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Antidiabetic and Thrombolytic Activities of Some Selected Medicinal Plants in High Fat Diet and Dexamethasone-Induced Type 2 Diabetic Rats

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ABSTRACT

Background: Type-2 diabetes (T2D) is a hypercoagulable and hypofibrinolytic condition that predisposes to cardiovascular and thrombotic complications. We screened three medicinal plants (*Albizzia chevalieri, Newbouldia laevis and Leptadenia hastate)* for their antidiabetic and thrombolytic activities.

Methods: T2D was induced with high fat diet and dexamethasone. Following induction, rats were grouped into 6 (n=8 rats); control, untreated, treated (500mg/kg body weight (BW) metformin only) or treated (300mg/kg BW leaf extracts). The rats were treated for two weeks and euthanized. About 2ml of the collected blood was used for thrombolytic activity assay while the rest was processed, and the recovered serum utilised for biochemical and hormonal assays.

Results: Rats treated with extracts had significantly (p<0.05) lowered concentrations of serum glucose, TG, LDL-cholesterol, VLDL-cholesterol and increased HDL-cholesterol compared to untreated rats. Extracts also lowered (p<0.05) the serum insulin concentration on day 14 compared to untreated rats. *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* showed 48.90%, 39.20% and 37.69% clot lysis activities respectively which was significantly higher (p<0.001) than distilled water, while streptokinase produced a substantial clot lysis of 93.70%.

Conclusions: The leaf extracts of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* have antidiabetic and thrombolytic activities in type-2 diabetic rats and thus, can be potentially used as combined antidiabetic and thrombolytic agents with *in vivo* effects in type-2 diabetic patients.

1. Introduction

There is currently an epidemic of diabetes mellitus across the globe (Sun et al., 2022). The statistics have multiplied several fold over the past few decades and are projected to increase further. Data from the International Diabetes Federation (IDF) showed a global prevalence of 10.5%, representing about 537 million adults with diabetes and an alarming projection that this figure would rise to about 643 million and 783 million by 2030 and 2045 respectively (IDF, 2021). The figures from the World Health Organization (WHO) also portray a gloomy picture. From 108 million people with diabetes in 1980 to about 422 million people in 2014, with the increase in prevalence being more pronounced in the developing compared to the developed nations (World Health Organisation, 2016).

Diabetes mellitus (DM), a chronic endocrine disease results from either a deficiency of, or a lack of effective insulin action (Glovaci et al., 2019). Because the body cells require insulin for the uptake and utilization of glucose, its deficiency results in persistently elevated levels of glucose in the blood (Cole and Florez, 2020). These high levels of blood glucose eventually lead to adverse consequences on vital organs such as the eyes, kidneys, heart and brain (Tomic et al., 2022). Depending on its aetiology, DM is classified into either type 1 (Insulin-dependent) or T2DM (Kononenko et al., 2020). T1DM results from the auto-immune destruction of the β cells of the pancreas consequently leading to the absence of insulin secretion (Syed, 2022), while in T2DM insulin secretion may be normal but there is a decreased sensitivity to its action. T2DM is more prevalent, accounting for more than 80% of diabetic patients (Ong et al., 2023). It is closely associated with environmental factors and obesity, sedentary lifestyle and diets rich in carbohydrates and fats.

The sustained elevated levels of glucose, damage the vascular endothelium and alter several proteins thereby predisposing to thrombotic tendencies, making DM a hypercoagulable condition (Vaidya et al., 2021). Consequently, DM patients are frequently treated with thrombolytic agents (Siasos et al., 2020) in addition to the usual treatment with lifestyle modification, oral hypoglycaemic agents and insulin. But the cost of this polypharmacy is prohibitive and constitutes a heavy financial burden especially in poor communities. Orthodox medications are also associated with side effects. These necessitate the search for agents, such as natural products, with medicinal value and activity against T2DM and its complications.

Natural products are identified to be safe, more affordable and widely available (Rahaman et al., 2023). The WHO advocates for the use of natural products in the management of ailments (Süntar, 2020). Several plants have been shown to have antidiabetic properties, among them the trio of *Leptadenia hastata*, *Newbouldia laevis* and *Albizzia chevalieri* which were previously reported to have antidiabetic action in type 1 diabetic rodent models (Saidu et al., 2010; Bello et al., 2011; Kabir et al., 2021; Mbagwu et al., 2021).

