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Screening of *Millettia aboensis* Leaf Extract for Antidiabetic and Antilipidemic Activities on Alloxan-Induced Diabetes: An *In-vivo* Study in Wistar Rats

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Abstract

Diabetes mellitus, a chronic metabolic disorder with increasing prevalence and associated complications, is a major cause of morbidity and mortality globally. Synthetic drugs used in its treatment are constrained by high cost and toxic effects, hence, necessitating the need to explore alternative remedies from plant origin. Plants have advantage of toxicity consideration due to their long term ancestral use as food in humans. Millettia aboensis is an edible plant in Southern Nigeria and widely employed in tradomedincinal practice in management of human ailments such as diabetes mellitus and heart-related disorders. This study was designed to screen Millettia aboensis leaf for antdiabetic and antilipidemic activities on alloxan-induced diabetes: an in-vivo study in wistar rats. This was done by extraction, phytochemical screening of Millettia aboensis extract (MAE) and randomization of wistar rats into seven groups (n=5). Alloxan monohydrate (150 mg/kg; i.p) was used to induce diabetes in groups 2-7, while group 1 served as non-diabetic normal control. Subsequently, different groups of rats received various treatments for 21 days. The results show that MAE (500 mg/kg) exhibited statistically significant ($p<0.05$)

dose- and time-dependent decrease in glucose level when compared with glibenclamide (5 mg/kg), and also dose-dependent decrease in "bad" lipids (TC, TG, LDL and VLDL) levels, with simultaneous increase in "good" lipid (HDL) level when compared with atovastatin (100 mg/kg). This study therefore concludes that Millettia aboensis leaf produces antidiabetic and antilipidemic activities.

Keywords: *Millettia aboensis*; Extraction; Phytochemical screening Antidiabetic; Antilipidemic

Introduction

Diabetes mellitus is a chronic non-communicable metabolic disorder [1], that constitutes global health concern [2] and psycho-socio-economic burdens [3] due to rise in its prevalence estimated to increase from 2.8% in 2000 to 4.4% by 2030 [4,5]. Globally, diabetes mellitus accounted for about 1.5 million deaths in the year 2019 [6] and has been predicted to reach 642 million by the year 2040 [7]. In Africa, it is estimated that about 14 million people suffer diabetes and this number is predicted to double [8].

Diabetes mellitus is characterized by persistent hyperglycemia resulting from lack or insufficient secretion of insulin, with or without concurrent insulin action. On the basis of etiology and clinical features, diabetes can be described and classified into two main types as insulin-dependent (type 1) and insulin-independent (type 2) diabetes [9,10]. However, other types of diabetes have been recognized by [11] and they include gestational diabetes, impaired glucose tolerance diabetes, malnutrition-related diabetes and diabetes associated with other conditions such as pancreatic disorder, hormonal disorder, drug or chemical induced or those arising from genetic and/or insulin receptor abnormalities.

Besides causing abnormal glucose metabolism, all types of diabetes result to development of microvascular (nephropathy) and macrovascular (dyslipidemia) complications which are the major cause of morbidity and mortality in diabetic patients [12,13]. Reports have shown coexistence of diabetes and dyslipidemia, hence, increasing the risk of coronary heart disease [14-16]. Therefore ideal antidiabetic agents should also have antidyslipidemic effect

Synthetic drugs used in treatment of diabetes are constrained by high cost and toxic effects. Plants have been reported to have advantage of toxicity consideration due to their long term ancestral use as food and medicines in humans, and therefore preferred to synthetic agents [17].

Millettia aboensis belonging to family Fabaceae, is found in rain forest zone of West African countries such as Nigeria, Cameroon and Equatorial Guinea [18]. It is characterized by dark reddish or chocolate colored wood [19] and used in tradomedicinal practice in Southern and Western Nigeria in treatment human ailments such as diabetes and heart-related disorders. Reported pharmacological actions of Millettia aboensis include antibacterial activity [20], hematopoietic activity [21], antidiabetic and antioxidant activities [22].

