

Evaluation of antidiabetic role of *Bridelia ferruginea* methanol leaf extract in streptozocin induced diabetic male wistar rats

Abstract

The aim of this study was to investigate the antidiabetic ability of *bridelia ferruginea* leaf extract on streptozocin induced diabetes mellitus in male wistar rats. A qualitative phytochemical analysis of *B. ferruginea* methanol leaf extract was performed using standard methods and the result was positive for six (6) out of nine (9) phytochemical tests. Saponins, Tannin, Flavonoids, Terpenoids, Alkaloids, and Catechol were present. following the confirmation of the bioactive component of the plant, a total of 20 male wistar rats between the weights of 140-160g were divided into four (4) groups (n=5), three (3) groups received I.P. STZ (50mg/kg) and the remaining group served as the normal control. Rats with values between (200 - 352 mg/dl) were considered diabetic and the range of (79.8-83.6 mg/dl) was considered normal in this study. Glycated hemoglobin (HBA1c) was measured by high performance liquid chromatography (HPLC) and blood sugar level using ACCU-CHEK Glucometer. The Results revealed that the extract significantly lowered ($P<0.05$) blood sugar levels similar to the reference drug as well as HBA1c compared to the negative control. Oral glucose tolerance test was carried out with a load dose of 2grams of glucose, and sugar levels were determined at 15, 30, 60, 90 and 120 minutes later, revealed a rapid blood sugar level clearance in group treated with 50mg/kg of *B. ferruginea* leaf relative to negative control. Kidney and liver function biomarkers (ALT, AST, ALP, bilirubin, urea and creatinine) were determined using standard method and the result shows that at 50mg/kg, *B. ferruginea* leaf exhibited a hepato-protective ability and kidney function restoration, by significantly lowering ($P<0.05$) levels of useful biomarkers. Therefore, *B. ferruginea* leaf extract may be considered as a safe and good therapeutic candidate at a dose of 50mg/kg for the management of diabetes and its related condition.

Keywords: *Bridelia ferruginea*, diabetes mellitus, glycated hemoglobin (HBA1c), liver function, kidney function

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Nwozo Sarah Onyenibe, Nwawuba Stanley Udogadi

Nutritional and Industrial Biochemistry Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria

Correspondence: Nwozo Sarah Onyenibe, Nutritional and Industrial Biochemistry Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria, 200005, Tel +234-9038098155, Email sonwozo@yahoo.com

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Introduction

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia.¹ DM occurs either when the pancreas does not produce insulin, or when the body cannot effectively use the insulin it produces.² Several findings has revealed that DM is a major global health concern with a projected rise in prevalence from 171 million in 2010 to 366 million 2030.^{2,3} Both the number of cases and the prevalence of DM has steadily being on a rise over the past few decades and it is regarded to be a silent killer disease, affecting millions of peoples in the world.^{4,5} In Africa, the number of people with diabetes will increase from 14.2 million in 2015 to 34.2 million in 2040 predominantly populated in some of the region's most populous countries: South Africa, the Democratic Republic of Congo, Nigeria and Ethiopia.^{3,4,6}

Regardless of the numerous conventional medications that have been reported to be in use for diabetes management, it inaccessibility has also been demonstrated to be huge concern as a result of the relatively high cost and sometimes unavailability particularly for the rural settlers.⁷ Owing to this, a switch to a readily available and cheaper alternative has become necessary in the form of phyto/herbal

medicine.⁷ Herbal medicine also regarded as phytomedicine refers to the use of plants seeds, flowers, roots for medicinal purpose.^{3,8} Currently, medicinal plants continue to play an important role in the management of DM, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies.⁹ Ethno-pharmacological surveys indicate that more than 1,200 plants are used in traditional medicine systems following claims of their hypoglycemic properties.^{10,11}

Bridelia ferruginea (Euphorbiaceae) is a shrub commonly growing up to a height of 45 feet in the Savannah or in open spaces of coastal districts. Bark, roots, fruits and leaves are used mainly as decoctions. They are ingredients traditionally used for the preparation of *agbo* by the Yoruba people of South-western Nigeria and are used in the preparation of mouthwashes as a remedy for thrush in children and the aqueous infusions of the leaves are used for the treatment of chronic diabetes.¹² Regardless of its popular traditional usage, strong scientific evidence on its medicinal potentials remains scarce. Therefore, further investigation, scientific justification and continual validation are required to highlight its importance in the management of diabetes and related conditions. In this light, the present study was designed to investigate the hypoglycemic and hepatoprotective ability

of methanol leaf extract of *bridelia ferruginea* on streptozotocin induced diabetic male wistar rats.

