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Antidiabetic, Antilipidemic and Antioxidant Properties of Aqueous Extracts of *Morinda Lucida* and *Nauclea Latifolia* Leaves in Alloxan Induced Rats

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Abstract: The study sought to evaluate the antidiabetic, antioxidants and hypolipidemic properties of *Morinda lucida* and *Nauclea latifolia* leaves in alloxan-induced diabetic rats. The rats used were grouped into six, including controls and standard drug groups. The body weights and blood glucose concentration of the animals were determined before and after induction of diabetes as well as after treatment with the aqueous extracts of the leaves. Superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, glutathione reductase, reduced glutathione, malondialdehyde, lipid profiles, and liver biomarker enzymes were also determined. The *Morinda lucida* and *Nauclea latifolia* extracts caused a significant reduction in the rats' glucose level after treatment. The status of reduced glutathione and antioxidants enzyme activities was significantly increased. However, the total cholesterol, low-density lipoprotein, triglyceride status showed reduction compare to the control. There was also a reduction in the concentration of malondialdehyde in the groups administered with *Morinda lucida* (2.88±0.00) and with *Nauclea latifolia* leaves extracts (3.85±0.12) compare to the diabetic control group (7.37±0.10). The biomarker enzyme activities were relatively reduced in the group administered with the extracts of both plants. However, the leaves extract of the plants exhibits hypoglycemic, hypolipidemic, and antioxidant properties.

Keywords: Diabetic mellitus; hyperlipidemic; free-radicals; *Morinda lucida*; *Nauclea latifolia.*

1. Introduction

Diabetes mellitus is a heterogeneous metabolic disorder characterized by insufficiency of insulin secretion and insulin receptor or post-receptor events with derangement in carbohydrate, protein, and lipid metabolisms resulting in chronic hyperglycemia with the fasting blood glucose concentration equal or greater than 140 mg/dl [1]. Insufficient insulin secretion caused by immune destruction of pancreatic β-cells leads to the development of insulin-dependent diabetes (Type 1). In the case of abnormal insulin cellular action, type-2 diabetes is developed due to the gradual development of insulin resistance and pancreatic β-cell dysfunction.

Diabetes mellitus is a rampant global epidemy of multifactorial etiology and risk predisposing factors such as the increasing aging population, genetic and environmental factors, fast-evolving, and increasing sedentary lifestyle and dietary changes [2,3]. Available statistics show that the global estimated prevalence for the disease stands at 285 million adults (6.4%) of the world population in 2010. This figure is projected to rise to 439 million adults (7.7%) by 2030 [4]. Diabetes prevalence is on the rise worldwide due to accumulating risk factors well pronounced in economically growing nations. An estimated 69% rise is observed for the prevalence of the disease in adults in developing countries versus 20% for adults in developed countries [4-6]. The percentage of deaths attributable to high blood glucose or diabetes that occurs before age 70 is higher in low and middle-income countries than in high-income countries [7]. Most deaths are estimated to result from diabetes complications such as heart disease and stroke, neuropathy, nephropathy, cataracts, microangiopathy, atherosclerosis, and retinopathy [8].

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control [9]. Mainstream drugs that are used to control diabetes fall into three main categories. The first category of drugs aims to enhance endogenous insulin availability. It includes agents that act on the sulfonylurea receptors in the pancreas to promote insulin secretion and others that impact the small intestinal mucosal epithelium. Medicines categorized as group two are directed to potentiate the insulin response, among them being thiazoline. This group of drugs seems to act as an initiator of peroxisomal receptors responsible for regulating the metabolism of carbohydrates, lipids, and proteins. The drugs categorized as group three are represented by α- glycosidase inhibitors and are targeted at reducing complex sugars' metabolism [10-12].

Current glycemic medications burden patients due to variable contraindications and interactions with other drugs, limited efficacy, limited tolerability, and significant side effects arising from their complex action mechanisms [13-15]. Existing pharmacotherapy is still far from achieving optimal blood sugar control in such patients, as an effect of dysfunction in insulin secretion, action, or both. Several reviews from different countries have highlighted the significance of medicinal plant application to control diabetes [16-18]. In response to this global health challenge, the WHO Expert Committee on diabetes mellitus recommended further evaluation of the folkloric methods of managing the disease because of high mortality and morbidity arising from its attendant complications and drawbacks associated with using conventional antidiabetic drugs [18]. In pursuit of this goal, several medicinal plants are being investigated for their hypoglycemic efficacies.

