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Modulatory Effect of *Morinda lucida* Aqueous Stem Bark Extract on Blood Glucose and Lipid Profile in Alloxan-Induced Diabetic Rats

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ABSTRACT

The present study evaluates the antidiabetic and antihyperlipidemic effects of 125 mg/kg, 250 mg/kg and 500 mg/kg of the *Morinda lucida* aqueous stem bark extract (MLASE) in alloxan-induced diabetic rats for 8 days based on the African traditional use of the water infusion of the stem bark of *Morinda lucida* (family: Rubiaceae) in the local management of diabetes mellitus and hyperlipidemia by the Yoruba herbal practitioners (Southwest Nigeria). In this study, experimental diabetes was induced in Wistar rats through intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline and sustained hyperglycemia was monitored over the successive three days. Diabetic rats [with the fasting blood glucose (FBG) levels greater or equal to 200 mg/dl] randomly divided into Groups II-VI of six rats per group were daily and orally treated with 10 ml/kg of distilled water, 5 mg/kg of glibenclamide, 125 mg/kg, 250 mg/kg and 500 mg/kg of MLASE, respectively, for 8 days after which the rats were sacrificed humanely under inhaled diethyl ether. FBG were determined in the treated diabetic rats on the 1st and 8th day of oral extract treatments while the blood samples for the lipid assays were obtained directly from heart chambers through cardiac puncture on the 8th day after an overnight fasting. In addition, preliminary qualitative and quantitative analyses of the aqueous stem bark of *Morinda lucida* were conducted using standard procedures. Results showed that the preliminary qualitative analysis of MLASE revealed the presence of flavonoids, alkaloids, saponin, terpenoids, phenols, tannins and phlobatannins while cardiac glycosides, steroid glycosides and anthraquinones were absent. Similarly, quantitative analysis of MLASE showed the presence of tannin, alkaloids, phenols, sugar and saponin at the concentrations of 29.73 ± 0.38 mg/100 g, 25.69 ± 0.10 mg/100 g, 37.15 ± 0.10 mg/100 g, 37.38 ± 0.08 mg/100 g and 54.84 ± 0.21 mg/100 g of MLASE, respectively. Repeated daily oral treatments with 125-500 mg/kg of MLASE significantly ($p < 0.01$ and $p < 0.001$) lowered the FBG dose dependently with highest antidiabetic effect recorded for the highest dose. Similarly, 125-500 mg of MLASE significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$) decreased serum TG, TC, LDL-c and VLDL-c levels while significantly increasing serum HDL-c levels. Overall, results of this study showed MLASE to possess antihyperglycemic and antihyperlipidemic effects in experimental diabetic rats which was probably mediated via enhanced peripheral glucose utilization and/or enhanced insulin secretion.

Key words: *Morinda lucida*, aqueous stem bark extract, alloxan-induced diabetes, antihyperglycemia, antihyperlipidemia

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INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder essentially characterized by insufficiency of insulin secretion

and insulin receptor or post-receptor events with derangement in carbohydrate, protein and lipid metabolism resulting in chronic hyperglycemia with the fasting blood glucose concentration equal or greater than 140 mg/dl taken at least on two separate occasions (Palsamy and Subramanian, 2010;

Nyamthabad and Umesh, 2014). DM is a rampant global epidemic of multifactorial etiology and risk predisposing factors such as the increasing ageing population, genetic and environmental factors, fast evolving and increasing sedentary life style and dietary changes, etc. (Weeratinga *et al.*, 2014). Available statistics show that the global estimated prevalence for the disease stands at 285 million adults (6.4% of the world's population) in 2010 and this figure is projected to rise to 439 million adults (7.7%) by 2030 (Shaw *et al.*, 2010). It was also estimated that a rise in the prevalence of diabetes and diabetes-related deaths is more marked in low- and middle-income countries (Mathers and Loncar, 2006). Diabetes mellitus, also, caused 1.5 million deaths in 2012. Higher-than-optimal blood glucose caused an additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases. Forty-three percent of these 3.7 million deaths occur before the age of 70 years. The percentage of deaths attributable to high blood glucose or diabetes that occurs prior to age 70 is higher in low- and middle-income countries than in high-income countries (World Health Organization, 2016). Most of the deaths are estimated to result from diabetes complications such as heart disease and stroke, neuropathy, nephropathy, cataracts, microangiopathy, atherosclerosis and retinopathy (Baynes and Thorpe, 1997; and non-alcoholic fatty liver disease (Katsiki *et al.*, 2017).

