Full Length Research Article

Pharmacological Evaluation of Oral Hypoglycemic and Antidiabetic Effects of Fresh Leaves Ethanol Extract of *Morinda Lucida* Benth. in Normal and Alloxan-Induced Diabetic Rats

Adeneye A.A.[1, *], Agbaje E.O.[2]

[1]*Department of Pharmacology, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria*

[2]*Department of Pharmacology, College of Medicine Of the University of Lagos, Idi-Araba, Lagos State, Nigeria*

ABSTRACT

In the present study, 50 – 400 mg/kg of body weight/day of 50% ethanol extract of the fresh leaves of Morinda lucida Benth. (MLE) was investigated for its hypoglycemic and antidiabetic effects in adult normal and alloxan-induced diabetic male rats for 7 days. Acute oral toxicity study of MLE at the limit dose of 2000 mg/kg of body weight using Up-and-Down Procedure on statistical program, AOT425Pgm, was also conducted. Results showed that MLE significantly (p<0.05) lowered the fasting blood glucose (FBG) in both normal and alloxan-induced diabetic rats in dose related fashion, and its effect was higher (p<0.001) than that of tolbutamide (Tolb.). Results suggest that MLE could be mediating its hypoglycemic effect via enhanced peripheral glucose utilization. Also, acute oral toxicity result showed MLE to be non-lethal at 2000 mg/kg of body weight. These results suggest that MLE could be relatively safe on acute exposure when administered to suspected diabetic patients.

(Afr. J. Biomed. Res. 11: 65 - 71)

Key words: *Morinda lucida Benth.; Fresh leaf methanol extract; Hypoglycemia; Normal and Alloxan-induced diabetic rats*

*Address for Correspondence : Telephone (mobile): +234-803-583-5589
Fax: +234-702-800-2758 ; E-mail: adeneye2001@yahoo.com

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, lipid, and protein metabolism characterized by persistent elevations of fasting blood glucose above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Murray and Pizzorno, 1997). DM is associated with increased risk of heart disease, stroke, kidney disease, retinopathy, neuropathy, ulceration and gangrene of extremities (Rotshteyn and Zito, 2004). Thus, DM and its associated complications have significant impact on health, quality of life and life expectancy of its sufferers. Recent statistics showed that the global epidemic of diabetes mellitus is worse or greater in developing than the developed countries (Oputa, 2002). For example, India is rated the leading country affected by DM epidemic with an estimate of 19 million diabetic subjects while China and United States of America are rated second and third, respectively (King *et al.*, 1998). However, approximately 120 million people are globally affected by its sufferers and this figure is estimated to double by the year 2025 (King *et al.*, 1998).

In response to this global health challenge, the WHO Expert Committee on diabetes mellitus recommended further evaluation of the folkloric methods of managing the disease because of high mortality and morbidity arising from its attendant complications and draw-backs associated with the use of conventional antidiabetic drugs (Adeneye *et al.*, 2006a). In pursuit of this goal, several medicinal plants are being investigated for their hypoglycemic efficacies. Of the several indigenous plants used in the local treatment of DM among Yorubas (South-West Nigeria) is *Morinda lucida* Benth.

Morinda lucida Benth. (Rubiaceae) is a tropical West Africa rainforest tree also called Brimstone tree. In Cote d'Ivoire, it is locally called Sangogo or Bondoukou alongua while in Ghana, it is known as Twi, Kòn kròmà or Ewe amake. Among the Togolese, the plant is popularly known as Ewe amake or Atak ake while among the Yoruba natives (South-West Nigeria), it is called Òruwó (Dalziel, 1937). Different parts of the plant are attributed with diverse therapeutic benefits. For example, in Southern Cameroon, cold decoction of the plant leaves is used for the treatment of fever (Dalziel, 1937). However, in most parts of West Africa, the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation (Dalziel, 1937). The Europeans sometimes use the decoction of the plant root or stem to make "bitters" (Dalziel, 1937).

Various extracts of the plant dried leaves are documented to possess trypanocidal (Asuzu and Chineme, 1990), antimalarial activities (Makinde and Obih, 1985; Tona *et al.*, 1999), and aortic vasorelaxant effect (Ettarh and Emeka, 2004). Among the Yoruba herbalists (South-West Nigeria), fresh leaves of the plant is often macerated in palm-wine and its bitter decoction is used in the oral treatment of suspected diabetic patients usually for a few days. In the present study, 50-400 mg/kg/day of MLE was investigated for its oral hypoglycemic activities in normal and alloxan-induced diabetic male Wistar rats for 7 days.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Morinda lucida* Benth. were harvested from an uncultivated farmland on the outskirt of Low Cost Housing Estate, Oke-Afa, Isolo, Lagos State, Nigeria in the month of October, 2006. Plant identification and voucher specimen (specimen no.: FHI 107459) referencing was done by Mr. T.K. Odewo, Chief Superintendent, in the Taxonomy Section of the Forestry Research Institute (FRIN), Ibadan, Nigeria.