Morinda lucida Benth. (Rubiaceae) is among several indigenous plants used in the local treatment of Diabetic Mellitus among Yorubas (South-West Nigeria). Different parts of the plant are attributed to diverse therapeutic benefits. *Morinda lucida* is a medium-sized tree used as a medicinal plant in West Africa (especially in Nigeria) in the local treatment of malaria and other febrile conditions, diabetes, hypertension, cerebral congestion, dysentery [19-26]. Different parts of the plant have been reported to possess antimicrobial [27]. The aqueous leaf extract of *M. lucida* has been reported to cause antidiabetic effects in streptozotocin-induced diabetic rats, while the ethanolic leaf extract of the same plant elicited antidiabetic effects in both normal and alloxan-induced diabetic rats [19].

Nauclea latifolia Smith (Rubiaceae) is a multi-stemmed shrub or small tree widespread in tropical Africa and Asia. The plant is used traditionally in the treatment of various diseases. In a part of Nigeria, the roots are used by some traditional medicine to treat hypertension [28].

The bark is used in the treatment of wounds, coughs, and gonorrhea in Nigeria. Biological activities reported on the roots include antibacterial, antifungal, anti-influenza, and anti-hyperlipidemic [29-32]. Therefore, this study sought to evaluate the comparative antidiabetic, antilipidemic and antioxidant properties of both *M. lucida* and *N. latifolia* aqueous extracts of their leaves in alloxan-induced rats.

2. Materials and Methods

2.1. Collection of plant sample.

Leaves of *Morinda lucida* and *Nauclea latifolia* were obtained from farmland and the Ifaki-Ikole town in Ekiti State, Nigeria, and authenticated with voucher number 2020044 and 2020045, respectively, at the University Herbarium, Department of Plant Science and Biotechnology, Ekiti State University, Nigeria. The leaves were air-dried and powdered using an electric blender.

2.2. Animal sample.

Adult male Wistar rats (100-150g) were obtained from Animals house of College of Medicine, Ekiti State University, Ado Ekiti, Nigeria. Animals were kept in separate animal cages, on a 12 hr light/dark cycle at room temperature with free access to food and water. According to the National Institute of Health guide for the care and use of laboratory animals, the animals were used. Animals were placed into six groups of six rats each:

Group 1- Normal Control, fed with rat-feed and distilled water only.
Group 2- Diabetic Control (alloxan-induced), fed with rat-feed and distilled water;
Group 3- Standard Drug (metformin), fed with rat-feed and distilled water;
Group 4- *Morinda lucida* Leaf
Group 5- *Nauclea latifolia* Leaf
Group 6- *Morinda lucida* and *Nauclea latifolia* Leaves

After induction with alloxan, animals were observed for 72 hours, after which the glucose concentration levels have increased significantly before treated with 20mg/kg body weight of aqueous leaves extracts of *M. lucida* and *N. latifolia* of both plants respectively for three weeks by oral administration. At the end of the experiment, animals were fasted 24 hours before sacrifice to obtain the blood for serum, liver, and kidney for organ homogenates.

2.3. Extraction of crude active components.

Dried powdered of *M. lucida* and *N. latifolia* leaves weighing 100g each was extracted with 500 mL of distilled water for 72 h. After that, the resulting mixture was filtered separately using a muslin cloth. The filtrates were left open for 72 h to allow for complete evaporation. The crude extract recovered after evaporation was stored separately in an airtight container in the refrigerator before use. Therefore, the crude extract was reconstituted in distilled water and administered to the animals orally for three weeks.

2.4. Preparation of serum and tissue homogenates.

At the end of three weeks, the animals were decapitated and dissected to obtain the blood, liver, and kidney. Whole blood was collected by cardiac puncture into a serum bottle. The serum was obtained by centrifugation at 3000 rpm for 15min. Simultaneously, the

homogenates of the liver and kidney were prepared by weighing 1g in 10 mL of 6.7 mM potassium phosphate buffer, pH 7.4, respectively, using Teflon homogenizer model 3431E10EA, Thomas Scientific, India. The tissue homogenate was centrifuged at 3,000 rpm for 10 min at 4^{o}C to obtain a clear supernatant, kept at 8^{o}C for subsequent analyses.

