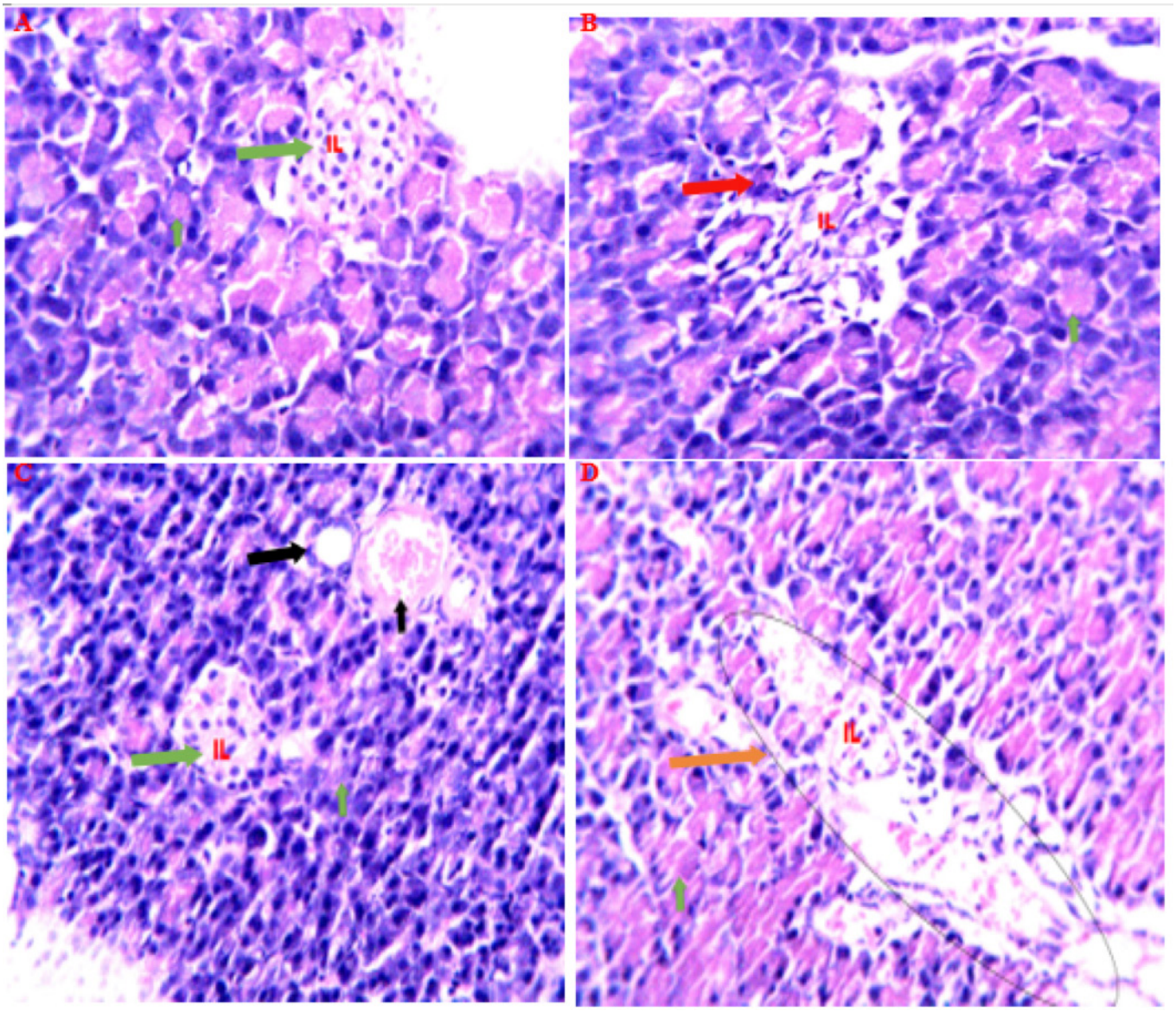

Figure 4: Photomicrograph of pancreatic sections (H&E ×200). A, Normal control group showed preserved islets of Langerhans and healthy acinar cells, indicating normal endocrine and exocrine structure. B (diabetic control) demonstrated degenerated islet cells with relatively preserved acinar cells, indicating diabetes-induced pancreatic damage. C (chloroform-treated with *Nm* 180 mg/kg). The pancreatic tissue exhibited preserved islet and acinar structures, although vascular congestion and dilated arterioles were observed. D (Metformin 200 mg/kg) showed focal islet necrosis with mild mononuclear.