Thus, we aimed to determine whether the methanolic extracts of these plants would have an antidiabetic and thrombolytic effect in a rat model of high fat diet and dexamethasone induced T2DM. This will help in the formulation of a safe, affordable combined medication for T2DM and thrombolytic agent.

2. Materials and methods

2.1. Ethical clearance

The protocols used in this study were approved by the Central University Animal Research Ethics Committee of Usmanu Danfodiyo University Sokoto (UDUS), Nigeria (UDUS/AEC/2020/0321) and conducted in line with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The ARRIVE guidelines were also used in the conduct and reporting of this study.

2.2. Plants collection

Samples were collected from plants grown on farms in Sokoto metropolis, Sokoto state, North-western Nigeria (13.0059^{o}N, 5.2476^{o}E), which falls within the savannah zone with mean annual rainfall ranging between 500mm to 1,300mm. We authenticated samples at the herbarium of the Botany unit, Biological Sciences Department, UDUS and deposited voucher specimens for each plant with voucher numbers as follows: *Leptadenia hastata* (UDUH/ANS/0847),

Newbouldia laevis (UDUH/ANS/0845) and *Albizzia chevalieri* (UDUH/ANS/0846).

2.3. Extracts Preparation

The plants leaves were washed under a running tap, air dried at 27°C for 7days and then ground using laboratory pestle and mortar into fine powder. Five hundred (500g) grams of each plant sample were separately macerated with 2.5L of methanol for 72h. The resulting homogenate was filtered twice, first with a piece of muslin cloth (white, clean and sterile) and then again with Whatman grade 1 filter paper (Sigma Aldrich Germany, Retention of 11µm at 98% efficiency). The filtrates were concentrated using a rotary evaporator device (RE 100, Stone Staffordshire, England) set at 40°C after which a freeze drier (BK-FD 10 series, Jinan, Shandong, China) was used to obtain a constant dry weight of the extracts. The dried extracts were weighed, covered with aluminium foil, labelled, and stored in a fridge at 4°C for use in anti-diabetic test and clot lysis determination. The yield following extraction was determined for each of the extracts (Eneh et al., 2018).

2.4. Experimental Animals

Wistar rats (n=50) of both sexes that weighed between 170-180g and aged between 10-12 weeks were used for this study. The rats housed in steel cages were acquired from the Department of Biological Sciences, UDUS, Nigeria. Prior to experimentation, rats were transferred and allowed to habituate for seven days at the animal house, Department of Biochemistry and Molecular Biology, UDUS. The rats were maintained at room temperature with 12 hr light cycle, adequate ventilation, fed normal rat chow with *ad libitum* access to clean drinking water. According to the manufacturers label (Vital feeds, Jos, Nigeria), diet composition provides 54 % carbohydrates, 13 % fat, 10 % proteins, 20 % fibre, 2 % normal supplement (calcium, phosphorus, ash & moisture) and 1% vitamin.

2.5. Study Design

Like previously reported, T2DM was induced in apparently healthy rats using high fat diet (58% animal fat, 25% protein-soya beans and 17% carbohydrates-maize) for 21 days and daily intraperitoneal injection of dexamethasone (1mg/kg BW) from day 15 to day 21 of the experiment (Martínez et al., 2016; Danboyi et al., 2020). Thereafter, rats were returned to standard feed for the remainder of the experiment. Blood was taken from rats' tail veins via a pin prick using a 25G needle and fasting blood glucose (FBG) levels of all the animals was determined using a calibrated portable glucometer (Accu-Chek®, Germany). FBG > 11.4 mmol/l was indicator of diabetes. Following the successful induction of T2DM, we randomly allocated the rats into six groups, each with at least eight rats.
Group 1: Non-diabetic controls administered distilled water
Group 2: Diabetic control, administered with a high fat diet and dexamethasone only with no treatment
Groups 3 to 6 were the diabetic groups treated as follows:
Group 3: Metformin 500mg/kg body weight
Group 4: *A. Chevalieri* 300mg/kg body weight (Saidu et al., 2010).
Group 5: *N. laevis* 300mg/kg body weight (Osigwe et al., 2017).
Group 6: *L. Hastata* 300mg/kg body weight (Bello et al., 2011).