2.5. Determination of alkaline phosphatase (ALP).

Alkaline phosphatase was assayed following the method described by Brichacek and Brown [33], using a standard Fortress kit from England. Five hundred microliter (500µl) of the substrate was equilibrium at 37^{o}C for 3 min. Fifty microliters (50µl) of the sample was added to the equilibrated substrate and incubated for 10 min at 37 ^{o}C. 2.5 mL of color reagent (mixture of NaOH, 0.09 M and Na_2CO_3, 0.1 M) was added. This mixture was thoroughly mixed. The absorbance of the sample against the blank was read at 590 nm. ALP (IU/L) was then calculated.

2.6. Determination of aspartate transaminase (AST).

Aspartate transaminase AST was assayed following the method described by Brichacek and Brown [33], using a standard Fortress kit from England. Briefly, 100µ of homogenate was added to 500 µl of AST buffer, thoroughly mixed, and incubated for 30min at 37^{o}C. After that, 0.5ml of dye reagent (2,4-dinitrophenyl hydrazine, (2.0 mM) was added, and the mixture was thoroughly mixed and allowed to stand for 20min at 20 ^{o}C. Five milliliters (5.0 ml) of 0.01 M NaOH was then added and thoroughly mixed. , The absorbance of the sample against the blank was read at 546 nm.

2.7. Determination of alanine transferase (ALT).

ALT was measured using a standard Fortress kit from England according to the method of the International Federation of Clinical Chemistry (1986) using a standard Fortress kit from England. One hundred microliters (100µl) of homogenates were added to 500µl of ALT buffer, thoroughly mixed, and incubated for 30min at 37^{o}C in a water bath. Five hundred microliters (500µl) of dye reagent (2, 4-dinitrophenyl hydrazine, 2.0 mM) was then added. This mixture was thoroughly mixed and allowed to stand for 20min at 20^{o}C. After that, 5.0ml of diluted NaOH was then added and thoroughly mixed. The absorbance of the resulting mixture sample against the blank was read at 546 nm.

2.8. Determination of Superoxide dismutase (SOD).

Superoxide dismutase activity in both serum and tissue homogenates was determined by the method of Molehin *et al*. [34], using a standard Fortress kit from England. An aliquot of the serum and/or tissue homogenates was added to 2.5ml of 0.05M carbonate buffer, pH 10.2. The mixture was allowed to equilibrate for 2 min in a spectrophotometer. The reaction was then started by adding 0.3ml of freshly prepared 0.3mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained all other assay components except the homogenate(s) and/or serum replaced with distilled water. An increase in absorbance at 480 nm was monitored every 30 sec for 150 sec.

2.9. Determination of catalase activities (CAT).

Catalase activity in the serum and tissue homogenates were determined as described by Igile *et al.* [35], using a standard Fortress kit from England. Two hundred microliters (200 µl) of tissue homogenates were mixed with 0.8ml distilled H_2O to give I in 5 dilutions of the sample. Five hundred microliters (500 µl) of tissue homogenate was rapid, mixed with the reaction mixture at room temperature by a gentle swirling. One milliliter (1 ml) portion of the reaction mixture was withdrawn and blown into 1ml dichromate/acetic acid reagent at 60 seconds. The hydrogen peroxide content of the withdrawn sample was determined by the method described above at 570 nm.

2.10. Determinations of HDL-cholesterol.

HDL-cholesterol was measured following the method described by Baggio and Bragagnolo [36], using standard Fortree from England. Briefly, 5µl of homogenate was added to 450µl of Good's buffer (4-amino antipyrine, POD, ascorbic oxidase, and anti-human lipoprotein Ab). This mixture was mixed well 5and incubated at 37ºC in a water bath for 5min. Thereafter, 150 µl of enzyme reagent (Good's buffer 1, cholesterol esterase, and cholesterol oxidase) was later added to the mixture and incubated for 5 min at 37ºC. The absorbance of the resulting mixture was then read against the blank at 600 nm.

2.11. Determination of LDL – cholesterol

Low-density lipoprotein – cholesterol (LDL-chol) was determined by the method earlier described by Baggio and Bragagnolo [36], using a standard Fortress kit from England. Briefly, 5µ of tissue homogenate was added to 450µl of buffer solution (Good's buffer, and peroxidase). This mixture was mixed well and incubated at 37ºC in a water bath for 5min. Absorbance 1 was measured at 600nm. One hundred and fifty microliters (150 µl) of enzyme reagent R2 (Amino antipyrine and POD) was later added to the mixture and incubated for 5 min at 37ºC. Absorbance 2 of the sample against the blank was read at 600 nm.