selected parameters. Pancreatic sections also showed substantial preservation of islet structure, with significant reductions in islet cell degeneration, necrosis, inflammatory infiltration, and architectural distortion ($F(3,20) = 57.07–185.20$, $P < 0.001$).

DISCUSSION

The findings of the present study demonstrate that the chloroform leaf fraction of *N. miranda* possesses considerable antidiabetic activity in Wistar rats rendered diabetic through HFD feeding and STZ administration. Treatment with the chloroform fraction at a dose of 180 mg/kg resulted in a marked and progressive reduction in FBG levels, with efficacy comparable to that of Metformin. These findings corroborate earlier reports that showed significant hypoglycemic effects of the chloroform fraction of *N. miranda* in experimental diabetic models, further reinforcing its potential as a promising therapeutic agent for the management of DM. [17]

The observed anti-hyperglycemic activity may be related to the presence of bioactive phytoconstituents previously reported in *Nm,* including flavonoids, phenolics, alkaloids, and triterpenoids. Chloroform is a moderately nonpolar solvent capable of concentrating lipophilic phytochemicals such as triterpenoids, sterols, aglycone flavonoids, and certain alkaloids. These compounds are frequently associated with antidiabetic activity because of their ability to improve insulin signaling, suppress oxidative stress, and regulate lipid metabolism. [26,27] The superior efficacy observed with the chloroform fraction compared with crude extracts

