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Ameliorative Effects of *Daniellia oliveri* Leaf Extracts on Streptozotocin-Nicotinamide-Induced Type II Diabetes in Wistar Rats

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ABSTRACT

Diabetes mellitus is becoming more prevalent, and well-known conventional therapies reportedly have adverse effects. Hence, in streptozotocin-nicotinamide-induced type II diabetic mice, the therapeutic efficacy and haematological implication of administering organic extracts of *Daniellia oliveri* leaf (Do) were investigated. The diabetic rats were treated with 250mg/kg body weight of organic extracts of *D. oliveri* obtained by cold macerations in diethyl ether, ethanol, ethyl acetate, and n-hexane and 10 mg/kg (metformin) daily for 14 days after being induced with a single dose of 60 mg/kg and 110 mg/kg body weight of streptozotocin and nicotinamide, respectively. Our study revealed that treatment with metformin and *D. oliveri* organic extracts significantly reduced elevated blood glucose levels and reversed the increased glucose-6-phosphate dehydrogenase (G6PD) and glycosylated haemoglobin values. Hepatic glucose concentrations, white blood cells (WBC), packed cell volumes (PCV), haemoglobin (Hb), and red blood cells (RBC) increased after the induction of diabetes were reduced following *D. oliveri* extracts treatment. In addition, the activities of glucose-6-phosphatase and neutrophil concentration were increased, while blood lymphocyte levels were significantly reduced in all groups except for the diabetic non treated group. Thus, this study suggests that while all solvent extracts of *D. oliveri* leaf were efficient in treating diabetes, the ethanol extract stands out as an excellent candidate. Additionally, the various solvent extracts studied are potential candidates for blood-boosting and may also aid in immune system enhancement.

Keywords: *Daniellia oliveri*, Nicotinamide, Streptozotocin, Glucose-6-phosphate dehydrogenase, Insulin, Glucose-6-phosphate.

Introduction

Diabetes mellitus (DM) is a term that refers to a group of metabolic diseases demonstrated by high blood glucose levels. It is a prevalent disease and one of the most complex global health issues. In 2015, the World Health Organization estimated 415 million diagnoses of diabetes, which later rose to 451 million (age 18-99 years) in 2017.[1-3] However, in 2019, an estimate of 463 million was made by the International Diabetes Foundation (IDF), with projections of 700 million cases by 2045.[4] Low or no insulin production (due to pancreatic beta-cell malfunction) and insulin sensitivity upon normal secretion are the causes of diabetes. Because of inefficient glucose storage and utilisation, this anomaly causes a spike in systemic glucose levels. As a result, diabetic treatment tries to reduce insulin resistance while also increasing insulin secretion.[5] There are four types of diabetes based on current classification based on the pathogenicity of the disease.[6] Type II Diabetes Mellitus (T2DM), a chronic metabolic disease with life-threatening complications due to hyperglycemia, insulin action, and excessive glucagon secretion.[7, 8] Various modified solutions should be developed to tackle the global type 2 diabetes epidemic, including high-quality, nutritious food, a physically active lifestyle, and other crucial lifestyle determinants such as alcohol consumption, smoking, and sleeping habits.[9]

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There are different orthodox treatment regimens available for T2DM, mainly through synthetic agents. These synthetic antidiabetic drugs, however, have several serious side effects.[10] Plant-based medicine has grown in popularity due to the adverse effects of the long-term use of synthetic medications. Plant-based medications have recently earned much attention as a legitimate therapeutic option for various illnesses and disorders. This is due to the unequaled availability of phytochemicals in medicinal plants, whether as standardised extracts or pure molecules, which can help drug discovery. More than 30% of therapeutic drugs approved for use worldwide between 1981 and 2019 were derived directly from natural products, with medicinal plant compounds dominating.[11] Several medicinal plants have been claimed to have anti-diabetic properties, but no empirical evidence has been found to support this assertion. However, many other plants have been reported by various researchers as plausible antidiabetic agents.[12] In type 2 diabetes patients, clinical trials on *Coccinia grandis*, a promising antidiabetic plant, were conducted to determine its therapeutic capabilities, and it was discovered that 500mg/day improved disease symptoms with well-tolerated safety.[13]

Daniellia oliveri is a member of the Caesalpinioideae subfamily (Leguminosae) and is also known as West African Copal tree, African copaiba balsam, Ilorin balsam, and Benin gum copal. *D. oliveri* is the only member of the Paradaniellia subgenus of the *Daniellia* genus's. The *Daniellia* species "*oliveri*" is the most prevalent throughout the lowland Savannah south of the Sahel.[14] Traditionally, *D. oliveri* aqueous leaf extract has been reported as antibiotic and antidiabetic.[15] The decoction of the root barks of *D. oliveri*'s, when combined with the roots of *Sarcocephalus latifolius* is used as antihyperglycemia in south-eastern Nigeria.[16] The antioxidant and cytotoxicity activities of oleoresin of *D. oliveri* were also reported.[17] Ringworm, scrotal elephantiasis, dysentery, syphilis, typhoid fever, and earache have all

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been treated with the *D. oliveri*'s roots, stem barks, and leaves.[18] The aqueous root bark extract of *D. oliveri* has been reported to treat neurological malfunctions in amnesic patients.[19] The leaf was reported to be a safe and effective treatment for type 2 diabetes.[20] Although medicinal plants are increasingly being used as antidiabetic agents to manage diabetes and its complications in most parts of the world,[21, 22] some medicinal plants are still understudied, one of which is *D. oliveri*. However, although the aqueous extract's potency has been reported,[20] it is expedient to evaluate various organic solvents for the best results. As a result, this study aimed to see how different organic solvents affect the efficacy of *D. oliveri* young leaves in the treatment of streptozotocin-nicotinamide-induced type II diabetic rats.

