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HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF METHANOLIC LEAF EXTRACT OF *Morinda lucida* ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT
Healthy male wistar rats(30) of average weight of 150 – 190gm were randomly distributed into 5 gr. oups of six each. Group 1, normal control; each given only 0.2ml distilled water throughout the period of study; group 2 diabetic control, induced 150mg/kg b.w., i.p., administration of alloxan monohydrate and thereafter given 0.2ml distilled water throughout the period of study; group 3 and 4 diabetic (i.p., 150mg/kg b.w. alloxan), rats were orally administered methanolic leaf extract of Morinda lucida at 100mg/kg and 200mg/kg respectively for 15 days. Group 5 diabetic rat (i.p., 150mg/kg b.w. alloxan) were treated with 84mg/kg /b.w. of chlorpropamide once daily for 15 days. Serum glucose levels were found to decrease significantly (p<0.05) both in groups 3 and 4 compared to the control groups. Hypolipidemic status was found to improved significantly (p<0.05) by decreasing the levels of serum total cholesterol (TC), triglycerides (TGs) and low density hypoprotein (LDL) in both the treated groups compared to the control groups. These reductions were dose dependant and compared well with the values obtained in the standard drug control group. Thus, results of the study indicate that Morinda lucida methanolic leaf extract can be potentially used for diabetics to control glucose and lipid levels.
Key Words: Morinda lucida, Diabetes mellitus, hypoglycemic effect, hypolipidemic effect, chlorpropamide, alloxan monohydrate.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both (WHO, 1999). It is one of the commonest endocrine metabolic disorders affecting mankind all over the world. It is ranked seventh among the leading causes of death and considered third on the account of its fatal complications (Trivedi *et al*., 2004). Its prevalence is on the rise world wide. The world prevalence of diabetes among adults (age 20 – 79 years) was 6.4% affecting, 285 million adults in 2010, and will increase to 7.7% and 439 million adults by 2030 (Shaw *et al*., 2010).

Complications of diabetes mellitus include hypertension, atherosclerosis and microcirculatory disorder. It is also associated with long term complications, including retinopathy, nephropathy, neuropathy, angiopathy and several others (Kristova *et al*., 2008).

Diabetes mellitus have been classified as growth or juvenile unset and maturity or adult unset. It has also been classified based on their requirement of insulin as insulin dependent diabetes mellutus (IDDM or type 1) and non insulin dependent diabetes mellitus (NIDDM or type 2). Insulin resistance and β cells dysfunction are the metabolic abnormalities in the type 2 diabetes (Sa'ad *et al*., 1991). The NIDDM account for 90 percent of diabetes cases (WHO 2002). In high II diabetes, the LDL particles stick to the arteries and damage blood vessels walls more easily (Sarti and Gallah, 2006).

Treatments of diabetes mellitus include life style modification such as diet and exercise and the use of insulin and or oral hypoglycemic drugs. For insulin, the major drawbacks include ineffective on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage - fatal hypoglycemia.

Oral hypoglycemia drugs currently used include sulfonylurea, biguanide, thiozolidinodione and glycosidase inhibitors. However the use of these agents has been restricted due to undesirable side effects such as propensity to gain weight (Rang and Dale 1990). These agents also fail to significantly alter the course of diabetic complications (Chandramohan *et al*., 2008).

A major focus of current anti-diabetic research is the development of anti hyperglycemic agents that are safe and free of negative side effect. There has also been increasing demand for the use of plant products with anti-diabetic activity due to their effectiveness, limited side effects and relatively low cost (Iwara *et al*., 2014)

In Nigeria, several thousands of plant species have been claimed to posses medicinal properties and employed in the treatment of many ailments. *Morinda lucida* is one of the over 30 medicinal plants used in rural areas, and also by some impoverished urban dwellers in the western and southern parts of Nigeria, to treat diabetes mellitus. This plants is a genus of flowering plant in the madder family *Rubiacae*. The present research program was undertaken to investigate hypoglycemic and hypolipidemic activity of the methanol leaf extract of *Morinda lucida* on alloxan induced diabetic Wistar rats.

The effect of *Morinda lucida* methanolic leaf extract on blood glucose and lipid profile viz total cholesterol, triglycerides (TGs), high density lipoprotein (HDL) and low density lipoprotein (LDL) on alloxan – induced diabetic rats were investigated.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Morinda lucida* were collected from Oyo State, Nigeria. The plant material was identified and authenticated by the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria where voucher number 1852 was deposited.

Extract Preparation

The leaves of the plant were dried under shade for one week and then pulverized into fine powder in a mortar and a pestle and passed through a mesh sieve. Extraction was performed using maceration process. This involves soaking 100g of the crude powder of *Morinda lucida* in 100ml of 70% methanol for 72 hours with intermittent shaking. Later the extract was filtered, then concentrated using rotary evaporator and was kept in desiccators until use.