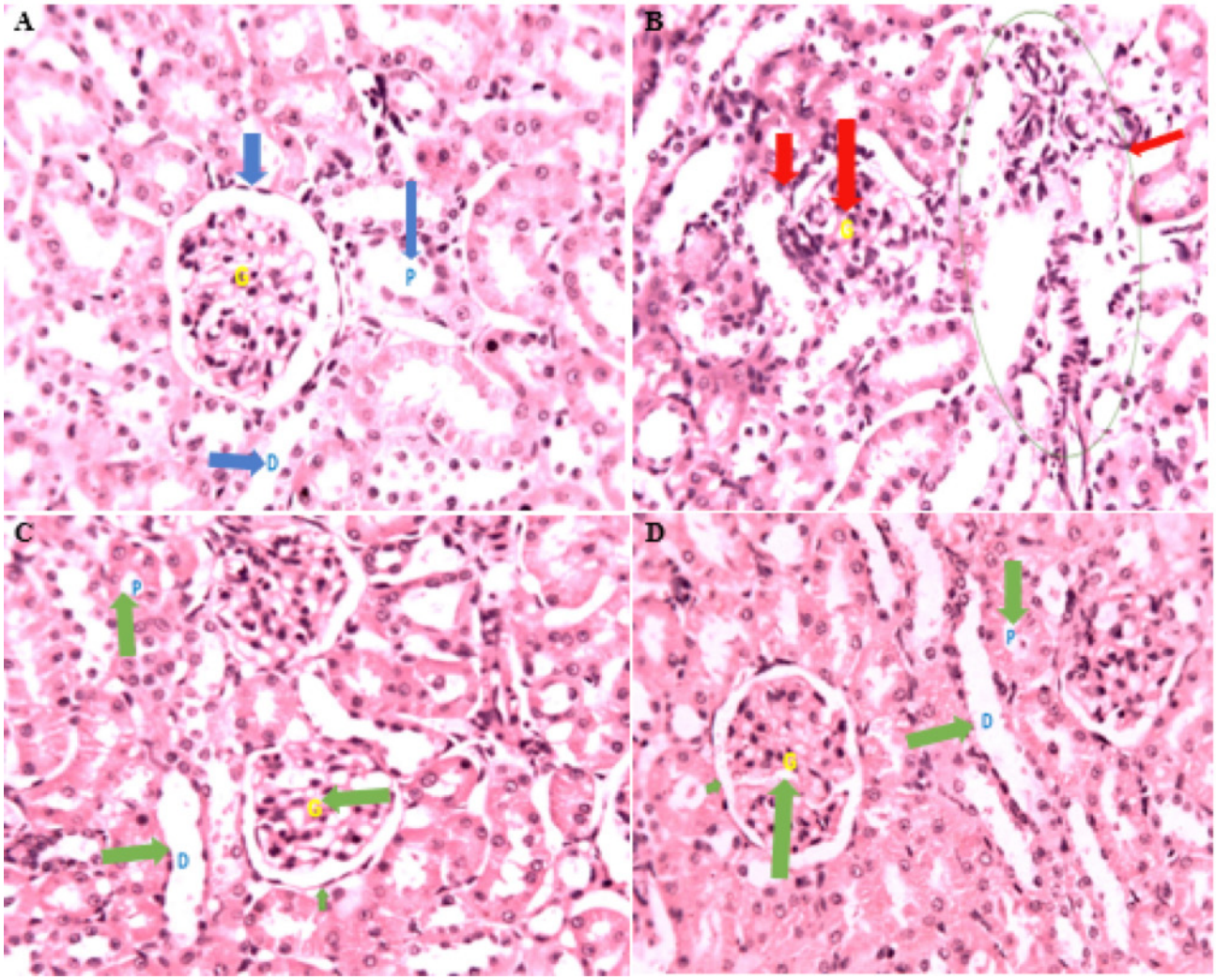

Figure 5: Photomicrograph of Liver (H&E ×200). A (normal control) showed a typical hepatic architecture with centrally located veins, radiating hepatocytes, and well-preserved sinusoids. B (diabetic control) exhibited marked hepatic abnormalities, including inflammatory cell infiltration within the portal triads and disrupted hepatocyte arrangement. C (chloroform-treated with *Nm* 180 mg/kg), showed a notable improvement in liver structure, with restoration of normal hepatocyte morphology and central vein integrity. D (Metformin 200 mg/kg) also showed improved hepatic architecture, though mild inflammatory infiltration persisted.

reported previously may therefore reflect enrichment of these bioactive constituents and reduction of inactive matrix components. The fraction may contain phenolic compounds that possess antioxidant properties capable of protecting pancreatic β-cells from STZ-induced oxidative injury, thereby preserving endogenous insulin secretion. [28,29] These findings are consistent with previous reports demonstrating the hypoglycemic activity of *Nm* in experimental diabetes, [17] which also observed lipid-regulating effects of *Nm* in diabetic rats, supporting its therapeutic potential in managing dyslipidemia and reducing cardiovascular risks associated with diabetes.

The chloroform fraction of *Nm* significantly improved glycemic control in diabetic rats by reducing FBG and improving HOMA-IR and HOMA-beta, indicating enhanced IS and partial β-cell recovery. Improvement in HOMA-IR may reflect enhanced insulin receptor signaling through activation of the IRS-1/PI3K/Akt pathway, which promotes GLUT4 translocation to skeletal muscle and adipose tissue, thereby facilitating glucose uptake. [30,31] In addition, phytochemicals may activate AMP-activated protein kinase (AMPK), a key metabolic regulator that suppresses hepatic gluconeogenesis and improves peripheral IS. [32] Improved β-cell function supports findings that plant compounds protect pancreatic cells from ROS-induced apoptosis. [32] However, incomplete restoration of HOMA-β may reflect limited regeneration, as commonly observed in short-term interventions.

Although rats treated with the chloroform fraction showed a trend toward lower BMI than the diabetic control group, the differences were not statistically significant. This suggests that the observed hypoglycemic effect was independent of changes in body mass. Similar findings have been reported in HFD/STZ-induced diabetic rats, where significant improvements in glucose homeostasis occurred without corresponding reductions in body weight, indicating that antidiabetic activity may be mediated through mechanisms other than weight loss, such as enhanced IS and improved glucose utilization. [33,34]

Table 7: Semi-quantitative histopathological scores of liver, kidney, and pancreatic tissues in experimental groups.