Materials and Methods

Chemicals and reagents
Metformin was procured from Tuyil Pharmaceutical Industry, Ilorin, Nigeria. Streptozotocin was a product of Sigma-Aldrich Chemicals Industry, St. Louis, Missouri, USA. Nicotinamide was a product of BDH chemicals LTD, Poole, England. Analytical-grade reagents and chemicals were utilized in the investigation, and buffers were made following published protocols.

Plant material
The fresh young leaves of *D. oliveri* were collected in December 2017 from their natural habitat in the University of Ilorin, Ilorin, Nigeria. The collected plant was authenticated at the species level in the Department of Plant Biology Herbarium, University of Ilorin, Ilorin, Nigeria. In addition, a voucher specimen (UILH/001/1291/2021) was deposited for reference.

Preparation of extracts
The leaves were sorted to make sure that only the fresh ones were selected. Then, to ensure that debris and dust particles were thoroughly removed from the sorted leaves, they were washed under running water. The sorted, washed leaves were dried under shade in a well-aerated room for three weeks and then processed into powder using an electric blender. Exactly 200g of the powdered leaves were weighed in four different containers, and each macerated in 1200 ml each of 70% Ethanol, Ethyl Acetate, n-Hexane, and Diethyl Ether, respectively, and kept in the dark while stirring intermittently for 72 hours. The extracts were filtered using Whatman filter paper (No. 1) after three days (72hrs) of cold maceration. Finally, the extracts were concentrated using a rotary evaporator and a water bath at 37^{o}C to ensure all the solvents were removed.

Experimental animals
The Animal Unit of the Department of Biochemistry at the University of Ilorin in Ilorin, Nigeria, provided thirty-five (35) adult Wistar rats weighing an average of 140.40±8.24g. The rats were acclimatised under laboratory conditions for two weeks (12 hours light/dark cycle; 25±2^{0}C). Water and feed pellets (Top Feeds, Premier Feed Mills Co Ltd, Ibadan, Nigeria) were available *ad-libitum.* The laboratory animal care principle was strictly followed and conformed throughout the research (NIH Publication No. 82-23). The University of Ilorin's Ethical Committee approved the research protocol.

Induction of diabetes
Acclimatised experimental rats fasted for 8 hours, after which the diabetic induction was carried out. Animals were subjected to a singly intraperitoneal injection of 60 mg/g body weight Streptozotocin (STZ), freshly prepared using cold Citrate buffer solution (pH 4.5) fifteen minutes after administration of a 200mg/kg body weight Nicotinamide (NAD) intraperitoneally to induce type 2 diabetes perfectly.[23] The fasting blood glucose of the experimental animals was determined 72 hours after the diabetic induction using AccuCheck active glucometer and compatible strips. Rats showing fasting blood glucose above 200 mg/dl were considered diabetic.

Animal grouping and extract administration
The acclimatised Wistar rats were divided into seven (7) groups, each with five rats. Animals in group I were non-diabetic, while those in group II-VII had diabetes and received 1 ml each of normal saline, 10 mg/kg body weight Metformin, 250 mg/kg body weight n-hexane, ethyl acetate, ethanol, and diethyl ether extract of *D. oliveri* leaf respectively. The administration was done once daily for 14 consecutive days.

i. Normal: Negative Control (Non-diabetic rats that received Normal Saline)
ii. Diabetic: Diabetic Control (Diabetic rats that received Normal Saline)
iii. Metformin: Diabetic rats administered with Standard Drug (10 mg/kg body weight Metformin)
iv. n-Hexane: Diabetic Treated with 250 mg/kg body weight of n-hexane extract *D. oliveri* leaf
v. Ethyl Acetate: Diabetic Treated with 250 mg/kg body weight of Ethyl acetate extract of *D. oliveri* leaf
vi. Ethanol: Diabetic Treated with 250 mg/kg body weight of Ethanol extract of *D. oliveri* leaf
vii. Diethyl Ether: Diabetic Treated with 250 mg/kg body weight of Diethyl ether extract of *D. oliveri* leaf

Determination of blood glycaemic indices
Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) of all experimental groups were determined at different phases of the experiment by collecting blood from the caudal vein of the animals.

Fasting blood glucose determination
After fasting the experimental animals for 8 hours and before induction of diabetes mellitus in experimental animals (day 0), the fasting blood glucose concentrations of the animals were monitored using a glucose oxidase-based commercial glucometer (AccuChek active, Roche Diagnostic) by withdrawing blood from the caudal vein of rat tail. After the induction of diabetes has been confirmed, FBG level was monitored on days 1, 4, 7, 10, and 14 during the experimental period.[24]

Oral glucose tolerance test
After an 8-hour fast, blood was drawn from the tail end 30 minutes before each group received *D. oliveri* leaf extracts or metformin. Thirty minutes later, all groups of rats were administered glucose (2g/kg body weight) orally. Blood was drawn from the tail vein before (0 minutes) 30 minutes, 60 minutes, 120 minutes, and 150 minutes after glucose loading.[25] The blood glucose level was determined at each time interval and compared with the control.

Body weight measurement
The body weight was measured five times during the experiment (days 1, 4, 7, 10, 14 of the treatment period) using a digital weighing scale.