Chemicals Used

All chemicals and drugs were obtained commercially and were of analytical grade. Alloxan monohydrate (Sigma – Aldrich, Inc. St. Louis, MO 63103, USA).

Experimental Animals

Thirty healthy male wistar rats of average weight of 150 – 190gm were used in the experiment. The animals were obtained from Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Kaduna.

The animals were acclimatized for a period of two weeks in the animal house of the Department of Applied Science, Kaduna Polytechnic. They were housed in well ventilated cages and maintained at a constant room temperature ($25^0C \pm 5^0C$) relative humidity (45% ± 5%) and 12 hour light/dark condition. The animals were fed on commercial feeds and were given water and libitum. The animals were fasted from feeds for 12 hours before the commencement of each experiment, but were allowed water ad libitum.

Induction of Diabetes

The rats were made diabetic by injecting alloxan monohydrate 150mg/kg body weight intraperitoneally with the exception of normal control group. Raised in blood glucose concentration level above 126mg/dl was considered diabetic after 7 days. After 7 days of alloxan injection the hyperglycemic rats were separated and divided into different groups comprising of 6 rats each for the anti diabetic study.

Experimental Procedure

For testing the anti-diabetic effect, the rats were then grouped into 5 of 6 each.

Group 1 (6) Normal rats
Group 2 (6) Diabetic control rats
Group 3 (6) Diabetic rats administered methanol leaf extract (100mg/kg) orally once daily for 15 days.
Group 4 (6) Diabetic rats administered methanol leaf extract (200mg/kg) orally daily for 5 days.
Group 5 (6) Diabetic rats given chlorpropamide, (84mg/kg) once daily for 15 days.

Determination of Blood glucose and lipid profile

Blood samples of the rats were collected by cutting the tail tip of the rats for blood glucose determination. This was done on the 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} days after the administration of the extract. Blood glucose of rat was measured by glucometer (one touch ultra).

Biochemical Assay

Immediately after the experiment, the rats were anesthesized with chloroform followed by decapitation. Plain sample bottles were used to collect whole blood and the following parameters were tested for lipid profile; total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein by method of Friedewald et al 1972.

Statistical Analysis

Experiment data were analyzed using the statistical package for Social Science (SPSS) software version 14 (SPSS Inc. Chicago, Illinois, USA). The results were expressed as mean (± SD) and to compare the difference between variables. Error bars were calculated by using standard deviations ANOVA followed by Bonferoni, Post hoc test were performed for statistical analysis of the result. A P-value less than 0.05 were considered significant.

RESULTS

Anti-diabetic Study

Table 1 showed the result of the effects of two doses (100mg/kg and 200mg/kg) of *Morinda lucida* methanol leaf extract, chlorpropamide and control groups of alloxan – induced diabetic wistar rats. A drop of blood samples was collected by cutting the tail-tip of the rats for the blood glucose determination by Oxidase method. It was done at intervals of 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th}, and 15^{th} days. Normal control group shows irregular raise and fall in blood glucose levels with increase in days. There was increase in fasting blood glucose levels of diabetic control group after the induction of alloxan and remain higher (as high as 16.45mmol/l) up to the end of the study.

The two groups orally administered with different doses of the extract (Table1) have their blood glucose levels significantly lowered ($p < 0.05$) when compared with the diabetic control but higher than that of the normal control rats. A more rapid decrease in blood glucose level was, however observed in animals treated with 200mg/kg of the extract. The blood glucose levels in chlorpropamide treated rats was found to be significantly lower ($p < 0.05$) within the first 10 days when compared to the two groups orally administered with different doses of the extract.

Hypolipidemic Study

The mean serum total cholesterol, triglycerides (TGs), low density lipoprotein (LDL) and high density lipoprotein (HDL) levels of normal control, diabetic control and diabetic treated group of the animals are shown in table 2. There was significant decrease ($p < 0.05$) in the levels of total cholesterol, triglycerides and low density liporptein in the *Morinda lucida* treated groups compared to the diabetic control group. The level of the HDL has also increased in the treated groups but the difference between that and the diabetic control group was not significant ($p > 0.05$.)

Table I – Effects of methanolic leaf extract of *Morinda lucida* on fasting blood glucose level in alloxain induced diabetic rats after two weeks treatment

