

Antihyperglycemic Studies on the Leaf Extract and Active Fractions of *Newbouldia laevis* (Bignoniaceae)

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Abstract

Optimal control of chronic hyperglycemia prevents both micro and macro vascular complications—a leading cause of morbidity and mortality in diabetic subjects. This study was undertaken to give credence to the traditional use of *Newbouldia laevis* leaves in the treatment of diabetes mellitus (DM). Dichloromethane-methanol (1:1) extract (DME) of *N. laevis* leaves was prepared by cold maceration. Separation of DME into column chromatographic fractions yielded the n-hexane fraction (HF), ethylacetate fraction (EF) and methanol fraction (MF). The extract and fractions were evaluated for antihyperglycemic activity in alloxanized diabetic rats. The results showed that the oral administration of extract and fractions (250, 500, 1000 mg/kg) caused a significant ($P < 0.05$) and dose-dependent reduction in blood glucose level in diabetic rats. The hypoglycemic potency after 24 h was in the order MF (methanol fraction; 56.31%) > DME (dichloromethane/methanol extract; 36.19%) > EF (ethylacetate fraction; 20.70%) > HF (n-hexane fraction; 10.09). The methanol fraction, which showed the highest potency in oral glucose tolerance test (OGTT), was further separated into column chromatographic sub-fractions—F₁, F₂, F₃ and F₄ fractions. These sub-fractions were evaluated for antihyperglycemic activity. Sub-fractions F₁, F₂ and F₃ (1000 mg/kg) did produce significant ($P > 0.05$) reduction in blood glucose level after 24 h. Sub-fraction F₄ (50, 100, 200 mg/kg), however caused a significant ($P < 0.05$) and dose-dependent reduction in blood glucose level. The reduction at 200 mg/kg dose of F₄ (74.57%) was significantly ($P < 0.05$) higher than that of glibenclamide (58.04%). These findings suggest that leaf extract and fractions of *Newbouldia laevis* possess antihyperglycemic activities and can be the basis for the folk use *N. laevis* in management of diabetes mellitus.

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Keywords

***Newbouldia Laevis*, Antihyperglycemic, Diabetes Mellitus, Blood Glucose, Alloxan, Hypoglycemia**

1. Introduction

Diabetes Mellitus (DM) is the commonest serious, complex and multifarious group of metabolic disorders of multiple etiology that disturbs carbohydrate, fat and protein metabolism, characterized by chronic hyperglycemia as a result of relative or absolute lack of insulin or mounting resistance to its action [1]. Chronic hyperglycemia gives rise to the risk of microvascular (retinopathy, nephropathy and neuropathy) and macro vascular (ischaemic heart disease, stroke and peripheral vascular disease) damage with attendant reduction in life expectancy and diminished quality of life [2]. The world prevalence of diabetes among the adult population aged 20 years to 79 years in 2010 was 6.4% affecting 285 million adults and expected to rise to 7.7% and 439 million adults by the year 2030 [3]. A report by the International Diabetes Federation said that more than 371 million people (8.3%) of the world's population had diabetes in 2012 [4]. This dramatic increase is noted to be more in developing countries particularly in Sub-Saharan Africa and Asia [5] as a result of on-going quest in adopting "western life style" and diet. The currently available orthodox medicines to control hyperglycemia in DM management include: "insulins, insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, thiazolidinedione), agents that enhance incretin secretion and action (incretin analogues, incretin mimetics, dipeptidyl peptidase IV (DPP-IV) inhibitors), agents that decrease gastrointestinal glucose absorption (alpha glucosidase inhibitors, alpha amylase inhibitors, sodium-glucose co-transporter (SGLT-1) selective inhibitors), agents that promote renal glucose excretion (sodium-glucose co-transporter (SGLT-2) inhibitors) and others (amylin analogue, bile acid sequesterants, bromocriptine) [6]-[8]. Despite the knowledge of the pathological processes involved in causation/progression of the disease and the wide range of therapeutic agents designed to fight hyperglycemia, the statistical projections are still alarming and the stability of communities is being threatened. Alternative strategies to the current pharmacological options of DM management are therefore urgently needed [9] to manage this crippling global health problem. The plant kingdom has become a target for the search of biologically active lead compounds for complementary/alternative management of diabetes mellitus. Medicinal plants are noted to play an important role in the management of diabetes. The effect of these plants may delay the development of diabetic complications and correct the metabolic abnormalities [10]. Similarly, it has been reported that some bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than hypoglycemic agents used clinically [11]. Ethnobotanical and ethnopharmacological surveys report that more than 1200 plants are being used in many ethnic societies around the world in traditional medicine for their alleged hypoglycemic activity [12]-[15] and *Newbouldia laevis* (Bignoniaceae) is one of such plants.