Preparation of plant extract

500 g of the harvested fresh leaves of *Morinda lucida* was completely extracted in 1L of 50% ethanol for 2 hours using Soxhlet extraction procedure. The Soxhlet extractive was filtered using a piece of sterile white, cotton cloth. The filtrate was completely dried into an aromatic green-to-brown solid residue over a water-bath,

giving a yield of 15.2% ($^w/_w$). This was then stored in water- and air-proof container, which was kept in the refrigerator at a temperature of 4ºC for about a week before the experiment begun. From this stock, fresh solution of the extract dissolved in DMSO (vehicle) was prepared.

Experimental Animals

Six and seven groups (of six rats per group each) of adult male Wistar rats, weighting 200-250 g were used for the first and second phases of the experiment, respectively. The rats were procured from the Animal House of the College of Medicine Of the University of Lagos, Idi-Araba, Lagos, Nigeria, after ethical approval was obtained from the *ad hoc* ethical committee of the Lagos State University College of Medicine, Ikeja, Nigeria. The rats were kept in polypropylene cages and maintained at standard laboratory conditions. The rats had free access to water and commercially available standard rat feed (Livestock Feeds, Ikeja, Lagos State, Nigeria).

Acute oral toxicity studies of MLE in rats using limit dose test of Up-and-Down Procedure

The Acute oral toxicity study was conducted using the limit dose test of Up-and-Down Procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Program - AOT425statPgm, version: 1.0, at a limit dose of 2000 mg/kg body weight/oral route and default of Sigma at 0.5. The study was conducted by method adopted by Adeneye *et al*. (2006b).

Experimental induction of diabetes in rats

Alloxan monohydrate (Sigma Chemical Co., St. Louis, U.S.A.) at a single intraperitoneal dose of 150 mg/kg of body weight dissolved in 0.1M freshly prepared cold citrate buffer of pH of 4.5 was injected into each rat of the 2nd phase of the experiment (as described by Al-Shamaony *et al*., 1994; Pari and Venkateswaran, 2002). Stable hyperglycemia was confirmed on the 5th day and rats with fasting blood glucose of greater than 200 mg/dl were considered diabetic and used in this study.

Drug administration

In the 1st phase of the experiment, fasted rats of group I received 10 ml/kg/day of distilled water (pH = 6.8) only, group II received 10 ml/kg/day of DMSO (the vehicle), groups III were gavaged with 250 mg/kg/day of tolbutamide while groups IV – VI were gavaged with 50, 100 and 400 mg/kg/day of MLE dissolved in 10 ml/kg/day of DMSO, respectively, for 7 days. In the 2nd phase of the experiment, same procedure was carried out in the alloxan-induced diabetic rats.

Blood glucose determination

Blood samples from experimental rats were collected from the tail vein after 14-16hrs of overnight fast. The fasting blood glucose was determined by glucose oxidase method of Trinder (1969), using One Touch Basic Blood Glucose Monitoring System (LifeScan Inc., Milpitas, California, U.S.A.).

Statistical analysis

Data were expressed as mean ± S.E.M. The statistical analysis was performed using two-ways analysis of variance and post hoc test was conducted using Newman-Keuls-Student test on SYSTAT 10.6. statistical program. The significance of the difference between the mean of the control and treated groups was considered at $p<0.05$.

RESULTS

Results and sequence of acute oral toxicity of MLE in rats using limit dose test of Up-and-Down Procedure

Table 1 shows the sequence and results of MLE on the 5 rats sequentially treated with a limit dose of 2000 mg/kg body weight/oral route of the extract. The extract caused no mortality in any of the treated rats. The dose, however, was associated with behavioral toxicities such as bilateral narrowing of the epicanthal folds, abnormal posture characterized by tugging of the head between the hind limbs in the first 4-6 hours of oral administration of the extract, decreased appetite, lethargy, tremor, diarrhea and sustained hypothermia lasting up to 4-6 hours post-oral

administration (rectal temperature of 35.2 ± 1.5 °C from 37.1 ± 0.8 ºC). Also shown in the table is the effect of the extract on body weight of treated rats. As noted, the high dose induced progressive and sustained weight loss in all the treated rats.