2.12. Determination of total cholesterol.

Total cholesterol in the serum and tissue homogenates was routinely determined following [35,36], using a standard Fortress kit from England. Ten microliters (10µl) of tissue homogenate was thoroughly mixed with 1ml of working reagent (4-amino antipyrine, cholesterol oxidase, cholesterol esterase, and peroxidase dissolved in 50ml of pipes buffer and phenol). The reaction mixture was incubated for 10 min at 25ºC. The absorbance of the sample against the blank was read at 546nm. Total cholesterol (nm) concentration was then calculated by the equation: [(Abs. x 200) ÷ (0.042)].

2.13. Determination of lipid peroxidation assay (TBARS).

Levels of the thiobarbituric acid reactive species (TBARS) in the tissues and serum homogenates were measured according to the method of Steghens *et al*. [37], using standard Fortress from England. An aliquot of 0.4ml of serum and other tissue homogenates was mixed with 1.6 ml of Tris-KCl buffer. It was added to which 0.5ml of 30% trichloroacetic acid (TCA). After that, 0.5ml of 0.75% thiobarbituric acid (TBA) was added and incubated for 45 min at 80 ºC. The resulting mixture was then cooled on ice and centrifuged at 3000 rpm. The clear

supernatant was collected and absorbance measured against a reference blank at 532nm. The MDA level was calculated according to the method of [38].

2.14. Determination of total protein.

Total protein content was determined using the Biuret method Al-Attar [39] using a standard Fortress kit from England. Briefly, 20 ml of homogenate was thoroughly mixed with 1ml of Biuret reagent and incubated at room temperature for 10min. The absorbance of the resulting mixture was read at 546 nm against the blank.

2.15. Determination of total phenolic contents.

The total phenolic contents were determined spectrophotometrically using the Folin-Ciocalteu reagent reported by Daay and Lattanzio [40]. The extracts were reacted with Folin-Ciocalteu reagent and then neutralized with sodium carbonate solution (25%). After 2 h, the absorbance of the resulting solution was measured at 765 nm. The concentrations of phenolic compounds were calculated according to the equation obtained from the standard as a gallic acid graph: Absorbance = [0.0007 x gallic acid (µg) + 0.0642], with R^2 = 0.9964.
All tests were carried out in triplicate, and the results are given as gallic acid equivalents (GAE) per g of dry extract.

2.16. Determination of total flavonoid contents.

Total flavonoid contents were determined using the Dowd method adapted by, Daay and Lattanzio [40]. 1 mL of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the methanolic extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 mL methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the equation obtained from the standard quercetin graph:

Absorbance = [0.0333 x quercetin (µg) + 0.0231], with R^2 = 0.9961.

2.17. Statistical analysis.

The data obtained from the study were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Duncan test and expressed as mean ± SD (standard deviation) with a p-value less than 0.05 ($p<0.05$) considered to be statistically significant.

3. Results and Discussion

In this present study, experimental diabetes was reliably established in the rats intraperitoneally when injected with alloxan monohydrate, which was characterized by hyperglycemia that was sustained throughout the three weeks of treatment as demonstrated in the untreated diabetic rats.

In Tables 1 and 2, the mean weight gain was high in groups that received both the extracts and the standard drug than the normal, which may be due to the extracts' beneficial effect. After the consumption of the extracts, animals showed a slight increase in weight gain. Diabetes was fully established in Table 2, as evidenced by the significant ($p<0.05$) elevation in the Fasting blood glucose concentrations in the negative control. However, oral treatment with *M. lucida* and *N. latifolia* extracts for twenty-one consecutive days significantly ($p<0.05$)

lowered the Fasting blood glucose level from 452.5 ± 2.50 *M. Lucida* group to 249.0 ± 1.00. *N. Latifolia* group extract also significantly reduced the blood sugar in group five from (502.0 ± 1.00) to (104.0 ± 1.00) close to the normal. The Fasting blood-glucose-lowering effect of *M. lucida* and *N. latifolia* was significantly ($p<0.05$) higher than that of metformin.

The observed hypoglycemic effect of *M. lucida* and *N. latifolia* is an indication that the two plants contain active components with the potent hypoglycemic property. In normal rats, they could be acting via increased insulin secretion or increased peripheral utilization of glucose, but in the *in vivo* type II diabetes model created in this study, the two extracts lower hyperglycemia by increasing the peripheral utilization of glucose in the diabetic rats.