The morphology, habitat and cultivation of *Newbouldia laevis* have been described [16]-[18]. In some parts of Southeastern Nigeria, it is usually planted at the grave to mark the position of the head of the deceased. This plant popularly known as boundary tree, chieftaincy tree, fertility plant or tree of life is locally called "Ogirisi" in Igbo, "Akoko" in Yoruba and "Aduruku" in Hausa languages [19]. The leaves, stem bark, roots and root bark of *N. laevis* have been reported to have versatile applications and are used in more than 25 medical purposes throughout the tropical Africa [17], including but not limited in Nigeria, Togo, Senegal, Ghana, Congo, Cote de Voire, and Cameroun. Some of the documented medical uses include in the folk treatment of fevers (including yellow fever), malaria, stomach ache, cough, sexually transmitted infections, skin infections, tooth ache, breast cancer, constipation, pain (pelvic pain in females, chest pain, ear ache), gonococcal orchitis, elephantiasis, sore-foot, ulcer, epilepsy, convulsion, migraine, sickle cell anaemia, as a febrifuge, as a vermifuge, in female reproductive healthcare (fibroids, infertility, hemorrhage), as aphrodisiacs, eye problems, snake bites, wound healing, diabetes, arthritis, rheumatism and other inflammatory conditions [20]-[28].

Pharmacological studies on extracts of different parts of *N. laevis* have revealed the antioxidant [29], free radical scavenging [30], antimicrobial [31] [32], antimalarial [33], sedative and anticonvulsant [34]-[36], analgesic, antinociceptive and antiinflammatory [37] [38], hepatoprotective [29], anticancer [39], uterine contraction [40], wound healing and antiulcer [41], antisickling [42], hypoglycemic [43], antihypertensive [44] [45], entomocide [46] activities among others. Scientific reports on the phytochemical constituents of different plants of

the plant revealed the presence of alkaloids, phenylpropanoid glycosides, flavonoids, tanins, saponins, phenols, essential oils, terpenoids, triterpenoids, quinoids, ceramides among others [29] [47]-[49].

In Southeastern and Midwestern Nigeria, a hydro-alcoholic decoction of *N. laevis* leaves has been used in folk medicine for the management of diabetes mellitus. Previously, there are preliminary reports on the antihyperglycaemic and antidiabetic studies which were on the crude extract of the leaves [4] [43] [50] [51]. In this study, we evaluated the crude leaf extract of *N. laevis* and its solvent fractions for antihyperglycemic activity in alloxan diabetic rats (acute study) in order to explore the fraction(s) that has/have the active principle(s) responsible for the antihyperglycemic activity.

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

Mature fresh leaves of *Newbouldia laevis* were collected from Igbo-Ukwu, Aguata L.G.A of Anambra State, South-Eastern Nigeria. The whole plant was identified and classified by Mr. J.M.C. Ekekwe, a plant analyst of the Department of Botany, University of Nigeria, Nsukka. The leaves were washed to remove contaminants, air-dried under shade and thereafter pulverized into a coarse powder.

2.2. Experimental Animals

Adult rats (150 - 250 g) and mice (15 - 30 g) of both sexes bred in the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were kept in still cages within the facility and fed with standard livestock feeds and allowed free access to water. The animals were allowed 14 days acclimatization period on transfer to the research area before the studies. All animal experiments were in accordance to our institutional ethics guidelines and in compliance with the National Institute of Health and Guide for care and use of Laboratory Animals (Pub No. 85 - 23 revised).

Preparation of extract:

The air dried and powdered material (5 kg) was macerated in a mixture of dichloromethane/methanol (1:1) for 48 hours and filtered. Removal of solvent *in-vacuo* in a rotary evaporator provided an organic extract of 14.2% yield (710 g) and labeled DME.

2.3. Acute Toxicity Test (LD₅₀)

The acute toxicity/lethality (LD₅₀) of DME in mice was determined using the method described by Lorke (1983) [52]. Adult albino mice of both sexes with a fasting body weight of 15 - 30 g were used. The LD₅₀ for the oral and intra-peritoneal routes were determined. The study was carried out in two stages. In the first stage 9 mice were randomly divided into 3 groups (n = 3) and treated orally with the extract at 10, 100 and 1000 mg/kg dose levels. The animals were observed for 24 h and the number that died in each group was recorded. Based on this, the doses for the second stage were selected. In this stage, 3 groups (as no animal died in the initial stage) of mice (n = 1) were treated with the extract at 1600, 2900 and 5000 mg/kg dose levels respectively. The LD₅₀ was calculated as the geometric mean of the minimum lethal dose and the maximum non-lethal dose. The same procedure was carried out for the intra-peritoneal route using a separate group of mice.

2.4. Solvent-Guided Fractionation of DME and Bioactivity-Guided Studies

The crude DME extract (500 g), was subjected to solvent guided fractionation in a silica gel (60 - 200 mesh size) column and successively eluted with n-hexane, ethylacetate and methanol in order of increasing polarity to yield n-hexane fraction (HF; 35.7 g; 7.14%), ethylacetate fraction (EF; 181.9 g; 36.38%) and methanol fraction (MF; 245.5 g; 49.09%). The fractions were concentrated using rotary evaporator (40°C - 50°C) under reduced pressure. The DME and fractions HF, EF, and MF were subjected to phytochemical analysis for identification of phytoconstituents and biological activity studies. The antihyperglycemic effect on alloxan diabetic rats was used to determine activity. Methanol fraction (MF) which showed the most potent antihyperglycemic activity was subjected to further fractionation. About 150 g of MF was fractionated by column chromatographic methods using gradient elution with n-hexane/ethylacetate (7:3) [53] to obtain twenty five (25) sub-fractions of MF. Thin layer chromatography (TLC) was employed to pull the column chromatographic fractions together on the basis of the R_f values of the similar spots to obtain four sub-fractions: F₁ (24.3 g; 16.2%), F₂ (27.9 g; 18.6%), F₃ (20.3 g;

13.5%) and F₄ (73.8 g; 49.2%) which were screened further. The sub-fractions were subjected to antihyperglycemic activity studies on alloxan diabetic rats and F₄ was significantly ($P < 0.05$) the most potent antihyperglycemic fraction. Sub-fraction F₄ was subjected to phytochemical analysis to determine the phytoconstituents present.