The treatments were constituted in distilled water and administered once daily via oral gavage for 2 weeks. FBG was measured on days 0, 7 and 14. The weights of the rats were recorded twice weekly to ensure their wellbeing and any impact the treatments may have on their body weights. Following an overnight fast (12 hr), the rats were euthanized using chloroform inhalation. Blood samples were collected through cardiac puncture using a 21G needle. About 2ml of the collected blood sample was transferred into plain blood collecting tubes for clot lysis determination. The remaining blood samples were subsequently transferred into plain tubes and left to stand for approximately 30 minutes and then centrifuged at 3000rpm for 15 minutes. Thereafter, serum was harvested and transferred into labelled micro tubes and stored in a freezer at -20˚C for downstream biochemical analysis.

2.6. Determination of Blood Glucose Concentration

This was determined with a calibrated portable glucometer (Accu-Chek®, Germany) with drops of blood obtained via a prick to the tail vein after sterilization of the area with alcohol impregnated swabs.

2.7. Estimation of lipid profile of the rats

The lipid profile of the experimental rats was determined using Randox lipid kits. The parameters measured were serum total cholesterol (based on the enzymatic method earlier described by Allain et al. (1974)) with the absorbance of this dye was measured spectrophotometrically at 500nm. Serum triglycerides were measured using the method of Tietz (1990). Serum HDL-cholesterol levels were determined using methods described by Burstein et al. (1970) with absorbance was measured spectrophotometrically at 500nm. Serum concentration of LDL-cholesterol and VLDL-cholesterol were derived using the Friedwald formula (Friedewald et al., 1972) as below:

LDL-C (mg/dl) = TC – (HDL-C) - $\frac{TG}{5}$

VLDL-C (mg/dl) = $\frac{TG}{5}$

Where: HDL-C= high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TC= total cholesterol, TG= triacylglyceride and VLDLC = very low-density lipoprotein cholesterol.

The plasma atherogenic index was determined using the formula:

AI= log (TG/HDL-C) (Niroumand et al., 2015).

2.8. Determination of serum concentration of insulin and calculation of HOMA-IR

The serum concentration of insulin was determined using a double sandwich rat insulin enzyme-linked immunosorbent assay (ELISA) Kits (Nanjing Pars Biochem CO., Ltd) according to the instructions of the manufacturer. The result of the fasting insulin concentration was then used together with the FBG to calculate the homeostatic model assessment of insulin resistance (HOMA-IR) as a measure of beta cell function and IR. This was determined using the formula provided by Matthews et al. (1985) as follows:

$$\text{HOMA-IR} = \frac{[\text{Fasting blood glucose (FBG) (mg/dl) x fasting plasma insulin (FPI) (µU/mL)}]}{405}$$

2.9. Thrombolytic assay

The method for examining clot lysis has been previously described by Prasad et al. (2007). Briefly, 200µl of blood was placed into 500µl micro-tubes that were weighed earlier. The tubes were allowed to stand for 45 minutes at 37°C to allow for clot formation. With caution, serum was aspirated out with a pipette leaving the clot intact. Each of the tubes was then weighed again to determine the weight of the clot, and then labelled. From each of the leaf extract powder, 5g was dissolved in 5ml of distilled water and then to each of the labelled tubes, 100 µl of leaf extract was added and then incubated at 37°C in order to observe for clot lysis. After 90 minutes, we removed the fluid obtained and weighed the tubes to calculate the change in weight following the disruption of the clot. The observed changes in the weights of the microtubes, before and after clot lysis, were then expressed as a percentage of clot lysis. Samples were compared to streptokinase (Sigma Aldrich, Missouri, USA; specific activity: ≥3500 unit/ mg solid) and distilled water. Values were analysed from 3 independent experiments. Percentage clot lysis (%) = (Weight of the released clot /Weight of clot) ×100.

2.9. Data Analysis

Datasets were analysed with GraphPad Instat statistical package (version 3.0) and presented as mean ± standard deviation (SD). Changes in body weights and serial blood glucose concentration measurements were analysed by repeated measures analysis of variance (ANOVA). Other datasets were analysed using a one-way ANOVA followed by Bonferroni *post hoc* test. A significance threshold of $p < 0.05$ (95% CI) was adopted.