In this study, continuous synergetic treatment with the leaves extract of M. lucida and N. latifolia for 3 weeks caused a significant decrease in the blood glucose level of treated rats compared to untreated diabetic rats seen in Table 3. A corresponding increase followed this in the bodyweight of the treated rats. Diabetes is characterized by a severe loss in body weight due to the loss or degradation of structural proteins [41]. This condition was alleviated by the treatment of diabetic rats with leaf extracts of *Morinda lucida* and *Nauclea latifolia*. Some plant extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin. In contrast, others act through extrapancreatic mechanisms by inhibiting hepatic glucose production or corrections of insulin resistance. This study's result is in consonance with that reported for the dried leaves extract of *Morinda lucida* Benth in normal and streptozotocin-diabetic rats [42].

Some phytochemical compounds such as saponin, terpenes and tannins, steroids, and alkaloids have been implicated in plants' antidiabetic activities. Phytochemical study of the leaves extracts revealed the presence of terpenes, saponins, tannins flavonoids, and alkaloids, as shown in Table 1.

Table 1. Phytochemical screening of aqueous leaves extracts of *M. lucida* and *N. latifolia*.

Plant Part	Tannin	Terpenoid	Alkanoid	Saponin	Glycoside
Morinda Lucida leaf	-	+	+	++	++
Nauclea Latifolia leaf	+	+	+	++	++

+ = Present - = Absent

Table 2. Effects of aqueous leaves extract of *M. lucida* and *N. latifolia* on diabetic Rats weights (g).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Before Alloxan Induction	161.50±0.50^{a}	144.50±0.50^{b}	173.50±0.50^{c}	150.50±0.50^{d}	164.50±0.50^{a}	156.50±0.50^{d}
After Alloxan Induction	166.50±0.50^{a}	154.00±1.00^{b}	159.50±0.50^{ac}	127.00±1.00^{d}	121.00±1.00^{d}	156.5±0.50^{b}
After Treatments	181.00±1.50^{a}	136.50±0.50^{b}	188.50±0.50^{a}	191.00±1.50^{c}	186.50±0.50^{a}	101.00±1.00^{d}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 3. Effect of aqueous leaf extracts of *M. lucida* and *N. latifolia* on fasting blood sugar concentration in diabetic rats (mg/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Before Alloxan Induction	107.50±2.50^{a}	83.50±1.50^{b}	112.50±0.50^{c}	94.00±1.00^{d}	104.00±1.00^{a}	88.50±1.50^{b}
After Alloxan Induction	103.00±2.00^{a}	328.50±1.50^{b}	283.50±0.50^{c}	452.50±2.50^{d}	502.00±1.00^{e}	471.50±1.00^{d}
After Treatments	102.00±1.00^{a}	209.50±0.50^{b}	108.00±1.00^{a}	249.00±1.00^{c}	104.00±1.00^{a}	327.50±2.50^{d}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

The presence of indole alkaloids in the root of *Nauclea latifolia* has been reported to exert antidiabetic activity [43]. In part, these constituents may be responsible for the observed significant activity of this extract either singly or in synergy with *M. lucida*. Equally, Olajide *et al*. [42] and Lawal *et al*. [44] independently reported *Morinda* leaves to contain high concentrations of flavonoids, alkaloids, tannins, and saponin. Hyperlipidemia contributes to major risk factors of cardiovascular diseases [45].

Diabetes has been related to oxidative stress either as a causative factor or because of its consequence [46]. Alloxan-induced diabetes is characterized by impaired glucose tolerance and hyperglycemia and the collapse in the antioxidant defense mechanism. Oxidative stress has been shown to be responsible, at least in part, for tissue damage and beta-cell dysfunction [47]. Reactive oxygen species (ROS) produced by alloxan administration may lead to DNA fragmentation and other deleterious changes in the cells [48]. In the present study, alloxan administration induced a collapse in antioxidant defense as observed in a decrease in SOD, CAT activities and an increase in lipid peroxidation product (MDA) in the diabetic control group as seen from Table 4 to 9. In order to reduce the effect of alloxan, SOD catalyzes the dismutation of the superoxide radical, and the CAT removes the product of the SOD (which is hydrogen peroxide) from circulation. The enzymes SOD and CAT are major antioxidant defense system of the body, which protect the cell membrane and other cellular constituents against oxidative damage by free ROS [49]. Both the extract and the standard drug (metformin) prevented the collapse in antioxidant defense by maintaining the SOD, CAT, and MDA towards normal. Earlier reports portrayed methanolic extract of the stem bark of *M. lucida* as having radical scavenging activities *in-vitro* [50].