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control (American Diabetes Association, 2017). An effective control of hyperglycemia is the hallmark in the management of DM and this is achievable through the use of standard conventional antidiabetic drugs such as the insulins and various classes of oral antihyperglycemic agents such as insulin secretagogues (sulphonyureas: glibenclamide, meglitinides), insulin sensitizers (biguanides: metformin; thiazolidinediones), α -glucosidase inhibitors (e.g. acarbose, miglitol), peptide analogues (exenatide, liraglutide and DPP-4 inhibitors) and insulin injections (which could be short-acting, intermediate-acting or long-acting insulin prototype) (Patel *et al.*, 2011; Patel *et al.*, 2012). Also, reports have it that DM is effectively managed using local medicinal/herbal remedies of different families and these include Cucurbitaceae, Chenopodiaceae, Acanthaceae, Apocynaceae, Myrtaceae, Asteraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Moraceae, Anacardiaceae, Amaranthaceae, Meliaceae, Rubiaceae, Poaceae and Rutaceae (Gunjan *et al.*, 2011; Patel *et al.*, 2012; Sidhu and Sharma, 2013).

Morinda lucida Benth, belonging to the Rubiaceae family, is a medium-sized tree used as a medicinal plant in West Africa (especially in Nigeria) in the local treatment of malaria and other febrile conditions, diabetes, hypertension, cerebral congestion, dysentery, stomach ache, ulcers, leprosy, and gonorrhoeal (Olajide *et al.*, 1999; Oliver-Bever, 2007; Adeneye and Agbaje, 2008; Adebayo and Krettli, 2011). Different parts of the plant have been reported to possess antimicrobial (Fakoya *et al.*, 2014) as well as antidiabetic properties. For example, while the aqueous leaf extract of *Morinda lucida* has been reported to cause antidiabetic effect in streptozotocin-induced diabetic rats (Olajide *et al.*, 1999), the ethanolic leaf extract of the same plant elicited antidiabetic effects in both normal and alloxan-induced diabetic rats (Adeneye and

Agbaje, 2008). In the same vein, repeated oral treatment with a single dose of 240 mg/kg of the aqueous and ethanolic extracts of *Morinda lucida* stem bark has also been reported to possess antidiabetic effect in alloxan-induced diabetic rats (Oduyiga *et al.*, 2010). However, the present study is designed at investigating the antidiabetic, antihyperlipidemic and the possible mechanism(s) of action of 125 mg/kg, 250 mg/kg and 500 mg/kg of the aqueous stem bark extract of *Morinda lucida* in alloxan-induced diabetic rats for 8 days. In addition, preliminary qualitative and quantitative analyses of the aqueous stem bark of *Morinda lucida* were conducted.

MATERIALS AND METHODS

Plant sample collection and preparation

Two kilograms (2 kg) of fresh stem bark of *Morinda lucida* were collected from an uncultivated farmland on the outskirts of Low Cost Housing Estate, Oke-Afa, Isolo, Lagos State, Nigeria in the month of April, 2014. The harvested plant materials were processed for voucher referencing as previously described by Adeneye and Agbaje (2008). The fresh stem bark peels were gently rinsed under tap water and dried under laboratory room temperature protected from direct heat and sunlight for 3 weeks. Afterwards, the dried samples were pulverized using laboratory hammer-mill in the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi-Araba, Surulere, Lagos State, Nigeria.

Aqueous extraction

Fifty gram (50 g) of the pulverized sample was boiled in 500 ml of distilled water under continuous stirring for 1 hour after which it was filtered using a piece of clean white 2-layer cotton cloth. The filtrate was then transferred to an aerated oven already preset at 40 °C and completely air-dried until solid residue was left behind. The procedure was repeated two more times. The residue obtained on each extraction process was kept in a water- and air-proof container in the refrigerator maintained at -4°C until required for experimentation.

Preliminary qualitative phytochemical analysis of MLASE

Preliminary phytochemical analyses of the *Morinda lucida* aqueous stem bark extract were done using standard procedure as Sofowora (1993). The procedures are summarized below:

Test for tannins

-Ferric chloride test: The dried powdered samples (0.5 g) was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for phlobatinins

Deposition of a red precipitate when MLASE sample was boiled with 1% aqueous hydrochloric acid on a water bath for 5 minutes was taken as evidence for the presence of phlobatinins.

Test for saponin

-Froth test: The powdered sample (2 g) was boiled in 20 ml of distilled water in a water bath and then filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken

vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for formation of emulsion.

Test for flavonoids

Three methods were used to determine the presence of flavonoids in the plant samples.

a. A portion of the extract solution was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution and color changes were observed. Production of yellow precipitate is considered as positive for flavonoids.

b. *Lead acetate test*: To a solution of 0.5 g extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

c. Few drops of 1% aluminium chloride solution was added to about 2 ml of the plant extract solutions then color changes were observed. Production of yellow precipitate is considered as positive for flavonoids.

d. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of MLASE followed by addition of concentrated H₂SO₄. A yellow coloration observed in the MLASE solution indicated the presence of flavonoids. However, the yellow coloration disappeared on standing.