3. Results

3.1. Yield of the Extracts

Following methanolic extractions, the extracts gave the following yields:

- yield for *Albizzia chevalieri* was 8.51%
- yield for *Leptadenia hastate* was 5.09%
- yield for *Newbouldia laevis* was 8.79%

3.2. Impact of Methanol leaf extracts on body mass changes of type 2 diabetic rats

Table 1 shows the changes in body mass of rats at several time points following the administration of methanol extract of *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastata* and Metformin for two weeks. The untreated diabetic rats showed significantly ($p<0.001$) decreased body weight when compared with normal control rats**.** This weight difference was present at all the time points the rats were weighed. Treatment with methanol extract of *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastata* for two weeks resulted in increased body weight in the first and second weeks of extract/drug administration as compared to diabetic control rats.

Table 1: Effect of treatments on weight across the experimental period.

Treatment (Per Kg BM)	Body mass before dexamethasone injection	Day 0	Day 7	Day 14
Control (distilled water)	173.4±2.73	178.8±1.96	179.4±1.57	183.0±1.64
Diabetic (Untreated)	175.0±1.84	166.6±1.44[a]	160.6±1.08[a]	156.4±1.21[a]
Metformin (500mg)	171.6±2.48	164.2±1.92	167.2±1.72 *	171.4±1.44 **
***A. chevalieri* (300mg)**	170.2±2.58	164.4±1.81	167.4±1.72 *	173.0±1.79 **
***N. laevis* (300mg)**	170.6±2.87	163.8±2.69	167.0±2.63 *	172.2±2.22 **
***L. hastate* (300mg)**	173.0±3.11	166.0±2.78	169.2±2.35 **	173.6±2.02 ***

*= $p<0.05$, **= $p<0.01$, *** = $p<0.001$ compared to diabetic controls, [a] = $P<0.001$ from normal controls. Data analysed using repeated measures ANOVA followed by Bonferroni *post hoc* test and expressed as mean ± Standard deviation (SD), n= 7-8 rats.

3.3. Effect of Methanol Extract of Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata on serial fasting blood glucose levels of type 2 diabetic rats

Table 2 highlights the effect of methanol leaf extracts on serial fasting blood glucose concentrations of high fat diet and dexamethasone induced type-2 diabetic rats. For all treatments, there was a reduction of blood glucose concentration as time progressed. At day zero (0), normal control recorded 5.41 mmol/l, diabetic control was 14.64mmol/l, metformin 14.92mmol/l, *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastate*, 13.87mmol/l, 14.79mmol/l and 14.84mmol/l respectively. On the 7th day, the following values were recorded; normal control (4.57mmol/l), diabetic control (16.48mmol/l), metformin (9.78mmol/l), *Albizzia chevalieri* (9.47mmol/l), *Newbouldia laevis* (10.71mmol/l) and *Leptadenia hastata* (11.04mmol/l). By day 14, relatively stable levels were observed in the normal control (6.95mmol/l) but a progressive decrease was recorded in all treated groups compared to the high resting values of diabetic controls (13.97mmol/l). However, the blood glucose concentration of the rats determined after 14 days of treatment with metformin, *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastata* were within the same range (7.1 - 8.4 mmol/l).

3.4. Effect of treatment with leaf extracts on the fasting blood glucose concentration, insulin concentration and HOMA-IR of diabetic rats

The results of the FBG, insulin levels and computed HOMA-IR of type-2 diabetic rats treated with methanol extract of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* are presented in Table 3. The diabetic untreated rats had significantly higher FBG ($P<0.05$), insulin concentration ($P<0.05$) and HOMA-IR ($p<0.05$) when compared to the controls. However, the administration of methanol extracts of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* and metformin significantly reduced the FBG ($P<0.05$), insulin concentration ($P<0.05$) and HOMA-IR ($p<0.05$) compared to the diabetic untreated group.

Table 2. Fasting Blood Glucose Concentration (mmol/l) at Time Interval.

