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Anti-Diabetic Effect of *Morinda lucida* Stem Bark Extracts on Alloxan-Induced Diabetic Rats

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Abstract: The present study was undertaken to investigate the potential hypoglycemic effect of aqueous and methanolic extracts of *M. lucida* stem bark in alloxan-induced hyperglycemia in rats. The study comprises three groups of diabetic rats administered 0.240 mg (aqueous extract) and 240 mg (methanolic extract) kg^{-1} body weight and a fourth group given 10 mL kg^{-1} body weight distilled water for 7 days. Body weight, fasting blood glucose as well as activities of some selected serum and liver enzymes were monitored before commencement of treatment and at the end of the treatment period. At the end of the 7 day treatment, fasting blood glucose of the animals were significantly ($p<0.05$) reduced by 73.5 and 39.0% of their initial values in diabetic group administered aqueous and methanolic extract, respectively. As it were before treatment, body weight was further reduced in the diabetic animal groups compared to normal rat group. Serum Alkaline Phosphatase (ALP) and liver aspartate Aminotransferase (AST) activities were not significantly ($p>0.05$) altered from their initial values after the 7 day treatment period. Serum AST and liver ALP were significantly ($p<0.05$) lowered in diabetic rats treated with aqueous and methanolic extracts compared to their initial values. Serum and liver Alanine aminotransferase (ALT) activity was significantly (serum)/non-significantly (liver) increased across the groups except for a significant decrease observed in liver ALT activity in diabetic rats administered the methanolic extract.

Key words: Aqueous extract, methanolic extract, *Morinda lucida*, phyto-chemicals, hypoglycemia, Nigeria

INTRODUCTION

Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent elevations of fasting blood glucose above 200 mg dL^{-1} due to insulin insufficiency or complete ceasation of insulin synthesis or secretion and/or insulin resistance (Murray and Pizzorno, 1997). Diabetes mellitus is associated with increased risk of heart disease, stroke, kidney disease, retinopathy, neuropathy, ulceration and gangrene of extremities. Thus, diabetes and its attendant complications have significant impact on health, quality of life as well as life expectancy of sufferers (Issa and Baiyewu, 2006).

Plants have always been utilized as sources of drugs and many of the currently available drugs have been directly or indirectly obtained from plant sources. In accordance with the recommendations of the World Health Organization (WHO) Committee on Diabetes Mellitus, it is important to investigate the hypoglycemic actions from plants which were originally used in traditional medicine. There are many reasons why the use of medicinal plants should be studied: herbal remedies may have recognizable therapeutic effects (Bailey and Day, 1989); they may also have toxic side effects (Keen *et al*., 1994). All parts of the *Morinda lucida* tree are used in natural medicine in the tropics including the bark, leaves, root and fruit-seeds.

Morinda lucida Benth. (Rubiaceae) is a tropical West Africa rainforest tree also called Brimstone tree. In Coted'Ivoire, it is locally called Sangogo or Bondoukou alongua while in Ghana, it is known as Twi, Kon kroma or Ewe amake. Among the Togolese, the plant is popularly known as Ewe amaka or Atak ake while among the Yoruba native of South-Western Nigeria, it is called Oruwo (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 astringent for dysentery, abdominal colic and intestinal

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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 tryponocidal (Asuzu and Chineme, 1990), antimalaria activities (Makinde and Obih, 1985; Tona *et al*., 1999) and aortic vasorelaxant effect (Ettarh and Emeka, 2004). Among the Yoruba herbalists, 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, 240 mg kg^{-1} day of aqueous and methanolic extracts of the plant were investigated for their oral hypoglycemic activity in alloxan-induced diabetic rats for 7 days.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical grade and are products of British Drug House (BDH, Poole, England) unless otherwise stated.

Plant materials: *M. lucida* bark was obtained from *M. lucida* tree in a forest at Ikeji-Arakeji (Nigeria). The plant material was identified and authenticated by a botanist in the Department of Plant Science, Joseph Ayo Babalola University, Ikeji Arakeji.