Table 4. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Catalase activities in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	16.44 ± 0.00^{a}	2.96 ± 0.01^{b}	25.96 ± 0.01^{c}	15.77 ± 0.01^{a}	3.96 ± 0.01^{d}	4.09 ± 0.00^{d}
Liver	0.03 ± 0.02^{a}	0.49 ± 0.02^{b}	0.74 ± 0.01^{c}	0.14 ± 0.02^{d}	1.01 ± 0.02^{e}	0.16 ± 0.02^{d}
Kidney	0.28 ± 0.01^{a}	0.20 ± 0.02^{a}	0.63 ± 0.01^{b}	0.67 ± 0.01^{b}	0.36 ± 0.00^{a}	0.14 ± 0.02^{a}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 5. Effect of aqueous extract of *M. lucida* and *N. latifolia* on Superoxide Dismutase (SOD) activities in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	4.80 ± 0.01^{a}	1.80 ± 0.00^{b}	3.20 ± 0.02^{c}	3.40 ± 0.01^{c}	2.20 ± 0.00^{b}	3.40 ± 0.01^{c}
Liver	5.60 ± 0.02^{a}	0.60 ± 0.00^{b}	4.20 ± 0.01^{c}	4.40 ± 0.01^{c}	2.20 ± 0.02^{d}	2.10 ± 0.01^{d}
Kidney	3.80 ± 0.00^{a}	1.20 ± 0.01^{b}	3.80 ± 0.02^{a}	1.80 ± 0.02^{c}	1.40 ± 0.01^{b}	0.90 ± 0.01^{d}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 6. Effect of aqueous extract of *M. lucida* and *N. latifolia* on Malonyldialdehyde (MDA) activities in diabetic rats (µM).

Category	Group 1	Group 2	Group 3	Group 4	GrouP 5	Group 6
Serum	2.56 ± 0.17^{a}	7.37 ± 0.10^{b}	1.92 ± 0.10^{c}	2.88 ± 0.00^{a}	3.85 ± 0.12^{d}	2.18 ± 0.15^{a}
Liver	2.56 ± 0.00^{a}	14.23 ± 0.10^{b}	14.13 ± 0.10^{b}	17.79 ± 0.00^{c}	26.28 ± 0.10^{d}	2.18 ± 0.15^{a}
Kidney	8.97 ± 0.17^{a}	16.25 ± 0.10^{b}	20.71 ± 0.10^{c}	23.39 ± 0.10^{c}	16.63 ± 0.10^{b}	16.19 ± 0.15^{b}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Thus, plant extract or metformin's presence prevented the build-up of oxidative stress and allowed for the recovery from antioxidant collapse. The ability of *M. lucida* and *N. latifolia*

leaves extracts to prevent the alteration of antioxidants status may be due to phenolic content and other phytochemical compounds present [51].

Table 7. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Gluthathione transferase activities in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	2.95±0.00^{a}	1.01±0.00^{b}	1.55±0.00^{c}	1.85±0.00^{d}	1.25±0.00^{e}	1.35±0.09^{e}
Liver	2.55±0.00^{a}	1.13±0.00^{b}	1.28±0.00^{c}	1.26±0.00^{c}	1.23±0.00^{c}	1.16±0.00^{b}
Kidney	2.90±0.00^{a}	1.07±0.00^{b}	1.17±0.00^{c}	2.14±0.00^{d}	1.10±0.00^{b}	2.00±0.00^{f}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 8. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Glutathione reductase activities in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	21.16±0.00^{a}	9.79±0.0^{b}	17.11±0.00^{c}	14.96±0.00^{d}	11.86±0.00^{e}	12.32±0.00^{e}
Liver	30.95±0.01^{a}	15.56±0.00^{b}	18.67±0.01^{c}	18.45±0.00^{d}	21.83±0.00^{e}	24.65±0.00^{e}
Kidney	41.52±0.00^{a}	35.22±0.00^{b}	37.73±0.00^{b}	38.48±0.00^{b}	40.96±0.00^{a}	39.63±0.00^{a}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 9. Effect of aqueous extract of *M. lucida* and *N. latifolia leaves* on Glutathione peroxidase activities in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	168.54±0.01^{a}	102.28±0.03^{b}	114.92±0.05^{c}	162.57±0.02^{a}	155.65±0.02^{a}	121.92±0.05^{c}
Liver	73.25±0.01^{a}	29.82±0.01^{b}	98.23±0.01^{c}	33.98±0.01^{b}	33.72±0.01^{b}	47.23±0.01^{b}
Kidney	271.64±0.04^{a}	89.46±0.01^{b}	91.15±0.01^{c}	95.07±0.02^{c}	110.04±0.02^{d}	130.15±0.01^{d}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves

Transaminases (ALT and AST) are important hepatic markers. Their presence in the serum at high concentration serves as indicators for liver pathology. This is the case in the administration of toxins to experimental animals, where there is a significant increase in these enzymes' serum levels. An increase in the activities of ALT and AST in Alloxan intoxication has earlier been reported [52,53]. Tables 10 and 12 showed similar results in the present study. However, both standard drug and *the* two plant extracts were able to inhibit changes in ALT and AST activity in experimental animals showing their protective potentials.

The serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), and Lactate dehydrogenase (LDH) activities increased, which was an indication of diabetes-induced hepatic parenchymal injury and hepatic dysfunction [54,55] and leakage of these enzymes from the liver cytosol into the bloodstream [55,56]. However, after administering the extracts in Table 10, 11, 12, and 13, the serum AST, ALT, and ALP concentrations decreased relatively with leaves of the *M. Lucida* and *N. Latifolia* extract and the standard drug.

Table 10. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Alanine Amino Transferase (ALT) in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	0.25±0.09^{a}	2.64±0.09^{b}	0.59±0.04^{c}	0.35±0.09^{d}	1.44±0.09^{e}	1.02±0.09^{d}
Liver	2.54±0.07^{a}	0.29±0.85^{b}	1.27±0.25^{c}	1.25±0.08^{c}	1.44±0.09^{d}	1.42±0.09^{d}
Kidney	2.84±0.09^{a}	0.34±0.17^{b}	1.36±0.17^{c}	0.59±0.09^{d}	0.68±0.51^{e}	0.79±0.02^{e}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 11. Effect of aqueous extract of *M. lucida* and *N. latifolia* Leaves on Alkaline Phosphatase (ALP) in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	4.14±1.38^{a}	9.66±4.14^{b}	5.14±1.38^{c}	4.14±1.38^{a}	6.52±2.76^{d}	7.14±0.00^{d}
Liver	6.90±1.38^{a}	5.76±0.00^{b}	6.14±1.38^{c}	6.76±0.00^{a}	7.76±0.00^{d}	6.90±0.00^{a}
Kidney	6.90±1.38^{a}	3.14±1.38^{b}	6.99±1.38^{c}	7.66±1.38^{d}	7.76±0.00^{d}	8.00±1.38^{d}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 12. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Aspartate Aminotransferase (AST) in diabetic rats (U/L).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	34.31±0.17^{a}	216.69±0.17^{b}	18.10±0.17^{c}	23.97±0.17^{d}	27.76±0.17^{d}	26.69±0.17^{d}
Liver	195.35±0.17^{a}	68.69±0.17^{a}	219.14±0.17^{b}	187.41±0.17^{b}	285.69±0.17^{ab}	191.90±0.17^{a}
Kidney	180.17±0.17^{a}	100.55±0.17^{b}	171.90±0.17^{c}	169.48±0.17^{a}	191.48±0.17^{d}	172.07±0.17^{c}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 13. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on Lactate Dehydrogenase (LDH) in diabetic rats (mg/dL).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	145.23±0.48^{a}	290.46±0.96^{b}	145.23±0.48^{a}	145.23±0.48^{a}	277.74±0.96^{b}	193.64±0.00^{c}
Liver	196.82±0.00^{a}	193.64±0.00^{a}	96.82±0.00^{b}	102.05±0.48^{b}	151.64±0.96^{ab}	120.46±0.00^{c}
Kidney	145.23±0.48^{a}	187.28±0.19^{b}	242.05±0.48^{c}	145.23±0.48^{a}	222.05±0.48^{d}	193.64±0.96^{b}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 14. Effect of aqueous extract of *M. lucida* and *N. latifolia* Leaves on Total cholesterol in diabetic rats (mg/dL).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	1.33±0.01^{a}	6.92±0.01^{b}	3.52±0.08^{c}	3.27±0.08^{c}	2.05±0.01^{a}	1.02±0.01^{a}
Liver	0.53±0.01^{a}	2.92±0.01^{b}	0.97±0.01^{c}	0.99±0.01^{c}	1.20±0.01^{d}	1.40±0.01^{d}
Kidney	0.77±0.01^{a}	2.45±0.01^{b}	1.18±0.01^{c}	0.23±0.01^{d}	1.58±0.01^{c}	1.20±0.01^{c}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 15. Effect of aqueous extract of *M. lucida* and *N. latifolia* leaves on High-Density Lipoprotein – cholesterol in diabetic rats (mg/dL).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	942.57±0.68^{a}	519.60±0.68^{b}	937.84±1.35^{a}	929.73±0.00^{a}	541.76±0.00^{b}	720.35±0.68^{c}
Liver	125.00±0.68^{a}	58.78±0.68^{b}	170.95±0.68^{c}	169.60±0.68^{d}	126.35±0.68^{e}	126.35±0.68^{e}
Kidney	260.14±0.68^{a}	106.08±0.68^{b}	180.14±0.68^{c}	165.54±0.68^{c}	121.62±0.00^{d}	156.35±0.68^{c}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves.