Treatment (Per Kg BW)	**Day 0**	**Day 7**	**Day 14**
Control (distilled water)	5.1±0.31	4.5±1.57	6.6±0.35
Diabetic (Untreated)	13.2±1.44[a]	15.44±1.08[b]	13.9±0.24[a]
Metformin (500mg)	14.2±0.92 [a]	8.78±1.72**	7.3±-0.18***
***A. chevalieri* (300mg)**	12.3±1.81 [a]	9.27±0.20*	7.1±0.26***
***N. laevis* (300mg)**	13.7±1.69 [a]	9.71±1.63*	8.4±0.18**
***L. hastate* (300mg)**	14.8±0.40 [a]	10.73±0.34*	8.2±0.32**

*= p<0.05, **= p<0.01, *** = p<0.001 compared to diabetic controls, [a] = P<0.001 from normal controls. Data analysed using repeated measures ANOVA followed by Bonferroni *post hoc* test and expressed as mean ± Standard deviation (SD), n= 7-8 rats.

Table 3: Fasting Blood Glucose Concentration, Insulin Concentration and HOMA-IR Following Oral Administration of Methanol Extract of *Albizzia chevalieri*, *Newbouldia laevis* and *Leptadenia hastata in* Diabetic Rats

Group	**Glucose (mmol/l)**	**Insulin (µU/ml)**	**HOMA-IR**
Control (distilled water)	6.6±0.35	3.9±0.22	1.14±0.21
Diabetic (Untreated)	13.9±0.24[a]	12.6±1.51[a]	7.78±0.06[a]
Metformin (500mg)	7.3±-0.18***	5.6±0.37***	1.82±0.06***
***A. chevalieri* (300mg)**	**7.1±-0.26*** **	**5.3±0.35*** **	**1.67±0.12*** **
***N. laevis* (300mg)**	8.4±0.18**	6.9±0.23***	2.57±0.08**
***L. hastate* (300mg)**	**8.2±0.32****	**7.4±0.23****	**2.69±0.08****

*= p<0.05, **= p<0.01, *** = p<0.001 compared to diabetic controls, [a] = P<0.001 compared to normal controls. **HOMA-IR**, homeostatic model assessment- insulin resistance. Data analysed using one way ANOVA followed by Bonferroni *post hoc* test and expressed as mean ± Standard deviation (SD), n= 7-8 rats.

3.5. The impact methanol leaf extract of Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata on serum lipid profiles of type 2 diabetic rats

Table 4 shows the serum lipid profile of type-2 diabetic rats treated with methanol extract of *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastate*. The diabetic untreated group had significantly higher (*P<0.05*) concentrations of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL)- cholesterol and very low density lipoprotein (VLDL)-cholesterol compared with normal control. Conversely, the concentration of HDL- cholesterol was decreased in the diabetic controls. Following the administration of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* and the standard metformin, we observed significantly (*P<0.05*) decreased levels of TC, TG, LDL- cholesterol and VLDL-cholesterol. These treatments also increased levels of HDL-cholesterol as compared with diabetic control. The atherogenic index of the rats administered with metformin (p<0.01) and *Albizzia chevalieri* (P<0.001) were significantly lower than that of the diabetic control.

Table 4: Serum Lipid Profile (mg/dl) of High Fat Diet and Dexamethasone Induced Type-2 Diabetic Rats Treated with Methanol Extract of *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastata*

Group	TC	TG	HDL-C	VLDL-C	LDL-C	AI
Control (distilled water)	82.6±3.64	89.8±1.77	63.6±3.05	17.9±0.35	20.0±1.28	0.4±0.03
Diabetic (Untreated)	137.4±3.97[a]	152.4±1.36[a]	44.6±2.70[a]	30.4±0.27[a]	43.3±1.72[a]	0.7±0.03
Metformin (500mg)	84.2±3.89**	86.2±1.39**	51.2±2.58**	17.2±0.28***	15.7±2.63***	0.3±0.06**
***A. chevalieri* (300mg)**	77.4±6.42***	80.6±1.57**	48.2±3.42*	16.1±0.31***	13.1±1.56***	0.2±0.03***
***N. laevis* (300mg)**	93.8±3.70**	110.4±1.81*	48.0±4.47*	22.1±0.36**	23.7±0.86***	0.5±0.03
***L. hastate* (300mg)**	88.2±5.07**	99.2±2.13*	45.0±4.74*	18.6±0.86**	23.6±3.79***	0.5±0.11

Abbreviations: TC-total cholesterol; TG-triacylglycerol; HDL-C-high density lipoprotein cholesterol; LDL-C- low density lipoprotein cholesterol; VLDL-C- very low density lipoprotein cholesterol; AI-atherogenic Index. *= p<0.05, **= p<0.01, *** = p<0.001 compared to diabetic controls, [a] = P<0.001 from normal controls. Data analysed using one way ANOVA followed by Bonferroni *post hoc* test and expressed as mean ± Standard deviation (SD), n= 7-8 rats.