Animal Sacrifice and collection of sample
The overnight fasted rats were ether anaesthetized twenty-four hours after the last treatment (Day 14, following diabetes induction). Blood samples were taken from rats' Jugular veins in clean sample bottles containing EDTA. Centrifugation at 3000 r/min for 15 minutes separated the serum. The resulting samples were then frozen. The rats were then dissected to expose the organs of interest (Pancreas and Liver), which were removed and promptly washed in ice-cold saline solution, weighed, and stored in 0.25 M sucrose solution in preparation for homogenisation and centrifugation. Following that, a portion of the suspension was homogenized in 0.1 mol/L potassium phosphate buffer (pH 7.4). The clear supernatant was kept for subsequent biochemical analysis following centrifugation at 3000 r/min for 10 minutes.

Determination of relevant parameters
Blood samples were collected after an overnight fast to determine plasma insulin levels, hepatic glucose levels, glycated haemoglobin levels, glucose-6-phosphate dehydrogenase (G6PD), and glucose-6-

phosphatase (G6P) activities, and haematological parameters using standard procedures after 14 days of treatment.

Haematological analysis

The blood samples obtained from the test groups were analysed within 24 h for haematological composition using BC-3200 Auto Hematology Analyzer. As previously described, the number of red blood cells (RBC) and white blood cells (WBC) was counted.[26] Haemoglobin (Hb) concentration was determined using the cyanmethemoglobin method.[27] Haematological indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the determined values of PCV, RBC, and Hb as described by standard procedure.[28]

Determination of plasma insulin level

Plasma insulin was determined by an enzyme-linked immunosorbent assay (ELISA) kit.[29]

Determination of activities of glucose-6-phosphatase and glucose-6-phosphate dehydrogenase

Glucose-6-phosphatase was assayed according to the method of Hikaru and Toshitsugu [30]. The activity of glucose-6-phosphate dehydrogenase in erythrocytic hemolysates was measured using a colorimetric method.[31]

Statistical analyses

The results obtained were statistically analysed using one-way ANOVA and Duncan's Multiple Range Test using SPSS (Version 20). Differences were considered statistically significant at $P \leq 0.05$. Data were presented as mean ± standard error of the mean (SEM) of five replicates except otherwise stated.

Results and Discussion

The non-diabetic and diabetic rats treated with 250 mg/kg body weight diethyl ether leaf extract of *D. oliveri*, had a significant increase ($P \leq 0.05$) in their weight throughout the experiment. Furthermore, the rats that received metformin (reference drug), and 250 mg/kg body weight ethyl acetate leaf extract of *D. oliveri*, had their weight significantly increased ($P \leq 0.05$) till day 10, while their weights subsequently decreased on day 14 to the weight at day 1 for the two groups. However, exposure of the rats to 250 mg/kg body weight of n-hexane and ethanol leaf extract of *D. oliveri* did not significantly alter ($P \leq 0.05$) their body weight during the experiment (Table 1). Bodyweight loss represents one of the most common signs of diabetes. Furthermore, despite the increased appetite, insulin deficiency reduces all anabolic processes and accelerates catabolic processes, contributing further to bodyweight loss, which is already occurring by glycosuria and polyuria.[30] Streptozotocin-induced diabetes causes weight loss, which is most likely due to muscle wasting, atrophy, and protein loss.[32] The hydrolysis of proteins and lipid reserves in muscles prevents these tissues from metabolising blood glucose for energy. This study showed this status since untreated diabetic animals showed body weight loss, while treatment with the different solvent extracts and metformin improved animals' bodyweight till the 10th day. However, on the last 14th day of treatment with *Daniellia oliveri* leaf extracts and metformin, diabetic animals experienced a loss of body weight, possibly due to factors worth investigating. As expected, streptozotocin caused a spike in the experimental animals' blood glucose levels, which was not significantly reduced ($P \leq 0.05$) upon administering normal saline for 14 days. However, treatment with metformin and all solvent extracts of *D. oliveri* leaf resulted in a significant decrease ($P \leq 0.05$) in FBG level with an increasing day of administration. Additionally, metformin and 250 mg/kg body weight ethanol leaf extract of *D. oliveri* showed the highest reduction (44.55% and 46.21%, respectively) in fasting blood glucose concentration at the end of the 14th-day treatment. In contrast, 250 mg/kg body weight diethyl ether and ethyl acetate leaf extracts of *D. oliveri* showed the least fasting blood glucose concentration reducing capacity (27.80% and 22.69%, respectively) (Table 2). The demonstrated effectiveness of the organic extracts of *D. oliveri*, especially the ethanol extract, is noteworthy. The reported phytochemicals such as alkaloids, phenols and flavonoids,[33] among others, may be responsible for the activity displayed, especially the ethanol extract.

The effects of various solvent extracts of *D. oliveri* leaf on diabetic rats' oral glucose tolerance are presented (Table 3). Administration of 250 mg/kg body weight ethanol and diethyl ether leaf extracts of *D. oliveri*, significantly increased ($P \leq 0.05$) glucose metabolic rate of rats after glucose overload with glucose reduction rates of 51.71% and 40.00%, respectively, when compared to the group with diabetic rats treated with normal saline (Table 3). However, glucose metabolism was relatively low in rats treated with 250 mg/kg body weight n-hexane leaf extracts of *D. oliveri* compared with diabetic rats treated with normal saline. The glucose metabolic/utilisation rate of rats that received metformin and 250 mg/kg body weight ethyl acetate leaf extract of *D. oliveri* leaf is not significantly different compared to diabetic rats treated with normal saline. No treatment group (i.e., metformin and 250 mg/kg body weight solvent extracts of *D. oliveri*) showed glucose-metabolising capacity compared with rats in non-diabetic, normal saline-administered rats (control).