Table 16. Effect of aqueous extract of *Morinda lucida* and *Nauclea latifolia Leaves on* Low Density Lipoprotein – cholesterol in diabetic rats (mg/dL).

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	519.6±0.68^{a}	1137.84±1.35^{b}	401.76±0.00^{c}	441.76±0.00^{d}	515.95±0.00^{e}	470.87±0.68^{f}
Liver	58.78±0.68^{a}	270.95±0.68^{b}	92.57±0.68^{c}	169.6±0.68^{d}	126.35±0.68^{e}	134.00±0.68^{e}
Kidney	106.08±0.68^{a}	160.14±0.68^{b}	101.62±0.00^{c}	105.54±0.68^{d}	121.62±0.00^{e}	130.55±0.00^{e}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: Morinda *Lucida* and *Nauclea Latifolia* Leaves.

This persistent hyperglycemia was characterized by corresponding high serum TG, TC, LDL-C (Tables 14, 15, 16, and 17). These biochemical alterations recorded in this study is in

complete agreement with earlier reported studies on metabolic abnormalities in diabetes-induced by alloxan [57,58].

Table 17. Effect of aqueous Leaves extracts of *M. lucida* and *N. latifolia* on Triglyceride in diabetic rats. (U/L)

Category	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Serum	5.33±0.01^{a}	17.75±0.00^{b}	8.76±0.01^{c}	6.05±0.01^{d}	10.82±0.14^{e}	5.50±0.14^{e}
Liver	16.21±0.01^{a}	28.71±0.01^{b}	20.42±0.01^{c}	17.10±0.01^{d}	22.99±0.01^{e}	24.06±0.01^{e}
Kidney	4.14±0.01^{a}	6.60±0.01^{b}	3.86±0.01^{c}	4.20±0.0^{d}	5.21±0.01^{e}	3.92±0.01^{e}

Values are expressed as mean ± SD. Values with different superscript letters indicate differences among the groups ($P < 0.05$). Group 1: Normal Control; Group 2: Diabetic Control; Group 3: Standard Drug; Group 4: *Morinda lucida* Leaf; Group 5: *Nauclea latifolia* Leaf; Group 6: *M. lucida* and *N. latifolia* Leaves

4. Conclusions

An *in silico* study has shown that 4 constituents of *Nauclea latifolia* leaf extracts (2-*O*-p- methylphenyl- 1-thio-β-d-glucoside, 3-tosylsedoheptulose, 4-benzyloxy-6 -hydroxymethyl - tetrahydropyran-2,3,5 -triol, and vitamin E) could inhibit iipeptidyl peptidase IV (DPP-IV), which is a pharmacotherapeutic target in type 2 diabetes [59]. It was reported that dichloromethane extract of *M. lucida* showed high inhibitory capacity on α-amylase and β-glucosidase activities [60]. The study has shown the protective role of 125-500 mg/kg/day of *Morinda lucida* aqueous stem bark extract in chronic hyperglycemia-associated renal and hepatic dysfunctions in the experimental diabetes model, which was mediated via antioxidant and free radical scavenging activities [61].

The ability of the aqueous leaf extracts of *Morinda lucida* and *Nauclea latifolia* to decrease hyperglycemic rats' blood glucose towards normal confirms its antidiabetic activity. The leaf extracts of both plants also inhibited diabetic complications by preventing an alteration in plasma concentration of lipoprotein, triglyceride, cholesterol, and AST and ALT activities. The aqueous extract of the two plants possesses good antioxidant potentials by inhibiting the collapse of antioxidant defense induced by alloxan. The bioactive substances present in this plant leaves extract might be responsible for their medicinal and antioxidant properties.

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Conflicts of Interest

The authors declare no conflict of interest.

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