Plant preparation and extraction: *M. lucida* stem bark was dried under shade for three weeks and blended into powder using an industrial blender. About 50 g of the dried powder was soaked in 500 mL 98% methanol and another 50 g in 500 mL distilled water separately; each was left for 48 h and filtered separately using Whatman (number 1) filter paper. The filtrates were completely dried into brownish solid residue in water bath. The percentage yields of the methanolic extract and aqueous extract were 9.3 and 14.7% (w/w), respectively. Each residue was kept separately in air and water-tight container and stored in refrigerator at -4°C. From these stocks, fresh solution of each extract was prepared.

Proximate composition analysis: The proximate composition analysis of *M. lucida* stem bark powder was carried out using the standard procedures described by AOAC (2001).

Phyto-chemical screening: Phyto-chemical screening of the extract for secondary metabolites in the aqueous and methanolic extracts was carried out by Mayer's and Dragendorff's test (alkaloids), Shinoda's test (flavonoids), Libermann-Burchard's test (steroids) and froth formation test (saponins) (Evans,1989; Sofowora, 1992; Harbone and Baxter, 1993).

Experimental animals: Albino rats (*Rattus norvegicus*) of both sexes weighing 105-115 g and 12 weeks old obtained from Ayo Ola Farms, Ilorin were used for the study. The rats were housed in metallic cages with raised wire floor at 27°C and kept in a well ventilated room and allowed to acclimatize to the laboratory condition for 2 weeks before being used. They were fed standard commercial rat pellets (Pfizer Feeds Plc, Nigeria) and had free access to water *ad libitum*.

Maintenance and treatment of animals were in accordance with the principles of the guide for care and use of laboratory animals in research and teaching prepared by the National Academy of Science and published by the National Institute of Health (NIH) publication 86-23 revised in 1985.

Acute toxicity study: About 24 rats were randomly selected and used to evaluate the acute toxicity of the extracts. The rats were divided into 6 groups (n = 4) and administered 500, 1000 and 1500 mg kg^{-1} body weight aqueous extract and 500, 1000 and 1500 mg kg^{-1} body weight methanolic extract of *M. lucida* stem bark. The extracts were administered orally. The rats were then observed for 48 h for signs of toxicity or death.

Experimental induction of diabetes: Diabetes was induced in the rats by a single intraperitoneal (ip) injection of 240 mg kg^{-1} body weight alloxan monohydrate (Sigma Chemicals Co., St Louis, USA) dissolved in 0.01 M citrate buffer (pH 3.0). After 48 h, the animals were fasted overnight and blood was taken from the lateral veins of the tail and blood sugar levels were determined with a glucometer (ACCU-Chek, Roche Diagnostics). Rats with blood sugar level >200 mg dL^{-1} were considered diabetic and included in the experiment. Diabetes was allowed to stabilize over a period of 3-5 days.

Experimental design: The diabetic animals were randomly distributed into three groups (n = 5) designated: B, C and D. Group A consisted of five normal rats. Rats in group A were administered 10 mL distilled water orally for 7 days. Group B rats consisted of diabetic untreated rats while groups C and D were diabetic rats administered 240 mg kg^{-1} body weight aqueous and methanolic extracts of *M. lucida* stem bark, respectively orally for 7 days. Before the commencement of the respective treatments rats in each group were fasted overnight but allowed access to water *ad libitum* and weighed. Within the treatment days, all the animals had free access to food and water *ad libitum*.

Serum and tissue preparations: At the end of the treatment period, rats in each group were starved

overnight but had access to water *ad libitum*, weighed and sacrificed by cervical dislocation while under mild anesthesia, 2 mL blood was collected from each rat by cardiac puncture into plain tube. The blood was allowed to stand for 5 min and then centrifuged at 3500 rpm (Beckman GS -6R, Germany) for 10 min at 4°C. Serum was obtained at the supernatant for measuring fasting blood glucose level and enzyme (AST, ALT and ALP) levels. Liver and kidneys were quickly excised, rinsed in Isotonic Sterile Saline (ISS), blotted dry on a filter paper and weighed. Each tissue was then placed in a separate plastic vial containing ice-cold ISS and stored at -4°C until required for further analysis.