The activity of glucose-6-phosphate dehydrogenase (G6PD), which was initially raised in diabetic rats, was significantly reduced ($P \leq 0.05$) upon administration of the reference drug (metformin) and 250 mg/kg body weight of various solvent extracts of *D. oliveri* leaf (Table 4). The reduction was obvious in the group treated with 250 mg/kg body weight ethanol extracts of *D. oliveri* leaf. All solvent extracts significantly increased the hepatic activity of glucose-6-phosphatase.

Furthermore, a reduced level of hepatic glucose in diabetic rats was not significantly altered ($P \leq 0.05$) by the reference drug (metformin) and all solvent extracts of *D. oliveri* leaf. The blood level of conjugated haemoglobin (HBA1c) was raised in diabetic rats that received normal saline. Glycosylated haemoglobin (HbA1c) is one of the reliable parameters for the prediction of diabetes. Persistent or prolonged increase in blood glucose leads to nonenzymatic adduction of glucose to the free amino groups at the N-terminal of the beta chain of haemoglobin, thereby forming glycosylated haemoglobin.[34] Hb1Ac is a measurement that reflects both fasting and postprandial glucose concentrations over three months.[35] In our study, the raised level of HBA1c was significantly reduced ($P \leq 0.05$) by metformin and all solvent extracts of *D. oliveri* leaf in a manner that compared with rats in the control group. Reductions in the levels of HbA1c by the treated groups indicate the hypoglycaemic effects of the extracts. The concentration of insulin in rats was significantly ($P \leq 0.05$) altered by the metformin and 250 mg/kg body weight of all solvent extracts of *D. oliveri* leaf when compared with the diabetic and non-diabetic rats that received normal saline (Table 4). Also, levels of glucose-6-phosphate (G6P), which was not significantly altered ($P \leq 0.05$) in non-diabetic rats, were significantly increased ($P \leq 0.05$) by all solvent extracts of *D. oliveri* leaf (Table 4).

The primary enzyme essential in the pentose phosphate pathway is Glucose-6-phosphate dehydrogenase (G6PD), which produces 1mol of NADPH, which is crucial for removing ROS prevention of cell damage.[36, 37] Reactive Oxygen Species (ROS) is stimulated by high glucose levels, damaging the nucleus by linking with DNA and RNA. By controlling the glucose level in the blood, the number of ROS in cells is decreased.[38] The superoxide anion level is enhanced by a high glucose level, leading to increased G6PD and NADPH oxidase activities.[39] Therefore, the increase in activity of G6PD in diabetic rats in the present study is expected.

However, administration of various solvent extracts of *D. oliveri* leaf and metformin resulted in a decrease in G6PD activity, implying that the plant may mitigate cellular damage caused by free radicals and reactive oxygen species produced in diabetes patients. Glucose-6-phosphatase is a liver enzyme that catalyzes the final stage of glycogenolysis and gluconeogenesis, which involves the hydrolysis of glucose-6-phosphate to glucose and phosphate.[40] The metabolism of carbohydrates, fats, and protein is regulated by insulin, a natural peptide hormone produced by the β-cell of the pancreas. It promotes glucose absorption from the blood into the liver, fat, and skeletal muscle cells and promotes its storage as glycogen.[41] Thus, both insulin

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deficiency and resistance are responsible for the pathogenesis of DM. However, insulin resistance is dependent on several factors. Therefore, increasing and maintaining insulin levels within the normal physiological range for antidiabetic treatment is very important.[42] The activity of enzymes such as glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase is inhibited by insulin which all contribute to gluconeogenesis.[43] The increase in activity of these enzymes after administering the extracts could be due to a decrease in insulin secretion, which is required to activate key gluconeogenesis enzymes. Therefore, our results suggest that the plant extracts work as normoglycaemic agents not through insulinogenic activity (insulin response) since all treatments reduced insulin levels, but maybe other mechanisms, especially by altering the activities of rate-limiting enzymes of biochemical pathways of carbohydrate metabolism.

In diabetic rats, the PCV, Hb, RBC, and WBC were significantly reduced ($P \leq 0.05$). They were, however, significantly higher ($P \leq 0.05$) in the groups treated with metformin and all solvent extracts of *D. oliveri* leaves compared to the control group (Table 5). The harmful effects of foreign substances, even plants, can be assessed with haematological indicators in the blood.[44] Therefore, PCV and RBC are essential tools to ascertain the extent of anaemia in blood cells. In diabetes mellitus condition, the increase in nonenzymatic glycosylation of proteins of the erythrocyte membrane causes anaemia.[45] The oxidation of these proteins, combined with elevated blood sugar levels in diabetes mellitus, increases the production of lipid peroxides, which results in RBC haemolysis.[46] In this study, a reduction in RBC level in diabetic rats may result from the anaemic influence of the administered STZ. However, a gradual increase in RBC upon treatment with various extracts may suggest improving haemolysis and glycosylation of RBC. The bone marrow produces blood cells such as RBC, WBC, and platelets.[47, 48] The Hb, RBC, and PCV are related to the total population of red blood cells in the blood.