Based on this background and on the paucity of information on the potentials of Millettia aboensis as anidiabetic and antilipidemic agent, this study sought to agree or disagree with tradomedicinal claim and existing reports on the plant, and therefore, was designed to screen the Millettia aboensis leaf for antidiabetic and antilipidemic activities of on alloxan-induced diabetes: an in-vivo study in wistar rats.

Materials and Methods

Collection, Identification and Authentication of Plant Material

Matured fresh leaves of the plant were collected from a garden in Okigwe, South Eastern, Nigeria in the morning hours between 9-10 am. The plant material (leaves) was identified and authenticated as *Millettia aboensis*, and was assigned voucher specimen number, UPH/P/1470, in the Herbarium of Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria.

Drugs and Reagents

Alloxan monohydrate (Sigma Aldrich Chemie, Germany), Glibenclamide (Sanofi-Aventis, Nigeria Ltd.), Atorvastatin (Rambaxy Laboratories, India) Ethylacetate (Rankem, Mumbai, India), Hydrochloric acid (Nice Laboratories Reagent, Kevala, India), Sodium Tetraoxocarbonate IV (Sigma Aldrich Chemie, Germany), Tetraoxosulphate VI acid (Hi Media Laboratories Pvt Ltd, India), 96% Ethanol (Gungsdong Guandgua Chemical Factory ,China), Sodium Hydroxide (Rankem Mumbai, India), Ferric Chloride (Super Tek Chemical, Germany). Glacial acetic acid (Sigma Aldrich Chemie, Germany)

Animal Ethics Approval

Senate Research and Ethics Committee of Madonna University, Nigeria, granted the animal ethics approval for this study. Guidelines for care and handling of animals as prescribed by [23] were strictly followed.

Extraction of Plant Material

About 3.0 kg of *Millettia aboensis* leaves were dried at room temperature and milled into coarse powder. About 250 g of the milled leaves was macerated in 2.0 liter 80% ethanol (prepared by dilution from 96% ethanol) for 72 hours and agitated at 6 hourly intervals. Using Whatmann No.1 filter paper, the resulting solution was filtered. The marc was re-macerated and re-filtered (2x). The obtained filtrates were pooled together and concentrated in a flask using rotary evaporator operated at temperature of 40-45°C. Determination of yield was done by calculating the difference between the initial weight of empty flask and final weight of the flask containing the solid residue. The extraction and concentration processes were repeated severally (5x) to obtain sufficient quantity of solid crude extract. The solid extract was stored in air-tight container, and then labeled as MAE (*Millettia aboensis* extract) for subsequent use.

Phytochemical Screening

Phytochemical screening was conducted on MAE using the procedure described by [24] to test for the presence or absence of various phytochemicals. The intensity of color and/or precipitate formation indicates the abundance of phytochemical present.

Acute Oral Toxicity (Lethal Dose50) Screening

A method proposed by [25] which involved two phases was used to ascertain safe doses of MAE that could be used in subsequent animal experiment.

Phase I: In this phase, nine (9) mice were used and randomized into three groups (1-3) of three animals per group. Doses of 10, 100, and 1000 mg/kg MAE were administered per oral to group one, two and three mice respectively. The mice were thereafter monitored for signs of toxicity and/or death within 24 hours

Phase II: This phase was conducted using the rest of the animals, which were divided into four groups (1-4) of one animal per group. From the result obtained in phase I, the animal groups (one, two and three) were given 1600, 2900 and 5000mg/kg body weight of the extracts respectively then monitored for 24 hours for signs of toxicity and/or death. Group four mouse served as the control.