Effects of distilled water, DMSO, tolbutamide and graded doses of MLE on FBG in normal rats

Figure 1 shows the effect of 7 days of oral administration of distilled water (DW), DMSO, tolbutamide and 50-400 mg/kg/day of MLE on fasting blood glucose in normal rats. As shown, MLE induced a progressive, and significant (p<0.05) dose related hypoglycemia in the tolbutamide- and 50-400 mg/kg/day MLE-treated rats. However, MLE hypoglycemic effect at 100 and 400 mg/kg/day was significantly (p<0.001) higher than that 250 mg/kg/day of tolbutamide.

Effects of distilled water, DMSO, tolbutamide and graded doses of MLE on FBG in alloxan-induced diabetic rats

Table 2 depicts effects of the stated drugs on the FBG of alloxan-induced diabetic rat model. As shown in the table, intraperitoneal administration of alloxan induced significant (p<0.05) progressive hyperglycemia in the treated rats between the 3rd and the 5th day of the experiment but diabetes mellitus became fully established in all the rats by the 5th day post-induction. However, daily administration of 50-400 mg/kg/day of MLE to the diabetic rats induced a significant (p<0.001) dose related reduction in the blood glucose concentration when compared to groups I and II. Although similar but less significant (p<0.05) hypoglycemic effect was recorded for tolbutamide when compared to the various doses of MLE.

Table 1:
Sequence, result and effect on the body weight of limit dose test of Up-and-Down Procedure of MLE in rats

Test Sequence	Animal ID.	Dose (mg/kg)	Body weight (g) on			Short term outcome	Long term outcome
			day 0	day 7	day 14		
1	01	2000	125	110	88	survival	survival
2	02	2000	120	110	90	survival	survival
3	03	2000	123	115	80	survival	survival
4	04	2000	130	119	96	survival	survival
5	05	2000	125	108	78	survival	survival

(ID. = Identification number)

Table 2:
Effect of oral administration of distilled water, DMSO, tolbutamide and 50-400 mg/kg/day of MLE on FBG in alloxan-induced diabetic rats after 7 days of treatment

Treatment groups	Fasting Blood Glucose concentration (mg/dl) on			
	Day 0	Day 3	Day 5	Day 12
I (normal + 10 ml/kg DW)	67.8±4.0	64.0±2.7	65.7±2.0	64.8±2.3
II (alloxan-treated + 10 ml/kg DW)	69.8±2.9	143.7±12.3[a]	255.0±18.1[c]	393.2±17.7
III (alloxan-treated + 10 ml/kg DMSO)	66.5±2.1	135.8±11.6[a]	285.8±22.3[c]	398.0±27.0
IV (alloxan-treated + 250 mg/kg Tolb.)	67.7±1.7	131.2±11.6[a]	264.2±25.3[c]	170.2±4.1[d]
V (alloxan-treated + 50 mg/kg MLE)	65.3±2.7	131.7±13.7[a]	271.0±26.3[c]	149.5±11.7[d]
VI (alloxan-treated + 100 mg/kg MLE)	66.0± 2.7	156.5±16.7[b]	250.8±20.1[c]	129.8±6.8[e]
VII (alloxan-treated + 400 mg/kg MLE)	67.5±2.0	165.7±17.7[b]	276.0±20.1[c]	117.2±6.8[f]

[a, b, c] *represent significant increase at p< 0.05, p<0.01 and p<0.001when compared to groups II and III on day 5 while* [d, e, f] *represent significant decrease at p<0.05, p<0.01, and p<0.001 when compared to groups II and III on day 12 , respectively.*

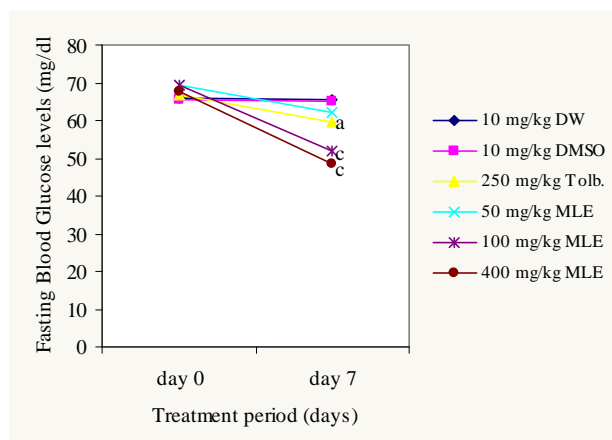*Antidiabetic effects of Morinda lucida*

Fig. 1: Effect of oral administration of distilled water (DW), dimethyl sulphoxide (DMSO), tolbutamide (Tolb.) and 50-400 mg/kg/day of MLE on fasting blood glucose concentrations in normal rats on days 0 and 7. [a] represents significant value at $p < 0.05$ while [c] represents significant values at $p < 0.001$