Histopathological parameter	Normal control	Diabetic control	Metformin (200 mg/kg)	*Nm* chloroform fraction (180 mg/kg)
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
Liver				
Hepatocellular degeneration	0.33 ± 0.08	3.50 ± 0.12	0.83 ± 0.12	1.17 ± 0.06^{a,b}
Sinusoidal congestion	0.17 ± 0.07	3.00 ± 0.21	0.67 ± 0.08	1.00 ± 0.26^{a,b}
Inflammatory cell infiltration	0.17 ± 0.05	3.33 ± 0.08	0.85 ± 0.09	1.33 ± 0.02^{a,b}
Vacuolar degeneration	0.00 ± 0.00	3.17 ± 0.31	0.73 ± 0.17	1.17 ± 0.11^{a,b,c}
Kidney				
Glomerular degeneration	0.13 ± 0.11	3.57 ± 0.12	0.83 ± 0.13	1.15 ± 0.07^{a,b}
Tubular necrosis	0.00 ± 0.00	3.37 ± 0.07	0.97 ± 0.09	1.20 ± 0.26^{a,b}
Interstitial inflammation	0.19 ± 0.17	2.90 ± 0.06	0.39 ± 0.01	1.57 ± 0.09^{a,b,c}
Vascular congestion	0.15 ± 0.07	2.83 ± 0.11	0.77 ± 0.21	1.80 ± 0.16^{a,b,c}
Pancreas				
Islet cell degeneration	0.12 ± 0.11	2.67 ± 0.33	1.30 ± 0.18	1.53 ± 0.21^{a,b,}
Islet necrosis	0.00 ± 0.00	2.83 ± 0.28	1.23 ± 0.15	1.47 ± 0.11^{a,b,c}
Inflammatory infiltration	0.14 ± 0.14	3.17 ± 0.15	0.87 ± 0.19	1.57 ± 0.14^{a,b,c}
Distortion of islet architecture	0.00 ± 0.00	0.12	0.88 ± 0.17	1.88 ± 0.14^{a,b}

Nm, *Neocarya macrophylla*; SEM, standard error of mean. Values are expressed as mean ± SEM (n = 6). 'a': Chloroform-treated group vs normal control. 'b': Chloroform-treated group vs diabetic control. 'c': Chloroform-treated group vs metformin-treated group.

Furthermore, the administration of the *Nm* leaf fraction of Chloroform demonstrated significant hepatoprotective effects in diabetic rats. Albumin levels, reduced in the diabetic control group, were restored following *Nm* fraction of Chloroform treatment, suggesting enhanced protein synthesis. Similarly, total protein levels improved in the *Nm*-treated group, indicating better liver function. The hyperglycemia-induced oxidative stress promotes hepatocellular injury through lipid peroxidation, mitochondrial dysfunction, and inflammatory cytokine production. [35,36] Restoration of albumin and total protein levels, together with reductions in AST and ALT following treatment, suggests preservation of hepatocyte integrity and synthetic capacity. These effects may be attributable to antioxidant phytochemicals capable of scavenging reactive oxygen species and attenuating NF-κB-mediated inflammatory signaling, thereby reducing hepatic injury associated with diabetes. [37] Additionally, ALP levels, which were reduced in diabetic rats, were normalized by *Nm*, further emphasizing its beneficial effects on liver health. This indicates the promise of *Nm* as a therapeutic agent to improve liver function in diabetes, a point also highlighted by, [16] who reported similar hepatoprotective effects in their studies on *Nm*.

The effect on lipid metabolism, *Nm,* shows a significant modulation of lipid profiles in diabetic rats. Elevated TCh and LDL levels in the diabetic control group reflect disrupted lipid homeostasis, a hallmark of diabetes. Treatment with *Nm* normalized these parameters, suggesting its ability to regulate cholesterol metabolism. Increased HDL levels in the *Nm*-treated group show its potential cardioprotective effects, while reduced triglyceride (Tg) levels point to improved lipid utilization.