Test for steroidal compound

The Liebermann's test was employed to determine the presence of terpenoids in the *Morinda lucida* aqueous stem bark extract. In a typical assay, 0.5 g of MLASE was dissolved in 2 ml of acidic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A color change from violet to blue or green indicates the presence of steroidal nucleus (i.e. aglycone portion of the cardiac glycosides).

Quantitative determination of secondary metabolites in MLASE

Preparation of fat free sample: 2 g of MLASE was exhaustively defatted with 100 ml of diethyl ether using a Soxhlet extractor for 2 hours as previously described by Edeoga *et al.* (2005).

Total phenols quantification: The total phenolic content in MLASE was determined by spectrophotometric method as described by Edeoga *et al.* (2005). The defatted MLASE sample was boiled in 50 ml of ether for 15 min to extract the phenolic component. Aliquot of the extract (5 ml) was pipetted into a 50 ml flask and made up to 15 ml with distilled water. Two (2) ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were left to react for 30 min for color development and later read at 505 nm on a spectrophotometer.

Alkaloid quantification: The alkaloid content of MLASE was determined using the method earlier described by Harborne (1973). 5 g of MLASE was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The resulting solution was covered and allowed to stand for 4 hours, filtered and the filtrate concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was

added drop-wise to the concentrate until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and rinsed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin quantification: Tannin content of MLASE was estimated using the method of Van-Burden and Robinson (1981). 500 g of defatted MLASE was weighed into a 50 ml plastic bottle. Distilled water (50 ml) was added and shaken continuously for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Following this, 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance of the resulting mixture was measured at 120 nm within 10 min.

Saponin quantification: Saponin was estimated by the method previously used by Obadoni and Ochuko (2001). Two (2) g of MLASE was placed into a conical flask and 10 ml of 20% aqueous ethanol was added. The resulting mixture was heated over a hot water-bath for 4 hours under continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 20 ml 20% ethanol. The combined filtrate was reduced to 4 ml over water-bath at 90 °C. The concentrate was transferred into a 50 ml separating funnel and 2 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The extraction process was repeated one more time and n-butanol was added to the combined aqueous portion. The resulting mixture was shaken and washed twice with 1 ml of 5% aqueous sodium chloride and filtered while the resulting solution was heated over a water-bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content calculated as percentage of the extract.

Reducing sugar quantification: Reducing sugar content in MLASE was determined using spectrophotometric method as described by Shaffer and Somogyi (1933).

Experimental animals

Fifty (50) healthy young adult male albino Wistar rats (weighing 110-150 g) used in this study were obtained from Bayo Farms, Sango-Otta, Ogun State, Nigeria, after an ethical approval for the study was obtained. The rats were housed in polypropylene cages and handled in accordance with international principles guiding the Use and Handling of Experimental Animals (United States National Institutes for Health, 1985). Rat feed (Livestock Feeds, Lagos, Nigeria) and tap water were provided *ad libitum*. The rats were maintained at an ambient temperature between 23-26 °C, humidity of 60 ± 5%, and 12 hour day/night photoperiod.

Induction of experimental diabetes: Experimental type 1 diabetes was induced in 35 young adult male Wistar rats using the method described by Venugopal *et al.* (1998) and as modified by Iwalewa *et al.* (2008). Rats were injected with freshly prepared 120 mg/kg body weight of alloxan monohydrates dissolved in sterile cold 0.9% normal saline, given via the intraperitoneal route. The rats were then orally treated with 5% dextrose solution for the next 24 hours in order

to prevent hypoglycemia which often accompanies alloxan-associated hyperinsulinemia resulting from massive pancreatic β -cells destruction (Gupta *et al.*, 1984). Fasting blood glucose levels in rats were measured on the 3rd day post-alloxan injection and diabetic rats with fasting blood glucose levels equal to or above 200 mg/dl were considered diabetic and used for the study.

Body weight measurement: Body weights of all rats were measured on the 1st and 8th day after establishing diabetes induction using digital mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 1st and 8th day in reference to the initial weight per group was calculated.