Materials and methods

Collection of plant and extraction

Bridelia ferruginea was collected from surrounding areas of University of Ibadan, Ibadan North LGA and was identified and identified at Botany Department, University of Ibadan. The fresh leaves were air dried for two weeks in Nutritional Biochemistry postgraduate students' laboratory and pulverized into fine powder. The powdered leaf (1 kg) was extracted with methanol at room temperature for 72 h followed by evaporation of the solvent under reduced pressure using a Büchi rotary evaporator (France) model. A crude extract (90g) was obtained placed in an air tight bottle and stored in a LG refrigerator.

Phytochemical screening

Phytochemical screening of *Bridelia ferruginea* methanol leaf extract was performed by standard methods.^{13,14}

Animals used

A total of 20 male wistar rats between the weights of 100-120g were procured from the central animal house, College of Medicine, University of Ibadan, Nigeria for the study and were allowed to acclimatize for two weeks before commencement of experimentation. They male wistar rats were kept in well kempt and ventilated cages and their beddings changed every three days and they were allowed free access to clean drinking water. All the processes involved in the handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the department.

Induction of diabetes with streptozotocin

Hyperglycaemia was induced by single dose intraperitoneal injection of streptozotocin (50 mg/kg) dissolved in a citrate buffer (0.1 M, pH 4.5) and after 48hours blood samples were collected from caudal vein for determination of fasting blood sugar level using ACCU-CHEK Glucometer. Rats with values between (200 - 352 mmol/dl) were considered diabetic and (79.8-83.6 mmol/dl) were considered normal in this study.

Oral glucose tolerance test

The oral glucose tolerance test was carried out on normal and streptozotocin-induced diabetic groups. The treatment groups were orally administered 50mg/kg of *Bridelia ferruginea* methanol leaf extract and 6mg/kg glibenclamide respectively after an overnight fast. Two hours later, glucose solution (2g/kg body weight) was administered orally. Blood samples were collected prior to the administration of the glucose load. Blood glucose values were determined at 15, 30, 60, 90 and 120 minutes later. The blood samples were collected from caudal vein for determination of blood sugar level using ACCU-CHEK Glucometer.

Experimental design

Group 1: normal control

Group 2: Negative control received 50mg/kg STZ and remained untreated

Group 3: Positive control received 50mg/kg STZ and treated with glibenclamide 6mg/kg as used by.¹⁵

Group 4: received 50mg/kg STZ and treated with 50mg of *Bridelia ferruginea* methanol leaf extract as used by.¹⁶

Biochemical analysis

Blood glucose level was measured every week for the period of 28days using ACCU-CHEK glucometer and Glycated hemoglobin (HbA1c) was measured by high performance liquid chromatography (HPLC) at the end of the study. Whole blood was used in the analysis of HbA1c with the aid of DCCT aligned clover A1c analyzer (Infopia Co. Ltd., Korea) that has a test range of 4-14%. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using RANDOX kit (AL 100) and (AS 101) a method described by.¹⁷ Alkaline phosphatase was determined using RANDOX kit (AP 542) by standard method according to the recommendation of.¹⁸ Urea was determined by colorimetric method using RANDOX kit (UR 1068). Creatinine level was determined using RANDOX kit (CR 510) according to the method of¹⁹ and Bilirubin level was determined using RANDOX kit (BR 411) according to the method of.²⁰

Statistical analysis

Data were treated by ANOVA (analysis of variance) and mean separation was done using Duncan multiple range test. $p < 0.05$ were considered significant. Data was expressed as means±standard deviation and pictorially presented in form of charts. All statistical analysis was done using IBM SPSS Version 22 and Microsoft Excel.

Results

Phytochemical analysis

Qualitative analysis of *B. ferruginea* methanol leaf extract (Table 1) showed positive results for six (6) out of nine (9) phytochemical tests. Namely: Saponins, Tannin, Flavonoids, Sterioids, Terpenoids, Cardiac glycosides, Alkaloids, and Catechol respectively.

Table 1 Qualitative analysis of *B. ferruginea* methanol leaf extract

Phytochemical	Observation
Saponins	++
Tannins	++
Flavonoids	+++
Steroids	-
Terpenoids	++
Cardiac Glycoside	-
Alkaloids	+++
Anthraquinone	-
Catechol	+

(+) indicates presence in trace amount, (++) indicates presence in moderate amount, (+++) indicates presence in strong amount, and (-) indicates not detected.