2.5. Phytochemical Analysis

Preliminary phytochemical tests were carried out on the extract, HF, EF, MF and F₄ using standard protocols [54], to qualitatively detect the presence, absence and relative amount of phytoconstituents.

2.6. Hypoglycemic Activity Test

Induction of diabetes: Diabetes was induced in adult albino rats of both sexes by a single intravenous injection of freshly prepared alloxan monohydrate (65 mg/kg) in 0.9% saline [53] [55]. Glucose solution (50%) was used to prevent the initial hypoglycemia caused by alloxan monohydrate [55] [56]. Blood samples were collected after 3 days from overnight fasted animals through the tail vein and blood glucose level was estimated using commercially available Accu-check Active (Roche Diagnostics) glucometer. Blood glucose levels above 250 mg/dl [56] were considered diabetic and selected for the study.

The diabetic animals were randomly divided into 14 groups ($n = 5$). Groups 1 - 12 received oral administration of DME, HF, EF, and MF at graded dose levels of 250, 500, and 1000 mg/kg. Group 13 received glibenclamide (Daonil™ Sanofi-Aventis, Nigeria) (5 mg/kg) [43] as a reference drug. Group 14 received 3% tween 80 (5 ml/kg) and served as negative control group. Blood samples were withdrawn through the tail vein at 0 (pre-treatment) and at 3, 6, 9, 12 and 24 h post treatment. The blood glucose levels were determined using the glucometer and percentage reductions in blood glucose levels were calculated relative to pretreatment values [57] [58].

2.7. Hypoglycemic Activity Test of Sub-Fractions of Methanol Fraction (F₁, F₂, F₃, F₄)

Six groups of diabetic rats ($n = 5$) were used. Groups 1 - 3 received 1000 mg/kg of F₁, F₂ and F₃ respectively. Groups 4-6 received 50, 100 and 200 mg/kg respectively of F₄. Blood samples were withdrawn and blood glucose levels were measured as described, at 0 (pretreatment), 3, 6, 9, 12 and 24 h post treatment. The percentage reductions in blood glucose levels were calculated relative to pretreatment values.

2.8. Effect of Methanol Fraction (MF) on Fasting Blood Glucose Levels of Normoglycemic Rats

Twenty five adult albino rats of both sexes were fasted overnight and randomly divided into 5 groups ($n = 5$). Groups 1 - 3 received oral administration of MF (250, 500, 1000 mg/kg respectively). Group 4 received glibenclamide (5 mg/kg) as reference drug. Group 5 received 3% tween 80 (5 ml/kg) and served as negative control group. The basal fasting blood glucose level was measured before treatment as described before and at 30, 60, 120 and 240 min post-treatment. The percentage reductions in blood glucose levels were calculated relative to pretreatment values.

2.9. Oral Glucose Tolerance Test (OGTT)

Twenty five adult rats of both sexes were fasted for 16 h but allowed free access to water. They were randomly divided into five groups ($n = 5$). Groups 1 - 3 received oral administration of MF (250, 500 1000 mg/kg respectively). Groups 4 and 5 received glibenclamide (5 mg/kg) and 3% tween 80 (5 ml/kg) respectively. After 60 min, the animals were fed with glucose (4 g/kg) [57] [59]. The blood glucose levels in each group were measured as described at 0 (before treatment) and at 30, 60, 120, 180 and 240 min after glucose challenge. The percentage change in blood glucose level was calculated relative to 0 min [57].

2.10. Statistical Analysis

The values obtained were analysed using one way analysis of variance (ANOVA) (SPSS Version 20) software and presented as mean \pm SEM. Differences between means were considered significant at $P < 0.05$ (LSD post hoc test).

3. Results

3.1. Result of Phytochemical Analysis

The preliminary phytochemical tests on the extract and fractions gave positive results of alkaloids, flavonoids, steroids, saponins, tanins, terpenoids, carbohydrates, proteins, oils, acidic compounds, reducing sugars and resins (Table 1).

3.2. Acute Toxicity (LD₅₀)

The oral LD₅₀ of the crude DME was >5000 mg/kg and was considered safe as no animal died at 5000 mg/kg. The intraperitoneal (i.p.) LD₅₀ was calculated to be 3807.9 mg/kg. The maximum non-lethal dose was 2900 mg/kg and the minimum lethal dose was 5000 mg/kg.

3.3. Hypoglycaemic Effect of Extract and Fractions

Administration of the extract (DME) and fractions (HF, EF, MF) produced significant ($P < 0.05$) lowering of mean blood glucose level to varying extent in diabetic rats at all the dose levels (Figures 1-4). The antihypergly-

Table 1. Result of phytochemical analysis.