Experimental design and oral treatment of alloxan-induced diabetic rats

Oral treatments of alloxan-induced diabetic rats for 8 days with oral cannula were as follows:

Group I: consists of six normal control rats that received 10 ml/kg and 1 ml/kg of distilled water via the oral and intraperitoneal routes, respectively

Group II: consists of six alloxan-diabetic rats that orally administered 10 ml/kg of distilled water

Group III: consists of six alloxan-diabetic rats orally administered 5 mg/kg of glibenclamide in distilled water

Group IV: consists of six alloxan-diabetic rats orally treated with 125 mg/kg of MLASE in distilled water

Group V: consists of alloxan-diabetic rats orally treated with 250 mg/kg of MLASE in distilled water

Group VI: consists of alloxan-diabetic rats orally treated with 500 mg/kg of MLASE in distilled water

Oral glucose tolerance test: Following an overnight fast but water made available and on the 5th day post-oral treatment with MLASE, the diabetic rats were subjected to 2-hour oral glucose tolerance test with 3 g/kg of D-glucose dissolved in distilled water. Initial baseline FBG was determined by tail tipping method after which the Groups II-VI diabetic rats were orally pre-treated with 10 ml/kg of distilled water, 5 mg/kg of glibenclamide in distilled water and 125 mg/kg, 250 mg/kg, 500 mg/kg of MLASE in distilled water, respectively, 1 hour before oral glucose load at 3 g/kg body weight. Following oral glucose load, serial blood glucose levels were measured at 0, 30, 60, 90 and 120 min using tail tipping method.

Blood glucose measurement: Whole fasting blood glucose (FBG) of treated rats was collected by tail tipping method and determined by the glucose oxidase method of Trinder (1969) using a One Touch Basic Blood Glucose Monitoring System[®] (Life Scan Inc., Milpitas, California, U.S.A.). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment.

Bioassay: On day 8, after an overnight fast, the final fasting blood glucose was determined before the rats were sacrificed after light diethyl anesthesia. After anesthesia, blood samples were collected directly from the heart chamber into 10 ml plain bottles. The blood samples obtained were cold centrifuged at -

70 °C at 3000 rpm for 20 min to separate out the serum that was then analyzed for the lipids [triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c)] using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.) on Automated Clinical System (Synchron Clinical System[®], model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland). Serum LDL-c was estimated using Friedlwann's equation: $LDL-c = [TC - (HDL-c + TG/5)]$

Serum very low density lipoprotein cholesterol fraction (VLDL-c) was calculated by deduction of the sum of HDL-c and LDL-c concentrations from that of TC as represented by the equation: $VLDL-c = [TC - (HDL-c + LDL-c)]$

Statistical Analysis

Results were presented as mean \pm S.E.M. for body weights and % weight changes while that of FBG and serum lipids were expressed as mean \pm S.E.M. of six observations. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-Newman-Keuls test, on SYSTAT 10.6. Statistical significance were considered at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

Extraction of MLASE: Extraction of MLASE yielded a deep brown, sticky, solid residue which is soluble in petroleum ether, methanol, ethanol, butan-1-ol and water. The calculated yield was $12.87 \pm 0.41\%$

Preliminary qualitative analysis of MLASE: Preliminary phytochemical analysis of MLASE showed the presence of flavonoids, alkaloids, saponin, terpenoids, phenols, tannins and phlobatinnins while cardiac glycosides, steroid glycosides and anthraquinones were absent.

Quantitative analysis of phytochemical constituents of MLASE: Quantitative analysis of MLASE showed the presence of tannin, alkaloids, phenols, sugar and saponin at 29.73 ± 0.38 mg/100 g, 25.69 ± 0.10 mg/100 g, 37.15 ± 0.10 mg/100 g, 37.38 ± 0.08 mg/100 g and 54.84 ± 0.21 mg/100 g of MLASE, respectively.

Effect of 125, 250 and 500 mg/kg of MLASE on average body weight and percentage body weight changes in alloxan-induced diabetic rats: Single intraperitoneal treatment with 120 mg/kg of alloxan monohydrate in cold 0.9% normal saline resulted in significant ($p < 0.001$) weight loss in the treated rats when compared to untreated normal rats (Table 1). This weight loss was significantly ($p < 0.001$ and $p < 0.0001$) attenuated dose-dependently by repeated daily oral treatment with 125 mg/kg, 250 mg/kg and 500 mg/kg of MLASE in alloxan-induced diabetic rats for 8 days (Table 1). However, the most significant ($p < 0.001$) weight loss attenuating effect induced by 500 mg/kg of MLASE was comparable to that of 10 mg/kg of glibenclamide oral treatment (Table 1).

Table 1:

Effect of repeated oral treatment with 125-500 mg/kg of MLASE on average body weight and percentage body weight change in alloxan-induced diabetic rats

Group		body weight (g)		percentage weight changes
		on day 1	on 8th day post-MLASE	on 8th day post-MLASE (%ΔWt)
I	Normal rats + oral 10 ml/kg of distilled water	194.3 ± 14.2	224.0 ± 9.2 ^c	15.6 ± 5.2
II	Alloxan-induced diabetic rats + oral 10 ml/kg of distilled water	194.4 ± 8.6	172.7 ± 15.8 ^f	11.3 ± 4.4 ^f
III	Alloxan-induced diabetic rats + oral 5 mg/kg of glibenclamide	213.3 ± 19.3	231.1 ± 14.4 ^c	6.8 ± 3.6 ^{c+}
IV	Alloxan-induced diabetic rats + oral 125 mg/kg of MLASE	201.6 ± 11.9	189.1 ± 8.6 ^d	-6.1 ± 2.5 ^{a+}
V	Alloxan-induced diabetic rats + oral 250 mg/kg of MLASE	226.1 ± 13.9	240.0 ± 13.1 ^c	1.5 ± 3.5 ^{b+}
VI	Alloxan-induced diabetic rats + oral 500 mg/kg of MLASE	223.6 ± 16.7	242.7 ± 17.1 ^c	8.6 ± 2.6 ^{c+}