Hyperglycemia

Figure 1 revealed the hypoglycemic ability of *B. ferruginea* leaf extract on male wistar rats. After 14days of acclimatization, the rats were intraperitoneally injected 50mg/kg streptozotocin (STZ) except for group 1 (normal control). Hyperglycemia was confirmed after 48hours of STZ injection, as they was a significant increase

($p < 0.05$) in blood sugar levels in the entire group that received STZ in comparison with the normal control (Day 0). Following confirmation of hyperglycemia, they rats were treated for a period of 28 days and blood sugar (mg/dL) levels measured weekly. There was a significant decrease ($p < 0.05$) in blood sugar levels in the groups treated with

B. ferruginea (group 3) and standard drug (group 4) relative to the negative control at day 7, 14 & 21 respectively. However, the hypoglycemic ability of *B. ferruginea* was more prominent on the 28th day.

Blood Sugar Level

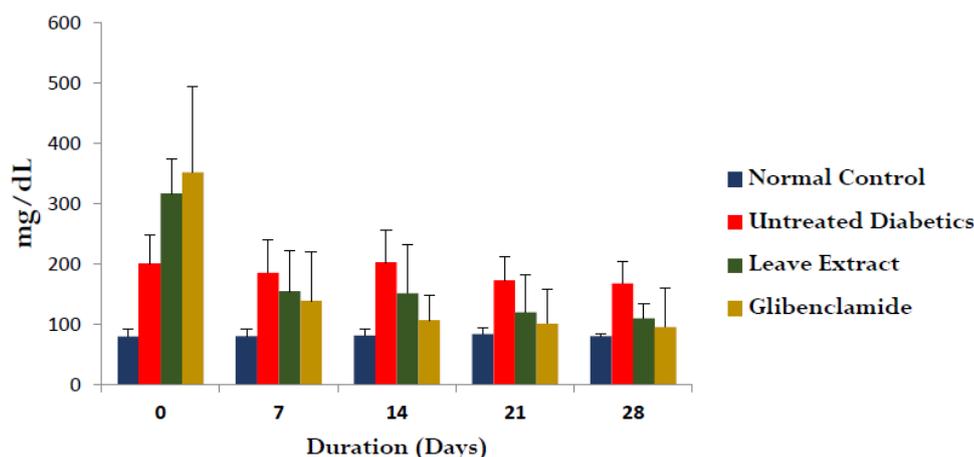


Figure 1 The effect *Bridelia ferruginea* methanol leaf extract on blood sugar level.

Oral glucose tolerance test

Figure 2 shows the effect of *B. ferruginea* methanol leaf extract on oral glucose tolerant test (OGTT). At day 28, serum blood was collected from caudal vein for determination of blood sugar level using ACCU-CHEK Glucometer post glucose load at timely interval (0, 15, 30, 60, 90 and 120 minutes) respectively. Following glucose

load dose, there was a significant elevation ($p < 0.05$) of blood sugar level after 15 minutes in (group 2) negative control (295 ± 56.6) relative to the groups treated with *B. ferruginea* leaf extract (157 ± 25.4) and glibenclamide (142 ± 4.2) respectively. However, from 30 minutes to 120 minutes, the blood sugar levels was significantly decrease ($p < 0.05$) in the normal control and the treated groups when compared to the negative control.

Oral Glucose Tolerance Test

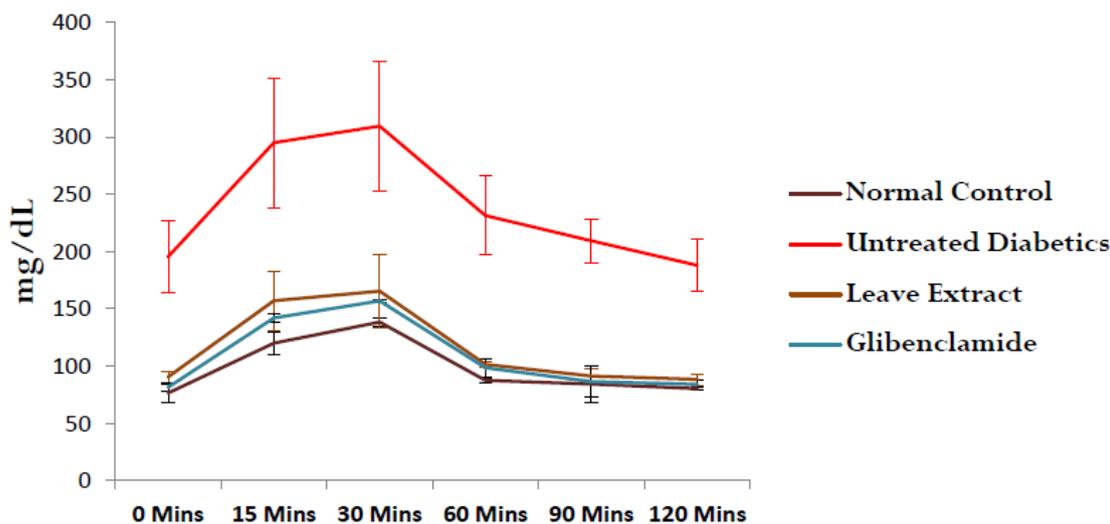


Figure 2 The effect *Bridelia ferruginea* methanol leaf extract on oral glucose tolerant test (OGTT).