Phytoconstituents	Relative abundances				
	DME	HF	EF	MF	F ₄
Alkaloids	++	-	++	+++	+++
Flavonoids	++	-	+	+++	+++
Glycosides	+	-	-	-	-
Steroids	++	+++	+	+	-
Saponins	+	-	-	++	+++
Tannins	++	-	++	++	+++
Carbohydrates	+	-	++	++	+
Proteins	+	-	+	++	-
Oils	+	+++	-	-	-
Acidic compounds	+	+	+	++	+
Reducing sugars	-	+	+	+++	+
Resins	+	++	++	++	+++
Terpenoids	++	++	+	+	-

Key: (-) → absent; (+) → present in small amount; (++) → present in moderate amounts; (+++) → abundantly present.

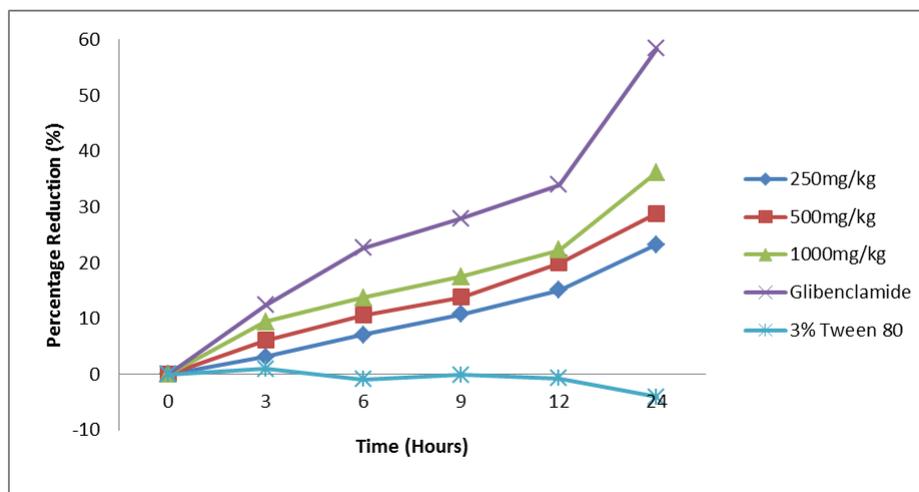


Figure 1. Effects of Dichloromethane/Methanol (DME) extract of *N. laevis* on diabetic rats.

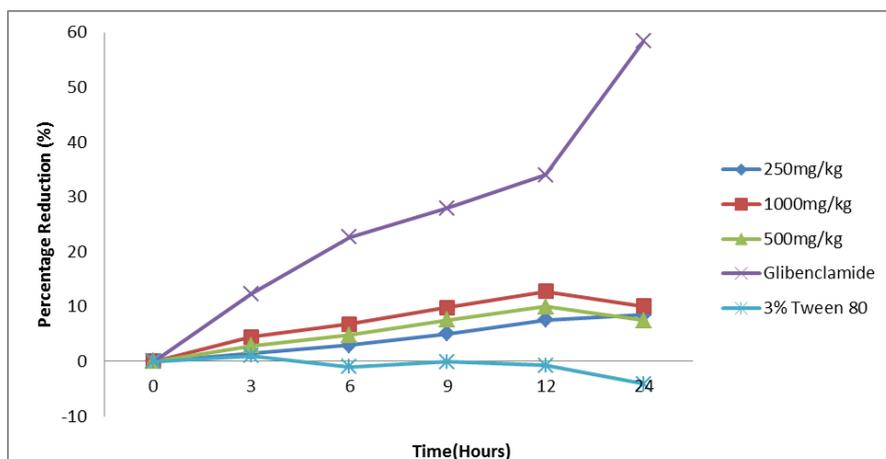


Figure 2. Effects of n-hexane fraction (HF) of *N. laevis* on diabetic rats.

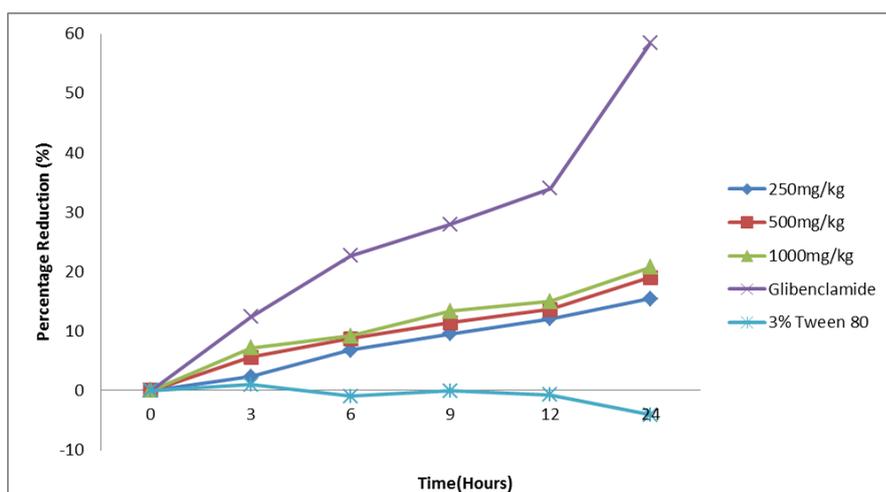


Figure 3. Effects of ethylacetate fraction (EF) of *N. laevis* on diabetic rats.