^c represents a significant increase in weight at $p < 0.001$ while ^d and ^f represent significant decreases in body weight on 8th day post-MLASE at $p < 0.01$ and $p < 0.001$, respectively, when compared to initial body weight on day 1. ^f represents a significant decrease at $p < 0.001$ when compared to Group I value while ^{a+}, ^{b+} and ^{c+} represent significant increases at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively when compared to Group II values

Table 2:

Effect of repeated oral treatment with 125-500 mg/kg of MLASE on the fasting blood glucose levels and its percentage change in alloxan-induced diabetic rats

Group	Treatment	Fasting blood glucose (FBG) levels (mg/dl)		
		on day 1	3 rd day PI (with %ΔFBG)	8 th day post-MLASE (with %ΔFBG)
I	Normal rats + oral 10 ml/kg of distilled water	51.9 ± 7.3	51.7 ± 1.6 (1.4 ± 6.2)	52.3 ± 0.9 (1.4 ± 1.9)
II	Alloxan-induced diabetic rats + oral 10 ml/kg of distilled water	52.3 ± 1.7	228.3 ± 4.7 (336.8 ± 8.5) ^c	247.6 ± 3.0 (8.6 ± 1.4) ^c
III	Alloxan-induced diabetic rats + oral 5 mg/kg of glibenclamide	59.1 ± 8.1	237.0 ± 4.8 (305.7 ± 17.2) ^c	107.1 ± 4.0 (-54.6 ± 2.2) ^f
IV	Alloxan-induced diabetic rats + oral 125 mg/kg of MLASE	54.0 ± 5.5	231.1 ± 4.2 (331.1 ± 14.6) ^c	169.1 ± 2.2 (-26.6 ± 2.1) ^e
V	Alloxan-induced diabetic rats + oral 250 mg/kg of MLASE	55.4 ± 10.1	228.6 ± 4.8 (323.5 ± 28.3) ^c	135.1 ± 2.52 (-40.6 ± 2.2) ^f
VI	Alloxan-induced diabetic rats + oral 500 mg/kg of MLASE	51.1 ± 6.0	228.0 ± 5.5 (349.5 ± 10.7) ^c	101.1 ± 3.0 (-55.5 ± 2.2) ^f

^c represents a significant increase in FBG value at $p < 0.001$ when compared to FBG value on day 1 while ^e and ^f represent significant decreases in FBG values at $p < 0.01$ and $p < 0.001$, respectively, when compared to the 3rd day post-alloxan induction (PI) values and Group II values on the 8th day post-MLASE treatment

Effect of 125 mg/kg, 250 mg/kg and 500 mg/kg of MLASE on post-treatment FBG and percentage post-treatment FBG changes in alloxan-induced diabetic rats:

Intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline significantly ($p < 0.001$) resulted in sustained hyperglycemia (above 200 mg/dl) by the 3rd day post-alloxan treatment (Table 2).

Subsequent repeated daily oral treatments with 10 mg/kg of glibenclamide and 125-500 mg/kg of MLASE for 8 days resulted in significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) dose-related reductions in the FBG as well as the post-extract treatment FBG changes (Table 2).

Effect of 5 mg/kg of glibenclamide and 125 mg/kg, 250 mg/kg and 500 mg/kg of MLASE on OGTT in alloxan-induced diabetic rats:

Oral administration of a single load of 3 g/kg of D-glucose to untreated alloxan-diabetic rats resulted in significant ($p < 0.001$) and sustained elevations in the post-pandrial blood glucose levels from 30 min to 120 min relative to the basal value at 0 min (Figure 1). However, in alloxan-induced diabetic rats pre-treated with 5 mg/kg of glibenclamide and 125-500 mg/kg of MLASE 1 hour before oral gavage with 3 g/kg of D-glucose dissolved in 10 ml/kg of distilled water, there was initial significant ($p < 0.001$) elevations in the 30 min-

postprandial glucose levels when compared to the basal glucose levels at 0 min (Figure 1). These profound increases in the blood glucose levels was subsequently succeeded by dose- and time-dependent significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) reductions in the blood glucose levels at 60-120 min when compared to 30 min values as well as Group II blood glucose values at 60-120 min (Figure 1).