Table 1: Effects of solvent extracts of *Daniellia oliveri* leaf on the bodyweight of streptozotocin-nicotinamide-induced diabetic rats

	Fasting Blood Glucose (mg/dL)					% FBG
Groups	**Day 1**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Reduction**
Diabetic + Normal saline	260.67±53.83^{a}	220.33±45.70^{a}	171.33±39.98^{a}	284.33±37.67^{a}	289.00±30.80^{a}	NR*
Diabetic + 10 mg/kg bwt Metformin	330.33±41.17^{a}	336.33±36.31^{a}	225.00±69.97^{a}	280.00±72.00^{a}	147.00±55.90^{b}	44.55^{a}
Diabetic + 250mg/kg bwt N-hexane	283.33±47.42^{a}	282.00±15.72^{a}	62.00±2.65^{b}	88.33±10.91^{b}	93.33±13.74^{b}	32.86^{b}
Diabetic + 250mg/kg bwt Ethyl Acetate	299.00±29.67^{a}	197.00±53.00^{b}	137.00±47.17^{b}	197.00±82.93^{b}	83.00±12.90^{c}	27.80^{c}
Diabetic +250mg/kg bwt Ethanol	277.00±29.72^{a}	320.00±21.36^{a}	172.00±55.06^{b}	128.33±28.11^{c}	128.67±44.80^{c}	46.21^{a}
Diabetic + 250mg/kg bwt Diethyl Ether	335.00±9.02^{a}	158.00±12.17^{b}	165.00±6.57^{b}	145.67±7.21^{b}	76.67±7.84^{c}	22.69^{c}

Values are mean of five replicates ± Standard Error of Mean (SEM)

Table 2: Effects of solvent extracts of *Daniellia oliveri* leaf on fasting blood glucose level of streptozotocin-nicotinamide-induced diabetic rats

	Weight of the Animals (g)				
Groups	**Day 1**	**Day 4**	**Day 7**	**Day 10**	**Day 14**
Diabetic + Normal saline	127.67±2.73^{a}	138.00±4.93^{b}	149.00±9.61^{b}	147.67±6.77^{b}	137.33±8.82^{b}
Diabetic + 10 mg/kg bwt Metformin	123.67±4.91^{a}	142.67±3.53^{b}	145.33±2.40^{b}	144.33±3.38^{b}	134.33±5.81^{a}
Diabetic + 250 mg/kg bwt N-hexane	136.00±7.21^{a}	152.00±9.02^{b}	146.00±8.19^{a}	145.00±9.02^{a}	136.67±9.03^{a}
Diabetic + 250 mg/kg bwt Ethyl Acetate	114.33±5.49^{a}	137.00±6.08^{b}	131.67±10.6^{b}	134.00±6.51^{b}	121.00±4.16^{a}
Diabetic + 250 mg/kg bwt Ethanol	134.00±4.58^{a}	149.00±3.06^{a}	152.00±5.03^{a}	140.33±2.73^{c}	137.00±3.22^{a}
Diabetic + 250 mg/kg bwt Diethyl Ether	127.67±1.20^{a}	151.67±0.67^{b}	156.00±5.86^{b}	151.33±3.93^{b}	149.33±4.81^{b}

NR = No reduction
Values are mean of five replicates ± Standard Error of Mean (SEM)
Values with the same superscript across the row are not significantly different ($P \leq 0.05$)

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However, the reduction in Hb, RBC, and PCV implies that the Streptozotocin administration decreased the red blood cells produced from the bone marrow. However, an increase in Hb, RBC, and PCV upon administering extracts in this study suggests that the extracts may not impaired red blood cell production in the bone marrow.[49]

MCV, MCH, and MCHC levels related to individual red blood cells were not significantly altered (P≤0.05) in animals treated with metformin and all extracts of *D. oliveri* leaf, compared with non-diabetic and diabetic non-treated groups (Table 5). The extracts of *D. oliveri* and metformin may not influence the oxygen-carrying capacity of red blood cells in the blood because MCH, MCHC, and MCV were not affected.[50] White blood cells and differentials are the immune-related components of the blood. Factors such as granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and the interleukins IL-2, IL-4, and IL-5 regulate the proliferation, differentiation, and maturation of committed stem cells for white blood cell production.[47, 51] An increase in the WBC concentrations, neutrophils, and lymphocytes following extract administration could indicate that some extract components increased the production of these regulatory factors or increased the sensitivity of committed stem cells (responsible for producing white blood cells) to these factors.

Furthermore, blood levels of platelets were significantly raised (P≤0.05) in normal saline-, n-hexane- and ethanol-treated diabetic rats compared to the non-diabetic control animals. Conversely, blood levels of platelets were significantly reduced (P≤0.05) in rats upon administration of metformin and 250 mg/kg body weight ethyl acetate extract of *D. oliveri* leaf, compared to the control (Table 5). The smallest blood component capable of thrombosis and hemostasis is platelets.[52] In addition, they initiate the repair of blood vessel walls and are considered acute phase reactants to infection or inflammation.[47] An increase in the level of platelets by the solvent extracts may suggest the presence of phytochemicals that could boost bone marrow production of platelets, thus enhancing wound healing and prevent bleeding.