Glycated hemoglobin

Figure 3 demonstrates the effect of *B. ferruginea* leaf extract on glycated hemoglobin. At the end of the study blood was collected and glycated hemoglobin (HBA1c) measured by high performance liquid chromatography (HPLC). The result showed that HBA1c (%)

ranged from (Normal Control) 4.6 ± 0.2 to 11.8 ± 0.1 (Negative Control-Untreated diabetics). Treatment with *B. ferruginea* (7.25 ± 1.6) and glibenclamide (7 ± 0.3) significantly lowered ($p < 0.05$) levels of HBA1c (%) relative to the negative control (11.8 ± 0.1). However, no significant difference ($p > 0.05$) was seen between the treated groups and the normal control.

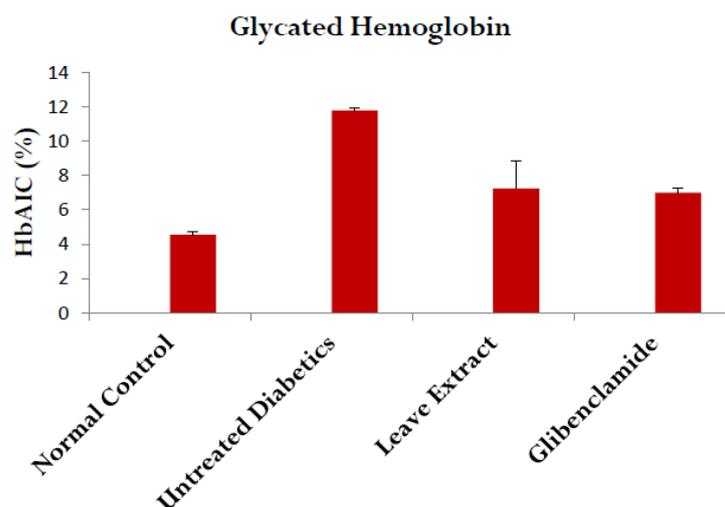


Figure 3 The effect of *Bridelia ferruginea* methanol leaf extract on Glycated Hemoglobin (HbA1C) levels.

Liver function

Table 2 shows the effect of *B. ferruginea* on liver function biomarkers. At the end of the study, blood was collected into a non-heparinized bottle centrifuged at 10,000rpm for 5minutes and serum collected for liver function enzyme determination. As seen, the group

that was intraperitoneal injected with 50mg/kg STZ and remained untreated (negative control) showed a significant elevation ($p < 0.05$) in the levels of liver function markers AST, ALT and ALP respectively relative to treated groups. However, they was no significant difference $p > 0.05$ between treated groups (50mg/kg *B. ferruginea* and 6mg glibenclamide) and the normal control.

Table 2 The effect of *Bridelia ferruginea* methanol leaf extract on liver function markers (AST,ALT, and ALP)

Groups	AST(U/l)	ALT(U/l)	ALP(U/l)
Normal Control	33.5±2.4 ^a	28.0±2.4 ^a	107.3±14.8 ^a
Negative Control	53.7±4.5 ^b	37.3±2.1 ^b	138.3±5.9 ^b
Positive Control	37.5±1.7 ^a	27.3±3.3 ^a	112.0±3.4 ^a
50mg <i>B. ferruginea</i>	38.40±2.8 ^a	28.2±1.1 ^a	112.0±3.36 ^a

Means with same alphabet as superscript within each column variable are non-significant ($P > 0.05$) different from each other. AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase

Kidney function

Table 3 revealed that the levels of serum urea, creatinine and total bilirubin were significantly ($p < 0.05$) higher in the untreated group (negative control) relative to the normal control. Treatment with

50mg/kg *B. ferruginea* methanol leaf extract and reference drug (6mg glibenclamide) showed a significant ($P < 0.05$) decrease in the level of BUN (mg/dl) and they were a noticeable decrease in the levels of creatinine (mg/dl) and total bilirubin (mg/dl) however not statistically significant as compared to the negative control.