Effect of 125 mg/kg, 250 mg/kg and 500 mg/kg of MLASE on post-treatment serum lipid profile in alloxan-induced diabetic rats: Intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline significantly ($p < 0.05$) elevated serum total cholesterol, VLDL-c and LDL-c while causing non-significant ($p > 0.05$) changes in the serum triglyceride and HDL-c values when compared to untreated normal rat values (Table 3).

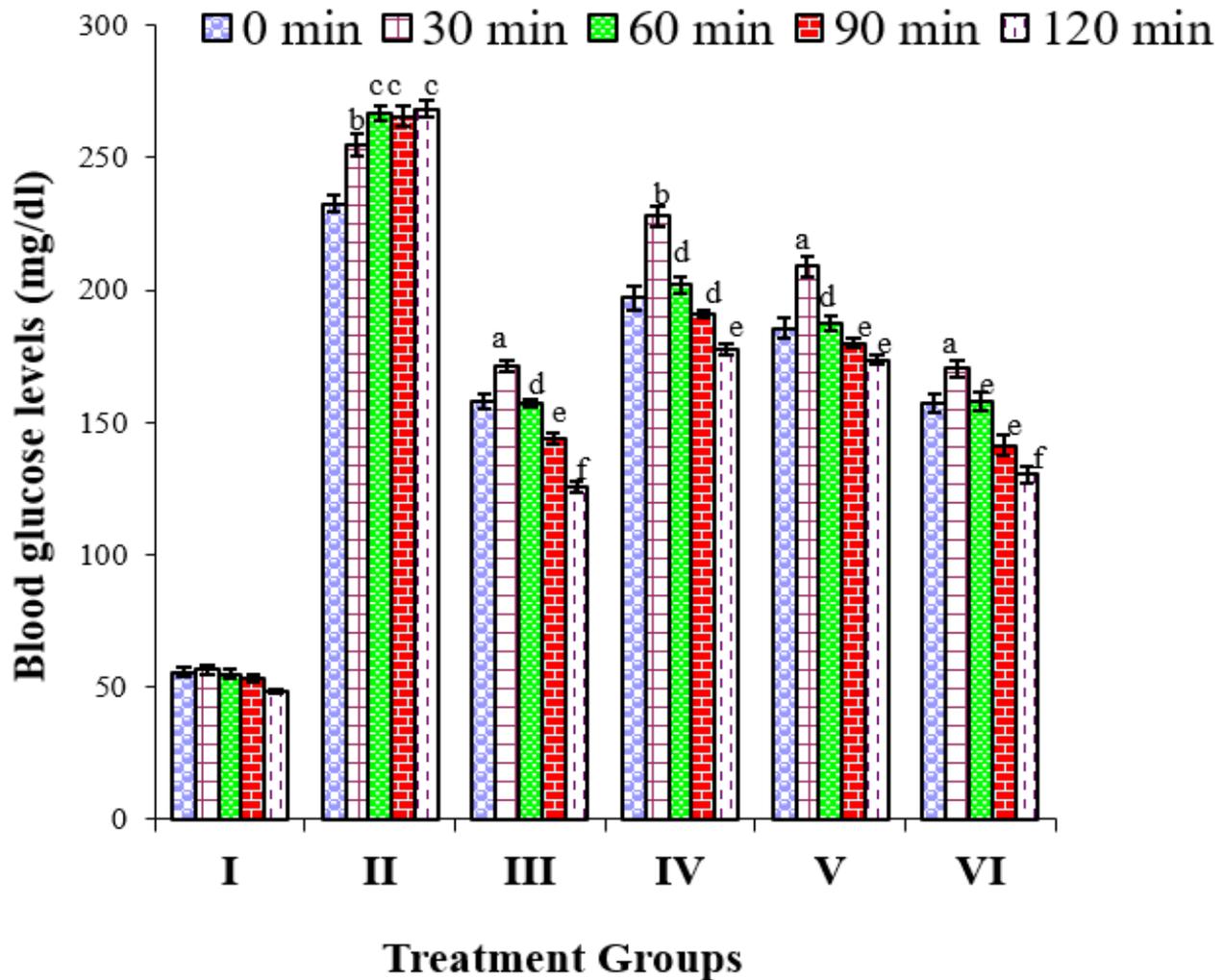


Figure 1:

120 min-oral glucose tolerance response to 3 g/kg D-glucose dissolved in distilled water administered to alloxan-induced diabetic rats pre-treated with 10 ml/kg distilled, 5 mg/kg glibenclamide and 125 mg/kg, 250 mg/kg and 500 mg/kg MLASE. ^a and ^b represent significant increases in postprandial blood glucose levels at 30 min, at $p < 0.05$ and 0.01 , respectively, when compared to 0 min values while ^d, ^e and ^f represent significant reductions in the postprandial blood glucose levels when compared to 30 min value and Group II values at 30-120 min.