Table 3: Effects of solvent extracts of *Daniellia oliveri* leaf on oral glucose tolerance status of streptozotocin-nicotinamide-induced diabetic rats

Treatment	Fasting Blood Glucose (mg/dL)	Glucose level at 30min (mg/dL)	Glucose level at 120min (mg/dL)	% reduction of blood glucose level
Non-diabetic + Normal saline	93.33±7.88^{a}	180.00±3.82^{b}	114.00±7.21^{a}	63.33
Diabetic + Normal saline	173.00±11.00^{a}	214.67±6.33	78.33±0.33^{a}	36.44
Diabetic + 10 mg/kg bwt Metformin	76.67±6.25^{a}	209.33±7.37	67.33±2.85^{a}	32.06
Diabetic + 250 mg/kg bwt N-hexane	87.00±8.15^{a}	256.67±7.30^{b}	61.33±10.35^{a}	23.83
Diabetic + 250 mg/kg bwt Ethyl Acetate	72.00±9.00^{a}	281.33±8.91^{b}	98.67±8.66^{a}	34.88
Diabetic + 250 mg/kg bwt Ethanol	82.00±4.00^{a}	350.67±8.20^{b}	181.67±4.92^{c}	51.71
Diabetic + 250 mg/kg bwt Diethyl Ether	76.67±7.84^{a}	420.00±6.46^{b}	168.00±4.00^{c}	40.00

Values are mean of five replicates ± Standard Error of Mean (SEM)
Values with the same superscript across the row are not significantly different (P≤0.05); bwt: body weight

Table 4: Effects of solvent extracts of *Daniellia oliveri* leaf on selected carbohydrate-metabolizing enzymes and other diabetic parameters in streptozotocin-nicotinamide-induced diabetic rats

Groups	G6PD (g/dL)	Hepatic Glucose (g/dL)	HBA_{1C} (%)	Insulin (µlU/mL)	G6P (g/dL)
Non-diabetic + Normal saline	16.82±2.82^{a}	3.09±0.22^{a}	13.69±0.39^{a}	6.33±0.17^{a}	7.40±0.25^{a}
Diabetic + Normal saline	32.16±0.37^{b}	2.79±0.05^{b}	18.09±0.56^{b}	6.32±0.17^{a}	7.68±0.47^{a}
Diabetic + 10 mg/kg bwt Metformin	8.97±1.30^{c}	2.77±0.11^{b}	15.36±1.18^{a}	7.81±2.86^{a}	7.61±0.18^{a}
Diabetic + 250 mg/kg bwt N-hexane	11.22±1.71^{d}	2.85±0.13^{b}	13.80±0.12^{a}	7.37±1.11^{a}	8.43±0.25^{b}
Diabetic + 250 mg/kg bwt Ethyl Acetate	11.59±0.74^{d}	2.62±0.26^{b}	13.92±0.39^{a}	5.64±0.15^{a}	7.90±0.19^{b}
Diabetic + 250 mg/kg bwt Ethanol	5.98±0.99^{e}	2.72±0.23^{b}	14.73±0.63^{a}	5.95±0.40^{a}	8.16±0.30^{b}
Diabetic + 250 mg/kg bwt Diethyl Ether	8.23±0.37^{c}	2.62±0.20^{b}	14.44±0.36^{a}	5.32±0.16^{a}	8.16±0.24^{b}

G6P: Glucose-6-phosphate; G6PD: Glucose-6-phosphate Dehydrogenase; HBA1c: Glycated Haemoglobin; Values are mean of five replicates ± Standard Error of Mean (SEM). Values with the same superscript down the group are not significantly different (P≤0.05); bwt: body weight.

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Table 5: Haematological status of streptozotocin-nicotinamide-induced diabetic rats after 14-day oral administration of solvent extracts of *Daniellia oliveri* leaf

Groups	PCV (%)	HB (g/dL)	RBC ($\times10^6$µL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^3$µL)	WBC ($\times10^3$µL)	NEUT (%)	LYMP (%)
Non-diabetic + Normal saline	45.00±0.00^{a}	14.33±0.00^{a}	6.72±0.82^{a}	65.67±0.67^{a}	20.67±0.33^{a}	31.67±0.33^{a}	751.33±34.33^{a}	10.83±1.03^{a}	11.00±1.00^{a}	88.00±1.00^{a}
Diabetic + Normal saline	24.33±0.33^{b}	7.70±0.06^{b}	3.64±0.20^{b}	67.00±3.06^{a}	21.33±0.33^{a}	29.67±0.33^{a}	790.00±20.82^{b}	6.60±0.79^{b}	12.33±2.96^{a}	84.00±4.36^{a}
Diabetic + Metformin	35.33±7.22^{c}	11.47±2.38^{c}	5.53±1.13^{c}	63.67±0.33^{a}	20.00±0.57^{a}	29.67±0.33^{a}	592.00±32.01^{c}	11.67±0.84^{a}	16.67±2.23^{b}	63.67±14.17^{b}
Diabetic + 250 mg/kg bwt N-hexane	35.67±2.33^{c}	11.47±0.81^{c}	5.69±0.49^{c}	64.00±0.58^{a}	19.67±1.20^{a}	29.67±0.88^{a}	804.67±55.10^{b}	11.27±1.99^{a}	16.67±0.88^{b}	71.33±21.17^{c}
Diabetic + 250 mg/kg bwt Ethyl Acetate	38.00±2.65^{cd}	12.37±0.74^{cd}	5.98±0.42^{cd}	64.67±0.88^{a}	20.67±0.67^{a}	29.33±0.88^{a}	641.00±62.08^{c}	12.10±1.15^{c}	16.33±1.57^{b}	53.67±12.73^{d}
Diabetic + 250 mg/kg bwt Ethanol	40.67±2.96^{d}	12.83±0.75^{cd}	6.22±0.38^{d}	63.00±0.58^{a}	19.67±1.20^{a}	30.67±0.67^{a}	845.00±29.67^{d}	7.43±1.53^{b}	16.67±2.60^{b}	82.67±3.18^{a}
Diabetic + 250 mg/kg bwt Diethyl Ether	40.67±2.33^{d}	12.90±0.75^{d}	6.57±0.38^{ad}	65.00±0.57^{a}	21.00±0.57^{a}	31.67±0.33^{a}	764.33±18.48^{a}	9.53±2.16^{a}	24.67±4.81^{c}	71.33±5.93^{c}