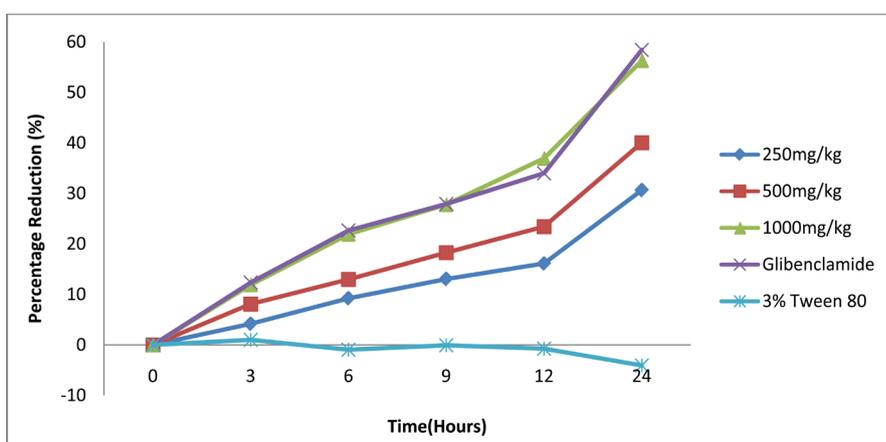


Figure 4. Effect of methanol fraction (MF) of *N. laevis* on diabetic rats.

cemic effect was observed to be dose-related and the order of potency was MF > DME > EF > HF (Figures 1-4). The antihyperglycemic effect of MF (1000 mg/kg) was comparable to that of the reference drug. The percentage reduction in blood glucose levels were calculated relative to pretreatment values.

3.4. Hypoglycaemic Effect of Sub-Fractions

Fractions F₁ and F₂ at the highest dose of 1000 mg/kg could not lower the blood glucose levels in alloxan diabetic rats after 24 h when compared to the control ($P < 0.05$). Fraction F₃ showed only weak (7.52%) reduction in blood sugar at the 6 h which could not be sustained at the 12 and 24 h time points. However, fraction (50, 100, 200 mg/kg) showed significant dose-related hypoglycemic activity (Figure 5). There was a marked decrease in blood glucose level of diabetic rats at 3 h time point; an effect that was sustained up to the 24 h. The reduction in blood glucose level of diabetic rats that received 50 mg/kg of F₄ was comparable with the glibenclamide treated group at 6 h, 9 h and 12 h time points. Group of diabetic rats treated with 100 and 200 mg/kg of F₄ showed significantly ($P < 0.05$) higher reduction in blood glucose level at 6 h, 9 h and 12 h time points when compared to the reductions in groups that received glibenclamide. Maximum reduction of blood sugar (74.57%) was observed at a dose of 200 mg/kg of F₄ after 24 h which was significantly ($P < 0.05$) higher than the reductions produced by glibenclamide treated group (58.04%) at the same time point (Figure 5).

3.5. Effect of Methanol Fraction (MF) on Fasting Blood Glucose Level of Normoglycemic Rats

Oral administration of methanol fraction (MF) at the three doses tested showed significant ($P < 0.05$) reduction in fasting blood glucose of normoglycemic rats at 2 h time point compared to the mean blood glucose level in the negative control group. The groups of rats that received 500 and 1000 mg/kg of MF showed a significant dose dependent reduction at 1 h and 2 h time points. The methanol fraction (250 mg/kg) did not reduce fasting blood glucose significantly ($P > 0.05$) at 1 h time point. The reduction at 2 h time point (3.17%) was however higher than that of 500 mg/kg (2.94%) although the difference was not significant ($P > 0.05$). The glibenclamide treated group however showed a significant reduction in the mean fasting blood glucose at all the time points (Figure 6). The fasting blood glucose level of all the rats treated with the methanol fraction returned to basal levels at 3 h time point. Rats treated with glibenclamide as standard oral hypoglycemic drug remained below basal level at 3 h time point.

3.6. Effect of the Methanol Fraction (MF) on Oral Glucose Tolerance Test in Normoglycemic Rats

Following oral administration of glucose, post-prandial blood glucose escalated to 104% in the control (untreated) group at 30 min post-treatment peak. Pre-treatment with MF (250, 500, 1000 mg/kg) suppressed this sudden glucose excursion by 43.26%, 39.12%, 24.50% respectively and produced a significant ($P < 0.05$) and progressive reduction in blood glucose level up to 180 min when compared to the untreated group (Figure 7). The MF (1000 mg/kg) treated group suppressed postprandial hyperglycaemia (24.50%) better than the glibenclamide treated group (28.61%) ($P < 0.05$). The blood glucose level of MF (500, 1000 mg/kg) treated groups remained below basal levels (pre-treatment values) between 120 and 150 min post treatment. The effect was comparable to the blood sugar reductions produced by glibenclamide treated group ($P < 0.05$) (Figure 7). However the blood glucose level in all the groups returned to pre-treatment values after 180 min.