The effect of the chloroform leaf fraction of *Nm* on renal function test parameters showed significant variations among groups (especially between the chloroform and diabetic control groups) for urea and creatinine, which are very important parameters of renal function tests. Post-hoc analysis indicates that the treatment with Chloroform significantly improved urea and creatinine levels compared with the diabetic control group. This aligns with other studies on the renal effects of Chloroform from medicinal plants, such as the study on the Chloroform extract of *Pedalium murex,* which has shown promising anti-urolithiasis potential. [38] Furthermore, this study has shown that the eGFR indicated significant improvements in kidney function with chloroform and metformin treatment compared to the diabetic control group. This finding suggests that eGFR levels, which are essential for assessing kidney health and function, may be improved by Chloroform, thereby enhancing renal function and helping manage diabetic kidney disease. [39,40]

Although treatment significantly improved RBC count, hemoglobin concentration, and PCV compared with diabetic controls, these values remained below those observed in the normal control group, indicating partial rather than complete restoration of hematological homeostasis.

The histological findings demonstrated preservation of pancreatic islet architecture following treatment with the chloroform fraction. Although regeneration of β-cells cannot be conclusively established without immunohistochemical or molecular markers of proliferation, the observed improvement may reflect protection of residual β-cells from oxidative injury and functional recovery of surviving endocrine cells. [16] STZ induces diabetes primarily through the generation of reactive oxygen species, DNA alkylation, and β-cell toxicity. Therefore, the preservation of islet morphology observed in the treated groups may be attributable to the antioxidant properties of flavonoids and phenolic compounds previously reported in *Nm*. Furthermore, flavonoids have been reported to upregulate

endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase while suppressing pro-apoptotic mediators, including caspase-3 and Bax. Such actions may contribute to the preservation of islet architecture and the maintenance of insulin secretory capacity in diabetic conditions. [41] In the kidney section, Chloroform-treated samples exhibited hypercellular glomeruli and enlarged tubules, indicating a positive impact on renal health. These findings suggest the therapeutic potential of the Chloroform fraction in minimizing diabetes-related histological defects in experimental animals. [16]

Although the chloroform fraction of *Nm* produced significant improvements in glycemic control, lipid profile, organ function markers, and histopathological parameters, its overall efficacy was generally comparable to, but not consistently superior to, Metformin. This finding is important because Metformin remains the standard first-line pharmacological agent for the management of type 2 DM. Nevertheless, the substantial therapeutic effects observed with the chloroform fraction suggest that *Nm* contains biologically active constituents capable of modulating multiple pathways involved in diabetes pathogenesis. The plant may therefore represent a promising source of lead compounds for the development of adjunctive or complementary antidiabetic therapies.

Limitation and Future Perspectives

Detailed phytochemical characterization and standardization of the chloroform fraction were not performed; therefore, the specific bioactive constituents responsible for the observed antidiabetic and organ-protective effects could not be identified. In addition, mechanistic biomarkers related to oxidative stress, inflammation, insulin signaling, and β-cell function were not evaluated, limiting confirmation of the proposed molecular mechanisms. Furthermore, only a single treatment dose and a relatively short treatment duration were investigated. Future studies should focus on phytochemical profiling and standardization, isolation of active compounds, evaluation of mechanistic biomarkers, dose–response studies, metabolomic analyses, and long-term safety assessments to further validate the therapeutic potential of *Nm.*

CONCLUSIONS

The chloroform fraction of *Nm* exhibited significant anti-hyperglycemic, hypolipidemic, hepatoprotective, renoprotective, and tissue-preserving effects in HFD/STZ-induced type 2 diabetic rats. Although its efficacy was generally comparable to that of metformin for several parameters, complete normalization of all biochemical and hematological indices was not achieved. These findings support the therapeutic potential of *Nm* as a source of antidiabetic agents and warrant further investigation.

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AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

None.

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