Values are mean of five replicates ± Standard Error of Mean (SEM). Values with the same superscript down the group are not significantly different ($P\leq0.05$); bwt: body weight. CV: Packed Cell Volume; HB: Haemoglobiin; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; PLT: Platelet; WBC: White Blood Cell; NEUT: Neutrophil; LYMP: Lymphocyte.

Conclusion

In this study, *D. oliveri* leaves, when extracted with different organic solvents, have antidiabetic potential, with ethanol extract giving outstanding performance and may serve as a plant source for managing complications relating to diabetes. It may also serve as a good source for boosting some haematological parameters, especially red blood cells and white blood cells. However, further study is needed to understand the toxicological implication of using *D. oliveri* leaves as an antidiabetic agent. Also, the bioactive principle responsible for the acclaimed activity of the ethanol extract should be isolated and characterised.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Ogurtsova K, da Rocha Fernandes J, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw J, Makaroff L. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017; 128:40-50.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013; 36(1):67-74.
3. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018; 138:271-281.
4. International Diabetes Federation. IDF Diabetes Atlas. IDF, 2019.
5. Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants used for diabetes mellitus. J Acute Dis. 2013; 2(3):196-200.
6. Maraschin JdF. Classification of diabetes. In: Ahmad SI, editor. Diabetes. Adv Exp Med Biol. 771. New York, NY: Springer; 2013. 12-9 p.
7. Lee Y, Berglund ED, Wang M-y, Fu X, Yu X, Charron MJ, Burgess SC, Unger RH. Metabolic manifestations of insulin deficiency do not occur without glucagon action. Proc Natl Acad Sci. 2012; 109(37):14972-14976.
8. Zaid H, Antonescu CN, Randhawa VK, Klip A. Insulin action on glucose transporters through molecular switches, tracks and tethers. Biochem J. 2008; 413(2):201-215.
9. Lemieux I. Reversing Type 2 Diabetes: The Time for Lifestyle Medicine Has Come! Nutr. 2020;12:1974.
10. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, del Cañizo-Gómez FJ. Update on the treatment of type 2 diabetes mellitus. World J Diabetes. 2016;7(17):354.
11. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020; 83(3):770-803.
12. Salehi B, Ata A, Kumar NVA, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, Ayatollahi SA, Fokou PVT, Kobarfard F, Zakaria ZA, Iriti M, Taheri Y, Martorell M, Sureda A, Setzer WN, Durazzo A, Lucarini M, Santini A, Capasso R, Ostrander EA, Atta-ur-Rahman, Choudhary MI, Cho WC, Sharifi-Rad J. Antidiabetic Potential of Medicinal Plants and Their Active Components. Biomol. 2019; 9(10):551.
13. Wasana KGP, Attanayake AP, Weerarathna TP, Jayatilaka KAPW. Efficacy and safety of a herbal drug of *Coccinia grandis* (Linn.) Voigt in patients with type 2 diabetes mellitus: A double blind randomized placebo controlled clinical trial. Phytomed. 2021; 81:153431.
14. De la Estrella M, Aedo C, Mackinder B, Velayos M. Taxonomic Revision of *Daniellia* (Leguminosae: Caesalpinioideae). Syst Bot. 2010; 35(2):296-324.
15. Bhat R, Etejere E, Oladipo V. Ethnobotanical studies from central Nigeria. Econ Bot. 1990; 44(3):382-390.
16. Iwueke AV and Nwodo OFC. Antihyperglycaemic effect of aqueous extract of *Daniella oliveri* and *Sarcocephalus latifolius* roots on key carbohydrate metabolic enzymes and glycogen in experimental diabetes. Biokemistri. 2008;20(2):63-70.
17. Atolani O, Olatunji GA. Chemical composition, antioxidant and cytotoxicity potential of *Daniellia oliveri* (Rolfe) Hutch. & Dalz. Turk J Pharm Sci. 2016; 13(1):41-46.
18. Nwaeze C and Abarikwu P. Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria. Nig J Microbiol. 2006; 6(12):32-40.
19. Beppe GJ, Kenko Djoumessie LB, Keugong Wado E, Ngatanko Abaïssou HH, Nkwingwa BK, Damo Kamda JL, Nhouma RR, Foyet HS. Aqueous Root Bark Extract of *Daniellia oliveri* (Hutch. & Dalz.)(Fabaceae) Protects Neurons against Diazepam-Induced Amnesia in Mice. Evid-Based Compl Altern Med. 2020; 2020:1-9.
20. Shauibu G, Abu AH, Agwu EO, Ehile V, Ezeudo ZE, Ogedemgbe B, Peter S. Effects of *Daniella Oliveri* Aqueous Leave Extract on Blood Glucose, Haematological and Biochemical Values in Streptozotocin-Induced Type 2 Diabetes in Male Albino Rats. Vom J Vet Sci. 2018;13(1):81-87.
21. Farswan M, Mazumder PM, Percha V. Protective effect of *Cassia glauca* Linn. on the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats. Indian J Pharmacol. 2009; 41(1):19-22.
22. Saidu A, Mann A, Onuegbu C. Phytochemical screening and hypoglycemic effect of aqueous *Blighia sapida* root bark extract on normoglycemic albino rats. Br J Pharm Res. 2012; 2(2):89-97.
23. Masiello P. Animal models of type 2 diabetes with reduced pancreatic β-cell mass. Int J Biochem Cell Biol. 2006; 38(5-6):873-893.
24. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, Vorstenbosch CVD. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001; 21(1):15-23.
25. Jalil AMM, Ismail A, Pei CP, Hamid M, Kamaruddin SHS. Effects of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese-diabetic (Ob-db) rats. J Agric Food Chem. 2008; 56(17):7877-7884.
26. Brown BA. Hematology: Principles and Procedures. 2nd ed. Philadelphia, USA: Lea & Febiger; 1976. 56-58 p.
27. Hewitt SG. Manual for Veterinary Investigation, hematology and Laboratory techniques. 3rd ed. Bulletin of Ministry of Agric. Fishery, Food and Hematology. 1984. 72-100 p.
28. Schalm OW, Jain NC, Carroll EJ. Veterinary hematology: Lea & Febiger.; 1975. 807 p.
29. Webster HV, Bone AJ, Webster KA, Wilkin TJ. Comparison of an enzyme-linked immunosorbent assay (ELISA) with a radioimmunoassay (RIA) for the measurement of rat insulin. J Immunol Meth. 1990;134(1):95-100.