DISCUSSIONS

Traditionally, various *in vivo* models (e.g. diazoxide, alloxan- or streptozotocin-induced diabetic rats) are used in evaluating medicinal plants with suspected hypoglycemic potentials. In this study, diabetes mellitus was induced using intraperitoneal alloxan at a single dose of 150 mg/kg of body weight. This dose reliably established DM in the treated rats 5 days post-induction. Literature shows that alloxan induces diabetes mellitus by selectively destroying the pancreatic β-cells, which are involved in the synthesis, storage and release of insulin, the peptide hormone regulating carbohydrate, protein and lipid metabolism (Malaisse, 1982; Shafrir, 1997). In the present study, diabetes was fully established as evidenced by the significant ($p < 0.001$) elevation in the FBG concentrations in the group II-VII rats on the 5th day of the 2nd phase of the experiment from their baseline values (Table 2). However, oral treatment with 250 mg/kg/day of tolbutamide, 50-400 mg/kg/day of MLE for 7 consecutive days significantly ($p < 0.05$) lowered the FBG from 264.2 ± 25.3, 271.0 ± 26.3, 250.8 ± 20.1, 276.0 ± 20.1 mg/dl in groups III-VI to 170.2 ± 4.1, 149.5 ± 11.7, 129.8 ± 6.8, 117.2 ± 6.8 mg/dl in group III-VI, respectively. The FBG-

lowering effect of MLE was dose related and significantly ($p < 0.001$) higher than that of tolbutamide. The observed hypoglycemic effect of MLE is an indication that MLE contains active principles with potent hypoglycemic property. In normal rats, MLE could be acting via increased insulin secretion or increased peripheral utilization of glucose but in the *in vivo* type II diabetes model created in this study, MLE lowers hyperglycemia by increasing the peripheral utilization of glucose in the diabetic rats. Result of this study is in consonance with that reported for the dried leaves extract of *Morinda lucida* Benth. in normal and streptozotocin diabetic rats, by Olajide et al. (1999). Equally, Olajide *et al*. (1999) and Asuzu and Chineme (1990) independently reported *Morinda* leaves to contain high concentrations of flavonoids, alkaloids, tannins and saponin. 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; Sudheesh *et al*., 2005). The presence of these two active biological principles in high concentrations in *Morinda* leaf extracts may be responsible for the oral hypoglycemic effects recorded in the present study. Also, literature has shown tannins to be responsible for most behavioral toxicities induced by medicinal plants (Muyibi *et al*., 1999). Thus, tannin and other biological principles may be responsible for the observed weight loss due to lethargy, anorexia in the MLE-treated rats. However, at its therapeutic doses, these behavioral toxicities were absent.

According to Bruce (1985, 1987) and American Society for Testing and Materials (1987), any chemical substance with LD_{50} estimate greater than 2000 – 5000 mg/kg/oral route could be considered of low toxicity and safe. Thus, lack of associated lethality with the high dose of this extract is an indication that MLE is relatively safe on acute oral exposure. This finding was corroborated by the intraperitoneal LD_{50} value of 2000 mg/kg obtained for the 50% methanol extract of the dried leaves of MLE obtained by Asuzu and Chineme (1990) in their earlier study. The high yield (15.2% $^w/_w$) obtained for the fresh leaves

suggests that MLE contains more extracted active principles than the 9.7% ($^w/_w$) obtained for the dried leaves of same plant by Asuzu and Chineme (1990).

In conclusion, results of this study show that MLE has potent oral hypoglycemic property which was mediated via increased peripheral utilization of glucose, thus, justifying its ancestral use in the management of suspected type II diabetic patients. Isolation of the active principle(s) would constitute areas of future research.

REFERENCES

Adeneye A.A., Amole O.O. and Adeneye A.K. (2006a): Hypoglycemic and hypocholesterolemic activities of the aqueous leaf and seed extract of *Phyllanthus amarus* in mice. Fitoterapia 77: 511-514.

Adeneye A.A., Ajagbonna O.P., Adeleke T.I. and Bello S.O. (2006b): Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. Journal of Ethnopharmacology 105: 374-379.

Al-Shamaony L., Al-Khazraji S.M. and Twaiji H.A. (1994): Hypoglycaemic effect of *Artemisia herba alba* II. Effect of a valuable extract on some blood parameters in diabetic animals. Journal of Ethnopharmacology 43: 167-171.