4. Discussion

Hyperglycemia is the defining feature of diabetes mellitus. Chronic hyperglycemia is associated with both microvascular and macrovascular complications in patients with diabetes [2], both of which are responsible for increased morbidity and mortality in diabetic subjects. Chronic hyperglycemia is directly and proportionately related to glycosylation of haemoglobin and lipid peroxidation, both of which are markers that herald the onset of diabetic complications [60]. Evidence has shown that tight and optimal blood glucose control eliminates diabetic complications [61]. The global incidence of the disease is geometrically rising to pandemic proportions. The bane of diabetes management has been to keep both short-term and long-term blood glucose levels optimized within acceptable limits in order to prevent or reduce these complications. This can only be achieved by tightly controlling fasting blood glucose levels as well preventing post prandial glucose excursions [62]. The later has been recognised lately as a better marker of glycaemic control than fasting blood glucose since glycation of haemoglobin is primarily favoured by post prandial hyperglycaemia [63]. Plasma glycated haemoglobin (HbA1c) level is an index of glycaemic control [60].

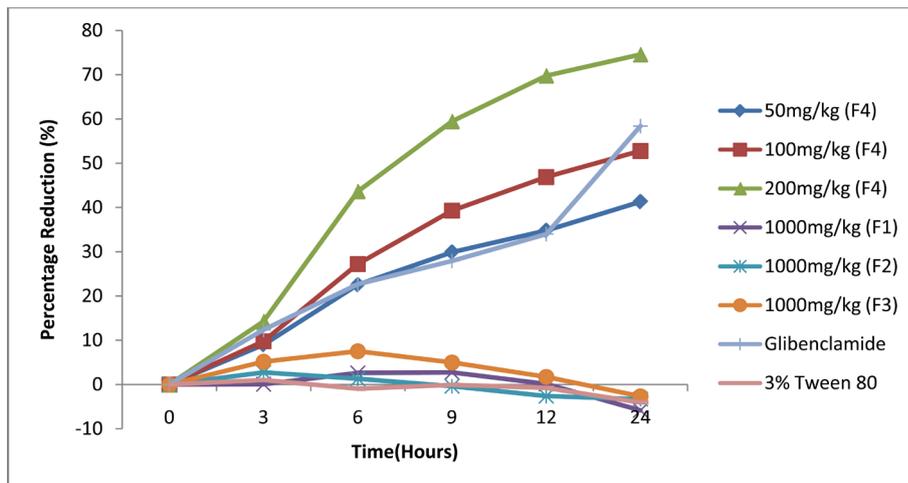


Figure 5. Effect of sub-fractions of methanol fraction of *N. laevis* on diabetic rats.

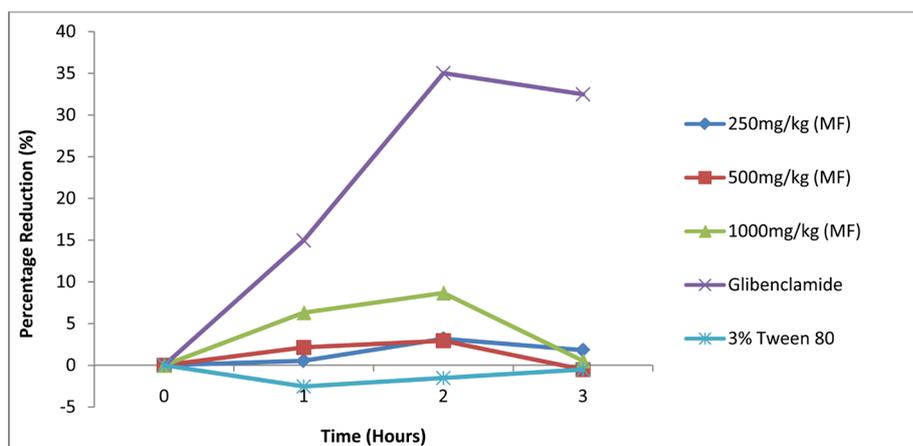


Figure 6. Effect of methanol fraction (MF) of *N. laevis* on fasting blood glucose level of normoglycemic rats.

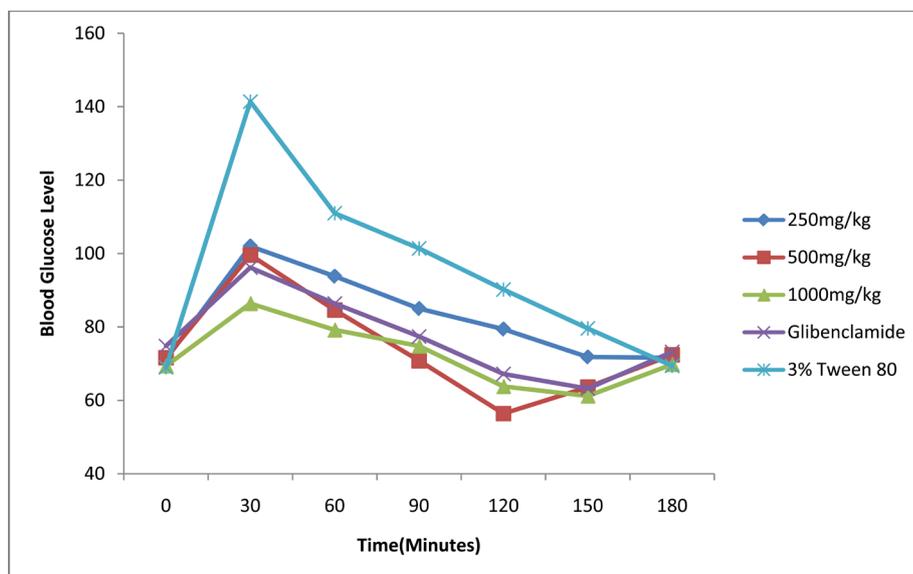


Figure 7. Effects of methanol fraction (MF) of *N. laevis* on oral glucose tolerance on non-diabetic rats.