30. Hikaru K and Toshitsugu O. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. Clin Chim Acta. 1959; 4(4):554-561.
31. Ells HA and Kirkman H. A colorimetric method for assay of erythrocytic glucose-6-phosphate dehydrogenase. Proc Exp Biol Med. 1961;106(3):607-609.
32. Gupta R and Sharma AK. Anti-hyperglycemic activity of aqueous extracts of some medicinal plants on wistar rats. J Diabetes Metab. 2017;8(752):000752.
33. Osuntokun OT, Fasusi OA, Ogunmodede AF, Thonda AO, Oladejo BO, Yusuf-Babatunde AM, Ige OO. Phytochemical Composition and Antimicrobial Activity of *Daniella oliveri* extracts on selected clinical microorganisms. Int J Biochem Res Rev. 2016;14(1):1-13.
34. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. Is haemoglobin A1c a step forward for diagnosing diabetes? BMJ. 2009; 339.
35. Sherifali D, Nerenberg K, Pullenayegum E, Cheng JE, Gerstein HC. The effect of oral antidiabetic agents on A1C levels: a systematic review and meta-analysis. Diab Care. 2010;33(8):1859-1864.
36. Ho H-Y, Cheng M-l, Chiu DT-Y. Glucose-6-phosphate dehydrogenase–from oxidative stress to cellular functions and degenerative diseases. Redox Rep. 2007;12(3):109-118.
37. Scott MD, Zuo L, Lubin BH, Chiu DT-Y. NADPH, not glutathione, status modulates oxidant sensitivity in normal and glucose-6-phosphate dehydrogenase-deficient erythrocytes. Blood. 1991;77(9):2059-2064.
38. Wright Jr E, Scism-Bacon J, Glass L. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int J Clin Pract. 2006; 60(3):308-314.
39. Halliwell B. Role of free radicals in the neurodegenerative diseases. Drugs Aging. 2001;18(9):685-716.
40. Arathi G and Sachdanandam P. Therapeutic effect of *Semecarpus anacardium* Linn. nut milk extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in mammary carcinoma rats. J Pharm Pharmacol. 2003; 55(9):1283-1290.
41. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005; 26(2):19.
42. Ali MD, Paul S, Tanvir EM, Hossen MD, Rumpa N-EN, Saha M, Bhoumik NC, Islam A, Hossain MD, Alam N. Antihyperglycemic, antidiabetic, and antioxidant effects of *Garcinia pedunculata* in rats. Evid-Based Compl Altern Med. 2017; 2017:1-15.
43. Rajagopal K and Sasikala K. Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. Singapore Med J. 2008; 49(2):137-141.
44. Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ. Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. Afr J Biotechnol. 2009;8(8):1425-1430.
45. Oyedemi S, Yakubu M, Afolayan A. Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. J Med Plant Res. 2011; 5(1):119-125.
46. Arun GS and Ramesh KG. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin-diabetic rats. Indian J Pharmacol. 2002; 34(3):156-164.
47. Ganong WF. Review of medical physiology. 20th ed. New York: Lange Medical Books/ McGraw Hill Companies Inc.; 1995. 500-515 p.
48. Grossmann A, Lenox J, Ren HP, Humes J, Forstrom J, Kaushansky K, Sprugel K. Thrombopoietin accelerates platelet, red blood cell, and neutrophil recovery in myelosuppressed mice. Exp Hematol. 1996; 24(10):1238-1246.
49. Cheeke PR. Natural toxicants in feeds, forages, and poisonous plants. Danville: Interstate Publishers, Inc., PO Box 50.; 1998.
50. Malomo S, Adebayo J, Olorunniji F. Modulatory effect of vitamin E on some haematological parameters in dihydroartemisinin-treated rats. Trop J Health Sci. 2002; 9:15-20.
51. Guyton AC and Hall JE. Textbook of Medical physiology. 11th ed. Philadelphia: W.B. Saunders Company; 2006. 382-401 p.
52. Periayah MH, Halim AS, Saad AZM. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11(4):319-327.