Induction of Diabetes

Fifty-five adult animals (rats) were allowed free access to clean drinking water and fed ad libitum with standard commercial animal feed (Top Feeds, Nigeria) but were fasted for 24 hours prior to induction of diabetes and collection of blood for fasting blood sugar determination. Diabetes was induced in fifty rats, each with single intra-peritoneal administration of 150 mg/kg alloxan monohydrate [26] reconstituted in 0.9% saline solution, and thereafter, the rats were allowed access to 10% glucose solution bottles for 24 hours to prevent initial hypoglycemia [27]. Nineteen rats died, perhaps due to hypoglycemia. Using glucometer (Accu-Check) test strips, fasting blood glucose of the survived rats was determined 72 hours post induction of diabetes. Rats with fasting blood glucose level greater than 200 mg/dl were considered diabetic [26] and thirty were selected further study in this work, while six rats showed fasting blood glucose level of less than 200mg/dl

Experimental Protocol

Five adult non diabetes-induced rats were placed as group 1, while the thirty selected diabetic adult rats were randomized into groups 2-7 (n= 5) in metabolic cages. The weight of rats in each group was between 200-220 g and was treated (per oral) as follows for 21 days:

Group 1 (non-diabetic normal control) received vehicle (3% v/v Tween 80), 10 ml/kg.

Group 2 (diabetic negative control) received vehicle (3% v/v Tween 80), 10 ml/kg.

Group 3 (diabetic positive control) received Artovastatin, 100 mg/kg.

Group 4(diabetic positive control) received Glibenclamide, 5 mg/kg.

Group 5 (diabetic test group) received MAE, 125 mg/kg.

Group 6 (diabetic test group) received MAE, 250 mg/kg.

Group 7 (diabetic test group) received MAE, 500 mg/kg.

Collection of Blood Samples for Estimation of Blood Glucose and Serum Lipid Levels

For estimation of blood glucose, blood was drawn from the tail tips of rats at day "0" and subsequently, at interval of 7 days for 21 days for determination of fasting blood glucose using glucometer test strips.

For estimation of serum lipid levels, the rats were euthanized and sacrificed under mild anesthesia with diethyl ether vapour. Blood samples were collected from various experimental animal groups by retro-orbital cardiac sinus puncture. The collected blood samples were allowed to clot and thereafter, centrifuged at 3000 rpm for 10 minutes. Lipid profile was determined by standard procedures: TC [28,29], TG [30], HDL [31], LDL [32] and VLDL [32].

Statistical Analysis of Data

Data obtained are presented in the tables as ± standard error of mean (i.e. ± SEM) of n=5, and were statistically analyzed by one-way analysis of variance (ANOVA). Duncan's Post-Hoc multiple comparison test was done using SPSS version 24. Differences between mean were considered significant at $p<0.05$

Results

Yield

The yield in each of the five rounds of extraction was low (9.87±2.61g) when compared to the quantity of plant material soaked

Phytochemistry

Phytochemical screening of MAE indicates presence of flavonoids, tannins, saponins and anthraquinones in large amounts while phenols and sterols were absent as shown in Table 1.

Acute oral toxicity test

This test reveals that up to 5000 mg/kg MAE, there were no signs of toxicity and/or lethality within 48 hours observation in the mice. In the present study, 125, 250 and 500 mg/kg were chosen to respectively represent low, medium and high dose.

Antidiabetic activity

As shown in Table 2, MAE produces dose- and time-dependent decrease in blood sugar level that can be compared to the standard drug, glibenclamide. MAE and glibenclamide respectively produce 40.86% and 60.34% decrease in blood sugar level at day 21, as shown in Table 2.

Antilipidemic activity

Administration of alloxan monohydrate significantly ($p<0.05$) increased blood sugar and serum lipid levels in the diabetic groups (2-7) when compared to non-diabetic normal control (group 1). MAE produces dose-dependent decrease and increase in level of "bad" and "good" lipids respectively as shown in Table 3.