Many of the available orthodox hypoglycaemic medications have not effectively addressed this global health problem. The reasons include affordability, accessibility, side-effects, acceptability, use restrictions in certain populations, and limitation of the agents in addressing underlying biochemical and pathological aberrations associated with the disease among many other reasons. Phytomedicines as an alternative have proven to be relatively safer, cheaper, more available and acceptable among the cultural society where they are found. Phytotherapy have demonstrated at least in animal models to prevent and/or halt the disease at different points of its natural history [64] [65].

Prior to evaluating the effectiveness of antidiabetic plants, World Health Organization (WHO) advocates that their safety be evaluated in order to standardize their use. Marles and Farnsworth (1994) [66] earlier noted that about one third of medicinal plants used in traditional ethnomedical treatment of diabetes mellitus are toxic. Therefore, the oral acute toxicity of the dichloromethane-methanol (1:1) leaf extract of *Newbouldia laevis* was evaluated and found to be greater than 5000 mg/kg body weight. This finding agrees with previous reports on the LD₅₀ of the leaf extract [43]. Toxicological assessment and safety of various parts of *N. laevis* has been reported [29] [49] [67]. Their findings suggest a high safety profile of *N. laevis* extracts and could be suitable for use in management of chronic diseases such as diabetes mellitus.

Alloxan is known to induce free radical generation of which the pancreatic beta cells are particularly vulnerable [68] leading to massive destruction of insulin secreting cells and subsequent hyperglycemia. In the present study, it was observed and demonstrated that oral administration of extract and fractions of *N. laevis* leaf exhibited effective and dose dependent reduction in blood glucose levels of alloxan diabetic rats and could have possibly reversed the effect of alloxan in causing and maintaining hyperglycemia in animal studies. Previous reports on antihyperglycemic effects of plant extracts on alloxan diabetic animal models favour regeneration of islet beta cells as the primary mechanism of recovery of alloxan injected animals [68]. The reported antioxidant and free radical scavenging properties of *N. laevis* extracts [30] [45] [69] [70] may have contributed to the recovery of the pancreatic beta islet cells and restoration of its secretory function. Methanol fraction slightly lowered the fasting blood glucose level of normoglycemic rats suggesting weak insulin secretagogue activity in nonglycemic state. The methanol fraction however, demonstrated a significant improvement in oral glucose tolerance in glucose fed hyperglycaemic non-diabetic rats which was comparable to that of the standard drug glibenclamide. This may suggest glucose dependent insulinotropic and insulinomimetic properties of the methanol fraction [10]. Inhibition of intestinal sodium-glucose co-transporter (SGLT 1) and/or inhibition of renal glucose reabsorption (SGLT 2) may contribute to the observed effects [71].

Although this study was not designed to investigate the antihyperglycemic mechanism of action of the extract and fractions in hyperglycemic non-diabetic and alloxan diabetic rats, a number of possible mechanisms (based on phytochemistry result) that may augment glucose disposal could help to explain the result of this study. Several phytoconstituents such as alkaloids, steroids, carbohydrates, glycosides, flavonoids, terpenoids, saponins, tannins, resins, peptides and amino acids, lipids, phenolics, glycopeptides and iridoids with putative antihyperglycemic activity have been reported [72] [73]. The preliminary phytochemical analysis of sub-fractions f₄ (showing highest activity of 74.57% reduction) showed high levels of alkaloids, flavonoids, saponins, resins and tannins. This suggests that the observed antihyperglycemic effects of the extract and fractions could have been mediated by one or more of these phytoconstituents.

Flavonoids have been shown to possess remarkable hypoglycaemic effects which have been linked to their capacity to avoid glucose absorption or improve glucose tolerance [74]. It has been demonstrated that flavonoids act as insulin secretagogues or insulin mimetics, attenuate diabetic complications, stimulate glucose uptake in peripheral tissues and regulate the activity and/or expression of the rate limiting enzymes involved in carbohydrate metabolism [75]-[77]. Flavonoids have been reported to show potent inhibitory activity against a wide range of enzymes such as lipo-oxygenases, cyclo-oxygenases, and prevent the generation or the action of free radicals which cause tissue damage during inflammatory processes [65] [78] [79]. They are therefore potent antioxidants and could modulate the activities of various enzymes involved in blood glucose homeostasis. This may have been responsible for the observed reduction of the blood glucose level by the extract and fractions of *N. laevis* leaf in alloxan-induced diabetic rats. Recently, there were similar reports on the antioxidant activities of the ethanol leaf extract of *N. laevis* and its ability to attenuate haemoglobin glycosylation and lipid peroxidation [51]. These reported antidiabetic potentials of *N. laevis* leaf extracts could only be explained wholly or in part by the result of this study since hyperglycemia favours these enzymatically induced changes in diabetic situations. Polyphenolic compounds have been implicated in the production of incretin hormone (glucagon-like