3.6. Thrombolytic effects of Albizzia chevalieri, Newbouldia laevis and Leptadenia hastate in type 2 diabetic rats

The thrombolytic activity of diabetic rats administered *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastate* is shown in figure 1. Treatment of the clot with streptokinase produced the maximum clot lysis (93.70%)). Whereas treatments with *A chevalieri, N laevis and L hastate* triggered 48.90%, 39.02% and 37.69% clot lysis, respectively, distilled water produced only 4.83% clot lysis. All the plant extracts produced significantly greater ($p<0.001$) thrombolytic activity when compared to distilled water and significantly lower ($p<0.001$) activity compared to streptokinase.

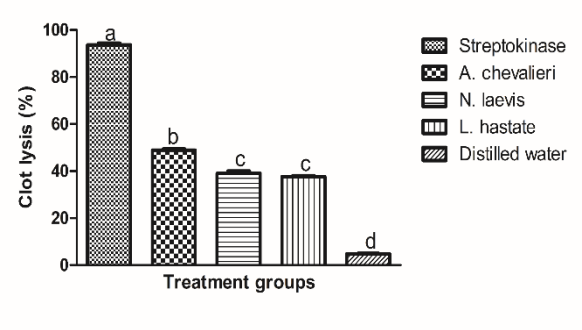

Figure 1: Thrombolytic potential of *Albizzia chevalieri, Newbouldia laevis* and *Leptadenia hastate* Leaf extracts. a,b,c,d= significantly different at $p<0.001$.Data analysed using one-way ANOVA, n=3 replicates.

4. Discussion

In the current study, we investigated some selected medicinal plants for their antidiabetic and thrombolytic activities of in type 2 diabetic rats. Following a successful induction of T2DM, treatment with the leaf extracts lowered the blood glucose level of type 2 diabetic rats. Interestingly, the leaf extracts also showed a substantial degree thrombolytic activities *in vitro*.

The combination of HFD and dexamethasone used in this study successfully induced T2DM and insulin resistance (IR) in the rats. IR is observed in conditions like T2DM, obesity and lipid disorders. Thus, agents that ameliorate IR can potentially mitigate against the development of T2DM and related conditions. Compared to current antidiabetic agents, natural herbs can impart lesser side effects. Among the potentially antidiabetic plants, leaf extracts of *Albizzia chevalieri* demonstrated significant ameliorative effect on type-1 diabetes (Saidu et al., 2010). *Newbouldia laevis* (Eneh et al., 2018) and *Leptadenia hastate* (Bello et al., 2011) have also shown anti-hyperglycaemic activity in T1DM rats. In this study, untreated diabetic rats showed significantly lowered insulin sensitivity, as indicated by HOMA-IR. This was an indication of successfully induced the pathogenesis that can lead to T2DM.

Weight loss seriously impacts the management of diabetes mellitus (Brown et al., 2019; Magkos et al., 2020). It may be due to degeneration in adipocytes or muscle tissues, which could be in compensation for the energy lost with frequent micturition and/or the excess conversion of glycogen to glucose. Our data showed marked differences between the body weight of normal and diabetic rats. Though both diabetic untreated and treated rats lost weight throughout the study, it was less severe in the treated rats. Individuals with diabetes mellitus may face alteration in body weight; for T1DM this comes as sudden weight loss but this scenario is seldom the case with T2DM. Over time, T2DM only triggers significant weight loss if left undiagnosed and untreated (Mbara et al., 2022). In this study, dexamethasone, and exposure to high fat diet for 21 days resulted in reduced body weight. A previous study by Shalam et al. (2006) also showed a similar finding of reduced body weight. Interestingly, methanol extracts of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* reversed this progressive loss in body weight caused by dexamethasone and high fat diet.