I = normal rats + oral 10 ml/kg of distilled water; II = alloxan-induced diabetic rats + oral 10 ml/kg of distilled water; III = alloxan-induced diabetic rats + oral 5 mg/kg of glibenclamide dissolved in distilled water; IV = alloxan-induced diabetic rats + oral 125 mg/kg of MLASE dissolved in distilled water; V = alloxan-induced diabetic rats + oral 250 mg/kg of MLASE dissolved in distilled water; VI = alloxan-induced diabetic rats + oral 500 mg/kg of MLASE dissolved in distilled water

Table 3:

Effect of repeated oral treatment with 125-500 mg/kg of MLASE on serum lipids in alloxan-induced diabetic rats

Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	VLDL-c (mg/dl)	LDL-c (mg/dl)
I	79.1 ± 3.2	121.9 ± 8.8	46.0 ± 1.2	23.9 ± 1.5	9.3 ± 2.4
II	92.7 ± 1.5a	129.7 ± 9.7	42.3 ± 1.9	28.7 ± 1.6a	21.4 ± 1.6c
III	86.9 ± 1.9	92.0 ± 2.6	50.0 ± 1.2	18.3 ± 0.5 ^f	18.6 ± 1.2
IV	91.1 ± 4.8a	95.4 ± 2.4	45.3 ± 2.1	19.6 ± 0.6e	25.4 ± 3.1
V	78.3 ± 6.0 ^d	81.9 ± 7.61 ^d	43.6 ± 3.2	18.3 ± 1.7 ^f	16.4 ± 4.2 ^d
VI	64.9 ± 5.8 ^e	75.9 ± 6.3 ^f	39.7 ± 2.8	19.1 ± 2.3 ^f	5.9 ± 1.8 ^f

a and c represent significant increases at $p < 0.05$ and $p < 0.0001$, respectively when compared to Group I values while d, e and f represent significant decreases $p < 0.05$ and $p < 0.001$ and $p < 0.0001$, respectively, when compared to Group II values

However, repeated daily oral treatments with 5 mg/kg of glibenclamide and 125-500 mg/kg of MLASE for 8 days resulted in significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) dose-related reductions in the serum TG, VLDL-c and LDL-c while the extract treatment caused non-significant alterations in the serum HDL-c when compared to the values obtained for the untreated alloxan diabetic rats (Table 3)

DISCUSSION

Diabetes mellitus is a chronic heterogeneous endocrine and metabolic disorder affecting carbohydrate, fat, and protein metabolism characterized by a general hallmark of persistent fasting and postprandial hyperglycemia (Silva-Sousa *et al.*, 2003; Joseph and Jini, 2011). For experimental basic research purposes, alloxan amongst other diabetogenic agents are often generally used to induce diabetes in experimental animals. Alloxan is known to selectively destroy pancreatic β -cell population (Malaisse *et al.*, 1982; Adeneye *et al.*, 2007).

A sudden selective destruction of pancreatic β -cells is known to occur when the free radicals generated by alloxan injection undergo dismutation to hydrogen peroxide with a concurrent massive increase in cytosolic calcium concentration and cellular death of the pancreatic β -cells (Stepple and Horton, 2004; Lenzen, 2008; Sollu *et al.*, 2010). Consequent upon β -cell destruction is hypoinsulinemia with attendant hyperglycemia, hyperlipidemia, hyperketonemia and lactic acidemia (Mansford and Opie, 1968; Szkudelski, 2001). One of the fundamental bases of hyperglycemia and hyperlipidemia in diabetic state is the overproduction of glucose by means of excessive hepatic glycogenolysis due to activation of glycogen phosphorylase and gluconeogenesis occasioned by the enhanced *de novo* glucose synthesis from non-glucose precursors (Hui *et al.*, 2009).

In the present study, experimental diabetes was reliably established in the rats intraperitoneally injected with alloxan monohydrate by the 3rd day post-treatment which was characterized by hyperglycemia that was sustained throughout the 8 days of treatment as demonstrated in the untreated diabetic rats. This persistent hyperglycemia was associated with hyperlipidemia which was characterized by corresponding high serum TG, TC, LDL-c and VLDL-c levels and profound decreases in the serum HDL-c levels in the untreated diabetic rats. These biochemical alterations recorded in this study is in complete agreement with earlier reported