peptide GLP-1) [65] that appears to act through many mechanisms towards effective glucose disposal, including stimulation of insulin secretion, suppression of glucagon release, slowing of gastric emptying, improving insulin sensitivity, and reduction of food intake. In rodents and cell line experiments, GLP-1 has been shown to promote β -cell regeneration, proliferation, mass, and function [65]. There is documented evidence that polyphenols slow down glucose absorption by competitive inhibition of the intestinal brush border membrane sodium-glucose co-transporter (SGLT 1) [80] as well as inhibition of disaccharidases [81]. Kolawole and Abanji (2013) [82] reported the inhibition of alpha glucosidase and alpha amylase enzymes by the ethanolic leaf extract of *N. laevis* on sucrose fed rats. Phenolic compounds are known to directly cause regeneration of alloxan damaged islet beta cells by their action as antioxidants and free radical scavengers causing restoration of pancreatic beta cell function [83].

Phlorizin-like effects of plant extracts have also been reported [84]. Phlorizin a non-selective, competitive inhibitor of sodium-glucose co transporter (SGLT-1 & SGLT-2), is a naturally occurring phenol glycoside, first isolated from the bark of an apple tree in 1835 [85] used to enhance glucose excretion through the kidneys. Modification of chemical structure of this prototype gave more selective and more potent SGLT-2 inhibitors, most of which are C-glycosides [85]. Studies have shown that oral administration of sergiflozin, a SGLT-2 inhibitor in normal and streptozotocin induced diabetic rats, increased urinary excretion in a dose dependent manner and attenuated the increase in blood glucose level following an OGTT without stimulating insulin release [86]. Similarly, dapagliflozin, a potent SGLT-2 inhibitor caused a dose dependent reduction in blood glucose level by as much as 60% in STZ-induced diabetic rats over a 5-hr period following oral administration [87]. These findings are consistent with the findings of this study. Obute and Adubor [88] reported that the flavonoid compound in *N. laevis* leaves is majorly quercetin which occurs naturally as glycosides [89]. Quercetin glycoside was noted to be a potential substrate for lactose phlorizin hydrolase (LPH) [89] and a similar pharmacological activity with phlorizin was suggested. Interaction of quercetin glycosides with intestinal SGLT-1 has also been reported [89] [90]. Flavonoids (quercetin glycosides), saponins (triterpenoid/steroidal glycosides) present in the extract and fractions may have acted in the above manner to cause the observed effects.

The saponin present in the fraction may have played a role to explain the improvement in the oral glucose tolerance of normoglycemic rats pretreated with methanol fraction of *N. laevis* leaf extract in this study. In other studies, saponins have been shown to possess antihyperglycemic activities or other beneficial activities relevant in the management of diabetes. For examples, charantin, a steroidal glycoside saponin from *Momordica charantia* is known to possess insulin-like effects, stimulates insulin release and exhibits rat lens aldose reductase inhibitory activity [91]. Similarly, saponin isolated from *Gymnema sylvestre* (Gymnemic acid) exhibited marked hypoglycemic effects in animal models showing stimulation of insulin secretion and release, regeneration of pancreatic beta cells and activation of glucose metabolising enzymes [92].

Alkaloids from plant extracts are reported to show antihyperglycemic activity through a number of mechanisms that regulate glucose homeostasis. Berberine effectively inhibited the activity of disaccharidases (alpha glucosidase) and decreased glucose transport through the intestinal epithelium [93]. Berberine was also reported to show antihyperglycemic activity by promoting the secretion of glucagon-like peptide-1 [94]. Catharanthine, vindoline and vindolinine are alkaloids isolated from *Catharanthus roseus* with potent hypoglycemic activity [95]. Arecoline, an alkaloid isolated from *Areca catechu* was reported to have hypoglycemic activity in animal model [96]. Aegeline, an alkaloidal amide from the leaves of *Aegle marmelos* was found to possess antihyperglycemic activity with suggested β_3 -adrenergic receptor activity [97]. Trigonelline isolated from a known hypoglycemic plant *Trigonella foenum graecum* could lower blood glucose level in alloxan treated diabetic rats through regeneration of new islets [98]. The β -carboline alkaloids obtained from *Peganum harmala* stimulates insulin secretion in a dose dependent manner [99] [100] and are known insulin secretagogues from plant sources.

5. Conclusion

The results of this study suggest that the extract and fractions of *N. laevis* leaves possess antihyperglycemic activity in alloxan diabetic rats. Alkaloids, flavonoids, tannins, resins and saponins may be responsible for activity. This study gave credence to the ethnomedicinal use of *N. laevis* leaves in the management of diabetes. Further work is suggested to isolate the fraction(s)/compound(s) responsible for the activity.

Conflict of Interest

The authors report no conflict of interest. The authors alone are responsible for the conduct and writing of this manuscript.

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