Table 1: Phytochemical Screening of *Millettia aboensis* Leaf Extract

Phytochemicals	*M. aboensis*
Flavonoids	+++
Tannins	+++
Saponins	+++
Alkaloids	++
Glycosides	++
Phenols	–
Anthraquinones	+++
Glucose	+
Sterol	–

– = absent

+ = present in small amount

++ = present in moderate amount

+++ = present in large amount

Table 2: Effect of MAE on Blood Glucose Concentration on Alloxan-induced Diabetes in Wistar Rats

Treatment Group	**Mean fasting blood glucose level ± SEM (mg/dl)**						
	Day 0	**Day 7**	**%Change**	**Day 14**	**%Change**	**Day 21**	**% Change**
1	95.26±3.61[b,c]	93.54±1.71[b,c]	1.81↓	96.68±2.26[b,c]	1.49↑	94.05±2.61[b,c]	1.27↓
2	300.18±2.02[a]	312.41±4.16[a,c]	4.07↑	328.06±3.45[a,c]	9.29↑	341.53±1.87[a,c]	13.76↑
3	297.72±2.16[a]	262.60±3.53[a,b,c]	11.80↓	249.31±1.82[a,b,c]	16.26↓	231.46±2.66[a,b]	22.26↓
4	290.64±3.84[a,b]	198.37±2.77[a,b]	31.75↓	161.61±3.18[a,b]	44.40↓	115.27±1.63[a,b]	60.34↓
5	304.16±4.10[a,c]	291.62±2.32[a,c]	4.12↓	275.43±1.35[a,b,c]	9.45↓	252.23±2.71[a,b,c]	17.07↓
6	298.44±2.66[a]	282.15±3.49[a,c]	5.46↓	244.60±2.82[a,b,c]	18.04↓	221.75±2.69[a,b]	25.70↓
7	294.82±3.33[a,b]	276.34±2.44[a,b,c]	6.27↓	183.85±3.75[a,b]	37.64↓	174.37±3.10[a,b]	40.86↓

Values represent ±SEM of n=5; [a]p<0.05 vs group1; [b]p<0.05 vs group 2; [c]p<0.05 vs group 4;

↑ Represents increase relative to initial fasting blood glucose (i.e. at day "0") of each group

↓Represents decrease relative to initial fasting blood glucose (i.e. at day "0") of each group

Table 3: Effect of MAE on Serum Lipid Level after 21 Days on Alloxan-induced Diabetes in Wistar Rats

Treatment Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL(mg/dl)
1	86.90±1.63[b,c]	63.56±3.11[b]	39.64±2.61[b,c]	57.03±1.70[b,c]	35.22±2.38[b]
2	112.36±3.11[a,c]	75.85±2.48[a,c]	29.50±2.85[a,c]	72.25±1.49[a,c]	51.48±2.55[a,c]
3	65.24±2.08[a,b,c]	52.92±3.23[a,b]	54.21±1.1.97[a,b,c]	42.35±2.12[a,b]	30.75±1.64[a,b]
4	71.40±2.35[a,b]	56.06±2.21[a,b]	49.62±3.37[a,b]	45.20±3.15[a,b]	33.43±2.41[b]
5	82.63±1.91[b,c]	63.15±2.53[b,c]	40.71±2.35[b,c]	55.65±2.03[b,c]	35.16±2.28[b]
6	77.51±2.42[a,b]	61.42±3.38[b,c]	42.81±1.58[b]	54.16±3.51[a,b]	35.07±1.17[b]
7	74.19±2.27[a,b]	57.11±3.26[b]	47.13±2.52[a,b]	49.42±1.86[abc]	34.51±3.05[b]

Values represent ±SEM of n=5; [a]p<0.05 vs group1; [b]p<0.05 vs group 2; [c]p<0.05 vs group 4

Discussion

Plants of natural origin are rich source of phytonutrients that possess interesting therapeutic potentials in ameliorating human ailments [20]. Result of phytochemical screening of MAE shown in Table 1 provides evidence of presence of flavonoids, tannins, saponins and anthraquinones alkaloids and glycosides, which have been reported to produce antidiabetic and antilipidemic properties [33] via several mechanisms such as modulation of activities related to glucose and lipid homeostasis [34], stimulation of insulin secretion/mimicry [35], eliciting insulin-like action by promoting glucose uptake by the muscles[36] and regeneration of pancreatic beta cell [35].