Table 3 The effect of *Bridelia ferruginea* methanol leaf extract on Kidney function Markers

Groups	BUN(mg/dl)	Creatinine(mg/dl)	Total Bilirubin(mg/dl)
Normal Control	15.1±0.5 ^a	0.5±0.08 ^a	0.2±0.2 ^a
Negative Control	18.6±1.2 ^b	0.8±0.1 ^b	0.5±0.1 ^b
Positive Control	15.80±0.6 ^a	0.6±0.1 ^{ab}	0.2±0.1 ^{ab}
50mg/k <i>B. ferruginea</i>	15.98±0.6 ^a	0.58±0.08 ^{ab}	0.26±0.13 ^{ab}

Means with same alphabet as superscript within each column variable are non-significantly ($p > 0.05$) different from each other. BUN, blood urea nitrogen

Discussion

Diabetes is a complex and a multi-variate group of disorder that disturbs the metabolism of carbohydrates, fats and protein; the disease is characterized by complex pathophysiology, increased fasting and post prandial blood sugar levels as a result of the loss of glucose homeostasis and shortage or lack of insulin secretion.^{21,22} The present study induced diabetes with a single dose of intraperitoneal injection of 50mg/kg STZ which is a nitrosourea analogue, and exerts its toxicity and diabetogenicity by selective accumulation in pancreatic beta cells via the low-affinity GLUT2 glucose transporter in the plasma membrane.²³ Consequently, the entire groups exposed to STZ showed a significant increase $p < 0.05$ in blood sugar levels relative to the normal control (day 0) as demonstrated in Figure 1. However, treatment with 50mg/kg *B. ferruginea* methanol leaf extract showed a promising ameliorative ability as there was a significant reduction $p < 0.05$ in blood sugar levels for the period of 28 days when compared to the negative control (Figure 1). This finding corroborates the report of.^{16,24} To further evaluate the hypoglycemic ability of *B. ferruginea* leaf extract, an oral glucose tolerant test (OGTT) was carried out and the results (Figure 2) showed that treatment with *B. ferruginea* leaf extract enhanced blood sugar clearance as there was significant decrease $p < 0.05$ in peak blood sugar level (15min) relative to the negative control following a load dose of glucose. Also, levels of glycated hemoglobin was significantly reduced $p < 0.05$ in *B. ferruginea* treated group when compared to the negative control. However, no significant difference $p > 0.05$ was observed when compared to glibenclamide treated group and normal control. Hypoglycemic ability of *B. ferruginea* may be attributed to presence of phytochemical as shown in Table 1 Saponin, Tannins, Flavonoids, Terpenoids and Alkaloids and the result is consistent with report of.²⁵ These classes of compounds have been demonstrated to confer antidiabetic ability.²⁶

The liver is among the primary organs susceptible to the effects of hyperglycemia-induced oxidative stress, which may lead to liver tissue injury and in some cases DM causes excessive accumulation of fat cells in the liver resulting in a fatty liver.²⁷ Liver enzymes (AST, ALT and ALP) are useful biomarkers of liver injury and the levels are usually raised in acute hepatotoxicity.²⁸ The result of the present study Table 2 showed a significant increase $p < 0.05$ in the levels of liver biomarkers (AST, ALT and ALP) in the group that remained untreated (negative control) following exposure to STZ relative to the treatment groups. This also highlights that STZ exerts some levels of liver dysfunction.²⁹⁻³¹ However, treatment with *B. ferruginea* leaf extract restored the function of the rats liver as there was a significant decrease $p < 0.05$ in the levels of liver function biomarkers as compared to the negative control. Additionally the toxicological analysis of *B. ferruginea* methanol leaf extract reported by,³² further assert its safety for treatment of a number of disorders.

One of the major concerns of DM is its related complications which can affect multiple vital organ systems.²⁷ Creatinine and urea are one of the markers of kidney function.³³ Thus, the result of this study Table 3 showed that the level of blood urea nitrogen (BUN), total bilirubin and creatinine was significantly higher $p < 0.05$ in the negative control relative to the normal control and this indicates a compromised functional kidney. However, treatment with *B. ferruginea* leaf extract significantly improve the rats kidney as they was a significant decrease $p < 0.05$ in the levels of BUN relative to the negative control. Also, they was a decrease in the levels of creatinine and total bilirubin but however not statistically significant.

Conclusion

The leaf extract of *Bridelia ferruginea* demonstrated a hypoglycaemic ability, hepato-protective capacity and restoration of the kidney function. Therefore, *Bridelia ferruginea* leaf extract may be considered as a safe and good therapeutic candidate at a dose of 50mg/kg for the management of diabetes and its related condition.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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