An initial increase in glucose and then circulating insulin levels can indicate a greater degree of insulin resistance. After treatment with dexamethasone and high fat diet, rats developed hyperglycaemia and reduced insulin sensitivity as determined by the HOMA-IR. However, our treatments of T2D rats with *Albizzia chivalieri,*

Newbouldia laevis and Leptadenia hastata leaf extracts restored the glucose concentration towards normal and improved insulin sensitivity. This is comparable to results obtained for *Albizzia chivalieri* (Saidu et al., 2010), *Newbouldia laevis* (Eneh et al., 2018) and *leptadenia hastata* (Bello et al., 2011) when they were administered to alloxan-induced type-1 diabetic rats. To our knowledge, there is currently no report on the potential of methanol extract of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastate* to exert antidiabetic activity in an animal model of T2DM, thus, making our findings novel. The antidiabetic activity demonstrated by the leaf extracts could be a result of their action on the B cells of the pancreas causing both an increased insulin secretion and enhanced sensitization of the insulin receptors (Semwal et al., 2021). Alternatively, this may be explained by enhanced blood glucose delivery to peripheral tissues or an inhibition of the action of alpha-glucosidase (Bhatia et al., 2019). Thus, the methanol leaf extracts of *Albizzia chivalieri, Newbouldia laevis and Leptadenia hastata* possess potent hypoglycaemic activity.

Dexamethasone stimulates lipolysis, which results in the production of free fatty acids, which then compete with glucose for intracellular oxidation (Olivas-Aguirre et al., 2023). This leads to the development of IR via the fatty acid cycle (Lee et al., 2022). Dexamethasone also increases triglyceride levels, causing a disordered lipid metabolism that triggers hyperlipidaemia (Dzinyela et al., 2021). In diabetes, free fatty acids are increasingly mobilised from peripheral fat depots because of the inability of insulin to inhibit hormone-sensitive lipase (Tella et al., 2019). As a result, the severe hyperlipidaemia that precedes diabetes may be seen as a side effect of unrestrained lipolytic hormone activities on fat depots. When compared to normal control, 21 days of a high fat diet and dexamethasone resulted in increased triglyceride, increased total, LDL- and VLDL-cholesterol but decreased HDL-cholesterol. Interestingly, Shalam et al. (2006) had previously reported similar findings. However, the administration of the leaf extracts in our study reversed the effects of the diabetogenic diet and increased the HDL-cholesterol level compared to the untreated diabetic rats. These findings emphasize the prospective role of plants in the prevention and treatment of cardiovascular disease. The extracts' potential to lower not only blood glucose but also total cholesterol and triglycerides may thus be of great benefit.

Various medicinal plants have been shown to possess anti-diabetic potential in T1DM and mild thrombolytic actions (Kianian et al., 2021; Yadav et al., 2022). To compare the clot lysis effects of our selected plant leaf extracts, we used Streptokinase, a novel thrombolytic drug, as a positive control. The novelty of this study lies in the fact that the thrombolytic activity of these plants, *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata,* has not been previously reported. T2DM increases the risk of building-up plaques in arteries. This can trigger harmful blood clots as a result of pro-thrombotic changes and contributes to the high incidence of thrombotic events in type 2 diabetic individuals. The activities exhibited by *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* may result in the stimulation of insulin sensitivity receptors and stimulation of pancreatic B-cells to produce enough insulin with additional benefit of lysing blood clots (thrombolytic activity). Therefore, if these results are validated then the plants extracts might prove useful as therapy for insulin resistance and artery-thrombotic events in type-2 diabetic patients.

5. Conclusion

In this study, we showed that methanol leaf extracts of the selected medicinal plants have anti-diabetic effects in high fat diet and dexamethasone induced model of type-2 diabetes. The extracts produced almost similar decrease in blood glucose concentration as the standard drug, metformin. This could be the explanation for the use of these plants to treat diabetes mellitus in some societies. Additionally, the extracts of *Albizzia chevalieri, Newbouldia laevis and Leptadenia hastata* have shown a great potential for blood clots lysis (thrombolytic) activity *in vitro*. Future studies to demonstrate *in vivo* clot dissolving properties and the bioactive components responsible for clot lysis should be considered. The goal of the management of

T2DM is not limited to reduction of the high blood glucose level, but also the prevention of complications such as hypercoagulation and mortality associated with it.

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