The antidiabetic activity of MAE was screened by measuring fasting blood sugar (FBS) at interval of 7 days for 21days after initial experimental induction of diabetes in adult wistar rats, using a single intrapritoneal injection of 150 mg/kg alloxan monohydrate [26,37]. FBS is a measure of blood glucose level after a 12-18 hour period of fasting. During this period, glucagon is released which in turn stimulates the release of glucose into the blood stream, hence, leading to elevated glucose level [38]. In non-diabetic condition, insulin is secreted to counter the elevated glucose level, but in diabetic condition insulin secretion is insufficient and/or impeded, therefore glucose level in the blood persistently remains high [38,39].

Alloxan monohydrate induces elevated glucose level through its selective cytotoxicity that destroys insulin-producing beta cells of islet of Langerhans of the pancreas [40]. The cytotoxicity of alloxan is mediated via formation of reactive oxygen species (ROS) particularly hydrogen peroxide, in association with massive elevated cytosolic Ca2+ level which cause beta cell DNA fragmentation, apoptosis and consequent reduction in the capacity of pancreas to secret insulin [37,41-43].

From the result of the study (Table 2), the diabetic rats treated with MAE showed dose- and time-dependent decreases in FBS when compared to non-diabetic normal and diabetic negative control groups, with maximum activity of 17.07%, 25.70% and 40.86% decrease observed after 21 days of treatment with 125, 250 and 500 mg/kg MAE respectively. The decrease in blood sugar level (40.86%) produced by MAE is comparable to that produced by glibenclamide (60.36%), hence showing its effectiveness as an antidiabetic agent in alloxan-induced diabetic rat: an action that may be attributed to the presence of some phytochemicals in the plant, which can be corroborated by other reports on flavonoids [44-46], saponins [47,48] and tannins [49], that by their antioxidant activities, can regenerate damaged beta cells of the pancreas thereby bringing about increased insulin secretion or insulin transport and utilization in tissues. Furthermore, studies have shown that antidiabetic principles such as phytochemicals are present in different plants in varying amounts, and can lower blood glucose by several mechanisms [36,50-52]. In Millettia aboensis leaf, these phytochemicals (Table 1) could be acting independently or in combination to produce additive or synergestic antidi-

abetic and antilipidemic actions.

Studies have shown that a positive relationship existing between gluconeogenesis and lipogenesis [53], and that one of the macrovascular complications of diabetes is dyslipidemia which include elevated serum levels of TC, TG, LDL, VLDL and decreased HDL level [15,54,55]. Diabetes-induced dyslipidemia results from excess fat mobilization from adipose tissues due to underutilization of glucose [56]. In diabetic state, an enzyme (lipoprotein lipase) that catalyses hydrolysis of triglycerides is inactivated, hence, resulting to elevated serum level of "bad" lipids (TC, TG, LDL, VLDL) and reduced level of "good lipid (HDL) [57]. The result in Table 3 indicates that the extract-treated diabetic rats exhibited significant dose-dependent reduction in serum levels of "bad" lipids, with simultaneous increase in "good" lipid serum level that can be comparable to glibenclamide ($p<0.05$) and atovastatin ($p<0.05$): actions that may be linked to the presence of phytochemicals in Millettia aboensis leaf. This finding is supported by other reports on the same plant [58] and other plants with antidiabetic and lipidemic properties [27,52,59], and also by the evidences that phytochemicals such as alkaloids [60] and saponins [47,48,61,62] present in plants produce antilipidemic activity by enhancing glucose utilization, which in turn suppresses lipid peroxidation and lipolysis, hence, ameliorating dyslipidemia.

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