American Society for Testing and Materials (1987): Standard Test Method for Estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials E 1163 87, Philadelphia, U.S.A.

Asuzu I.U. and Chineme C.N. (1990): Effect of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. Journal of Ethnopharmacology 30(3): 307-312.

Bruce R.D. (1985): An Up-and-Down Procedure for Acute Toxicity Testing. Fundamental Applied Toxicology 5: 151-157.

Bruce R.D. (1987): A confirmatory study of Up-and-Down Method of Acute Oral Toxicity Testing. Fundamental Applied Toxicology 8: 97 – 100.

Dalziel J.M. (1937): The Useful Plants of West Africa. 1st edition; pp. 403-404; Crown Agents, London.

Ettarh R.R. and Emeka P. (2004): *Morinda lucida* extract induces endothelium-dependent and –independent relaxation of rat aorta. Fitoterapia 75(3-4): 332-336.

King H., Albert R.E. and Herman W.H. (1998): Global Burden of Diabetes 1995 - 2025: Prevalence, Numerical estimates and Projections. Diabetes Care 21(9): 1414-1431.

Malaisse W.J. (1982): Alloxan toxicity to the pancreatic β-cell: A new hypothesis. Biochem. Pharmacol. 31: 3527-3534.

Makinde J.M. and Obih P.O. (1985): Screening of *Morinda lucida* leaf extract for malarial action on *Plasmodium berghei berghei* in mice. African Journal of Medicine and Medical Sciences 14 (1-2): 59-63.

Murray M. and Pizzorno J. (1997): Encyclopedia of Natural Medicine, 2nd edition; pp. 401; Prima Health Publishing, Rockling, U.S.A.

Muyibi S.A., Olorede B.R., Onyeyili P.A., Osunkwo U.A., Muhammed B.Y. and Ajagbonna O.P. (1999): Haematological and histopathological changes of *Cassia occidentalis* leaf extract in rats. Proceedings of the 8th Scientific Conference of the Nigerian Society of Pharmacognosy, 1st-4th December, 1999, held at the Ahmadu Bello University, Zaria, Nigeria.

Oputa R.N. (2002): Malnutrition related diabetes mellitus (MRDM). Nigerian Journal of Endocrinology and Metabolism 3(1): 33-35.

Oladele S.B., Ayo J.O. and Adaudi A.O. (1995): Medicinal and physiological properties of flavonoids, coumarin derivatives and anthraquinones of plant origin. West African Journal of Pharmacology and Drug Research 11: 134–144.

Olajide O.A., Awe S.O., Makinde J.M. and Morebise O. (1999): Evaluation of the antidiabetic property of *Morinda lucida* leaves in streptozotocin-diabetic rats. Journal of Pharmacy and Pharmacology 51(11): 1321-1324.

Pari L. and Venkateswaran S. (2002): Hypoglycaemic activity of *Scoparia dulcis* L. extract in alloxan-induced hyperglycaemic rats. Phytotherapy Research 16: 662-664.

Rotshteyn Y. and Zito S.W. (2004). Application

of modified *in vitro* screening procedure for identifying herbals possessing sulfonylureas-like activity. Journal of Ethnopharmacology 93: 337-344.

Shafrir E. (1997): Diabetes in animals: Contribution to the Understanding of Diabetes by Study of Its Etiopathology in Animal Models. In: Porte, D. (Jnr.), R.S. Sherwin eds. Ellenberg and Rifkin's Diabetes Mellitus. 5th edition; pp. 301-348; Appleton and Lange, Connecticut.

Sudheesh S., Manilal V.B., Vijayalakshmi N.R. (2005): Potential health promoting effects of flavonoids – A comparative study on hypolipidaemic and hypoglycaemic activities. In: Abstract of Posters, P 179, final program and book of Abstracts, 53rd Annual Meeting of the Society

Medicinal Plant Research (GA) and Joint Congress with the Italian Society of Phytochemistry (SIF), Florence, Italy, August 21st-25th 2005. http://www.famacia.unifi.it/congress2005.html.

Tona L., Ngimbi N.P., Tsakala M., Mesia K., Cimanga K., Apers S., De Bruyne T., Pieters L., Totte J. and Vlietinck A.J. (1999): Antimalarial activity of 20 crude extract from nine African medicinal plants used in Kinshasa, Congo. Journal of Ethnopharmacology 68(1-3): 193-203.

Trinder P. (1969): Determination of Blood Glucose using 4 – aminophenzone as Oxygen Acceptor. Journal of Clinical Pathology 22: 246-248