studies on metabolic abnormalities in diabetes induced by alloxan (Mansford and Opie, 1968; O'Meera *et al.*, 1990; Wasan *et al.*, 1998; Xie *et al.*, 2005; Adeneye, 2008; Ashok-Kumar *et al.*, 2012; Sreelatha and Inbavalli, 2012). With repeated daily oral treatments with 125-500 mg/kg of MLASE for 8 days, alterations in the glucose and lipid concentrations in the diabetic rats were profoundly reversed, indicating the effectiveness of MLASE in diabetes management. Results of this study are in strong consonance with that of Odutuga *et al.* (2010) where the aqueous and ethanolic stem bark extracts of *Morinda lucida* profoundly controlled hyperglycemia in alloxan-induced diabetic rats although this study was devoid of investigation into the effects on the serum lipid profile of the treated alloxanized diabetic rats. Previous *in vivo* pre-clinical studies have reported the effectiveness of *Morinda lucida* leaf extracts in the management of experimental diabetes (Olajide *et al.*, 1999; Adeneye and Agbaje, 2008; Ojewunmi *et al.*, 2013; Domekouo *et al.*, 2016).

Medicinal plants with oral hypoglycemic and/or antihyperglycemic activities have been reported to mediate their actions via increased insulin secretion, enhanced peripheral glucose utilization, decreased intestinal glucose absorption and decreased glucose production from the liver through the processes of gluconeogenesis and/or glycogenolysis (Hui *et al.*, 2009; Wang *et al.*, 2013; Fuller and Stephens, 2015). The fact that MLASE effectively controlled fasting hyperglycemia and dyslipidemia in the alloxanized diabetic rats strongly suggest that MLASE could be mediating its antidiabetic effect through enhanced insulin secretion from the remnant of the damaged pancreatic β -cells and/or enhanced peripheral glucose utilization although the possibility of decreased glucose production through gluconeogenesis and glycogenolysis could also not be ruled out. The fact that MLASE significantly reduced 60-120 min post-pandrial glucose levels in the OGTT model of diabetic rats suggest that MLASE (particularly at the oral dose of 500 mg/kg) could also be mediating its antidiabetic effect through intestinal glucose uptake inhibition mechanism. This assertion could be corroborated by the findings of other studies where *Morinda lucida* leaf extracts were reported to elicit α -amylase and α -glucosidase inhibition *in vitro* (Kazeem *et al.*, 2013) and intestinal glucose uptake inhibition *in vivo* (Adeneye, 2013). Thus, the antidiabetic effect of MLASE could be by way of inhibiting enzymes in the small intestinal brush border that are responsible for the degradation of oligosaccharides and

disaccharides into monosaccharide suitable for absorption. This action in turn halts glucose entry into the systemic circulation and lowers postprandial glucose levels. Odotuga *et al.* (2010) has reported the presence of flavonoids, saponins, glycosides and steroids in the aqueous stem bark of *Morinda lucida* which is in complete agreement with phytochemical results of our study, although in addition we found tannin to be present while cardiac glycosides and steroids were reportedly absent. This variance could be attributed to differences in the collection site and seasonal variation as the factors have been reported to significantly affect the bioactive compounds of the same plant found in different environments (Elujoba *et al.*, 1989). Literature has equally shown the biological activities of alkaloids and flavonoids to include hypoglycemia, hypolipidemia, hypoazotemia, hypotension among other biological effects (Oladele *et al.*, 1995; Marles and Farnsworth, 1996). Similarly, medicinal plants containing high alkaloid, flavonoid, phenolic contents have been reported to show antidiabetic activity mediated via insulinomimetic mechanism (Patel *et al.*, 2012). Thus, the presence of active secondary metabolites such as phenols, saponins, tannins and alkaloids in high concentration as demonstrated by the quantitative results of this study either alone or in combination could have accounted for the antidiabetic action of MLASE recorded in this study.

Another significant finding of this study is the effect of MLASE on the average body weight and percentage weight changes in alloxan-induced diabetic rats. Repeated oral treatment of the diabetic rats with MLASE significantly improved weight loss in the treated rats, dose-dependently, with the most profound improvement observed in rats treated with 500 mg/kg/day of MLASE, an effect that was comparable with what was recorded for 5 mg/kg/day of glibenclamide, a standard oral antihyperglycemic drug, used in this study. Typically, uncontrolled DM is known to cause significant weight loss in its sufferer and this is known to result from the hypercatabolic state and polyuria associated with DM (Hakim *et al.*, 1997; Charlton and Nair, 1998; Fararh *et al.*, 2002; Al-Amin *et al.*, 2006; Zafar and Naqui, 2010). In this study, significant weight loss was recorded in the untreated alloxan-induced diabetic rats, an observation that was in strong concordance with earlier reports of other investigators (Odotuga *et al.*, 2010). The fact that MLASE significantly improved weight loss in the diabetic rats could have resulted from improvements in the metabolic derangements often associated with uncontrolled DM.

Overall, the present study shows that oral treatment with 125-500 mg/kg/day of MLASE significantly ameliorates hyperglycemia and hyperlipidemia associated with type 1 diabetes mellitus which was partly mediated via improved intestinal glucose tolerance mechanism.

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