Days	Blood glucose level (mmol/l)				
	Normal control	Diabetic control	*MLEML*		84mg/kg chlorpropamide
			100mg/kg	200mg/kg	
Day 1	3.48 ± 0.05[a]	14.45 ± 0.86[b]	15.57±0.25[b]	14.93 ±1.16[b]	15.06 ± 1.30[b]
Day 3	5.30 ± 0.00[a]	14.68 ± 0.83[c]	14.2 ± 0.30[c]	12.03 ±1.77[c]	14.20 ± 0.90[b]
Day 5	4.58 ± 0.05[a]	14.95 ± 0.71[b]	12.97 ± 0.42	10.38 ± 0.96	9.10 ± 1.20
Day 7	4.13 ± 0.05[a]	15.33 ± 0.54[b]	11.83 ± 0.25	8.23 ± 0.56	7.20 ± 1.40
Day 9	3.80 ± 0.00[a]	15.60 ± 0.38[b]	9.60 ± 0.30	6.28 ± 0.57	7.10 ± 0.80
Day 11	3.80 ± 0.00[a]	15.80 ± 0.36[b]	7.77 ± 0.59	5.55 ± 0.31	6.34 ± 0.31
Day 13	3.78 ± 0.05[a]	16.05 ± 0.29	6.17 ± 0.42	5.30 ± 0.22	5.56 ± 0.60
Day 15	3.78 ± 0.05[a]	16.45 ± 0.30	5.47 ± 0.15	4.78 ± 0.22	5.12 ± 0.28

Values are expressed as mean ± SD N = 6.
Data in the same row carrying different superscripts (i.e. a, b, c or d) differ significantly form each other P < 0.05. MLEML - methanol leaf extract of *Morinda lucida*.

Table 2 – Effects of methanolic leaf extract of *Morinda lucida* on lipid profile of experimental rate models.

Groups	Total cholesterol (TC) mmol/l	Triglycerides (TGs) mmol/l	Low density lipoprotein LDL mmol/l	High density lipoprotein HDL mmol/l
Normal control	1.15 ± 0.54[a]	0.68 ± 0.94[ab]	0.03 ± 0.05[a]	0.55 ± 0.06[a]
Diabetic control	1.6 ± 0.48[ab]	0.93 ± 0.21[b]	0.13 ± 0.10[a]	0.30 ± 0.41[a]
Treated group (100mg/kg) MLEML	1.5 ± 0.44[ab]	0.83 ± 0.12[ab]	0.13 ± 0.05[a]	0.23 ± 0.12[a]
Treated group (200mg/kg) MLEML	1.13 ± 0.74[a]	0.58 ± 0.26[b]	0.05 ± 0.10[b]	0.33 ± 0.15[a]

Values are expressed as mean ± SD. Values carrying different subscripts in the same column differ significantly from each other (p < 0.05). Comparison was done using one-way ANOVA with post hoc boniferoni p – test.
All values are presented as mean ± SD, n = 6 in each group p < 0.05 compared with normal control group and p < 0.05 with diabetic control.

DISCUSSION

Diabetes mellitus is possibly the world's largest growing metabolic disease and as the knowledge the heterogeneity of this disorder is advanced, the need for more appropriate theraphy increases. The development of research into new hypoglycaemic and hypolipidemic agent is of great interest. Considerably, large number of anti-diabetic plant and herbs are known through forkore but their introduction into modern therapy waits pharmacological testing by modern methods.

The results obtained suggest that methanol leaf extract of *Morinda lucida* exhibited significant hypoglycemic and hypolipidemic activities in alloxan induced diabetic wistar rats. This was evidenced by reduction of fasting blood glucose level (Table 1) and serum lipids (Table 2).

The significant increase in serum glucose level in diabetic control rats compared to normal control rats is as a result of damage of the pancreatic β cells by the effect of alloxan. Alloxan monohydrate induces diabetes by damaging insulin secreting cell of the pancreas leading to hyperglycemia (Szudelski ,2001). There is selective necrosis of the β_ cell of the islet of langerhans on the pancreas so that insulin production is totally or partially inhibited, depending on the concentration of the alloxan (Etuk, 2010).

The significant decrease in the level of fasting blood glucose in diabetic rats treated with methanolic leaf extract of *Morinda lucida* may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose (Erah,*et al.,*1996). The hypoglycemic activity of the extract was due to regeneration of pancreatic cell that were partially destroyed by alloxan.

Serum triglycerides is also said to implicate in the relationship (Gotto, 1998). Elevated low density lipoprotein cholesterol and decrease low density lipoprotein cholesterol levels are well recognized CHD risk factors with recent evidence supporting the benefit of intensive LDL-C reduction in CHD risk (Evans *et al.,* 2004). A reduction is serum lipids particularly of the LDL and VLD fractions and triglycerides should be consider as being beneficial for the long term prognosis of diabetics patients (Chattopadhyay and Bandyopaglhyay, 2005).

Lowering of blood glucose and plasma lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease. The high lipid levels seen in diabetics rats (Table 1) was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones (Ei-soud *et al.,* 2007).

Moreover, daily oral administration of MLEML for two weeks resulted in decreased serum total (TG) cholesterol, serum triglyceride (TGs) and low density lipoprotein (Table 2). This effect may be due low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Sharma *et al,* 2003).

In conclusion, the methanol leaf extract of *Morinda lucida* exhibited promising hypoglycemic and hypolipidemic activities in alloxan induced diabetic wister rats. Hence the leaf can be helpful in the management of diabetes mellitus and its other associated complication. Further investigation using other part of the plant may be more useful to explicate the mechanism of action and other active principles to project this plant as a therapeutic target in diabetic research

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