Preparation of tissue homogenate: A weighed portion each of liver and kidneys was cut out chopped into small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenate (5%) in buffer solution (50 mM Tris-HCl, 0.25 M sucrose, pH 7.4) was prepared and stored at -4°C until further analysis was carried out.

Biochemical assays: Serum glucose concentration was determined by means of Bayer Elite® Glucometer and compatible blood glucose test strips. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) activities were determined using commercially available enzymatic test kits (Randox Laboratories Ltd., San Francisco, USA) methods following the manufacturer's instructions.

Statistical analysis: Results are means±SEM of triplicate determinations. Data were statistically analyzed using one way Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests (DMRT) using SPSS 11.0. $p<0.05$ than normal control was considered significant.

RESULTS

Results obtained from the proximate composition analysis of *M. lucida* stem bark showed the presence of high content of crude fibre (60% w/w). Total ash content, crude protein, total carbohydrate and crude fat content were 19.8, 8.2, 7.0 and 5.0%, respectively (Table 1). This shows that *M. lucida* stem bark is low in fat. The phytochemical screening analysis of both aqueous and methanolic extracts of *M. lucida* revealed the presence of glycosides, saponins, flavonoids and streroids (Table 2). About 7 days after the extracts administration, all alloxan-induced diabetic animals displayed moderate loss of body weight relative to normal control rats. The loss in weight in the diabetic rats became more pronounced even after treatment with the extracts. There was no significant difference observed in percentage change in weight loss in both aqueous extract treated rats and methanolic extract treated rats (Table 3).

Table 1: Proximate composition analysis of *Morinda lucida* stems bark

Nutrient composition	Weight (%)
Total carbohydrate	7.0
Crude fat	5.0
Protein	8.2
Total ash	19.8
Crude fibre	60.0

Table 2: Phyto-chemical constituents of aqueous and methanolic extracts of *M. lucida* stem bark

Secondary metabolite	Aqueous extract	Methanolic extract
Phenols	-	-
Flavonoids	+	+
Saponins	+	+
Tannins	-	-
Glycosides	+	+
Steroids	+	+

+ = Detected; - = Not detected

Effect on serum glucose level: At the end of the 7 days treatment period, blood glucose concentration was significantly higher in the diabetic animal groups compared to normal control group. However, a significant drop in blood glucose level was observed in diabetic rats treated with both aqueous and methanolic extracts compared with diabetic untreated rats (Table 3). The aqueous extract was particularly more potent in reducing blood glucose concentration as compared with the methanolic extract.

Effect on serum enzymes: From the results obtained on the effect of the extracts on serum enzyme activities (Table 4), there appeared to be no significant change in serum ALP activity across the groups except for a slightly significant decrease observed in diabetic rats treated with the methanolic extract compared to the diabetic rats treated with the aqueous extract. The results (Table 4) also show that there was no significant change ($p>0.05$) in serum AST activity across the groups. Serum ALT activity was not significantly altered from the initial level in normal control, diabetic untreated and diabetic but treated with aqueous extract groups at the end of the treatment period. A significant decrease was observed in ALT activity after treatment with the methanolic extract compared to the enzyme level at the initial period (Table 4).

Effect on liver enzymes: Liver enzymes activities were not significantly altered in both normal and diabetic rats except for a significant reduction in ALP activity observed in both aqueous and methanolic extracts treated diabetic groups compared to both normal control and diabetic untreated groups (Table 5).

Table 3: Effect of aqueous and methanolic extracts of *M. lucida* stem bark on body weight and blood glucose level in alloxan-induced diabetic rats

	Body weight (g)				Blood glucose (mg dL^{-1})			
Values	A	B	C	D	A	B	C	D
Final	151±29.8	114.6±18	122.1±1.2	125.9±19.8	121±11.3	289.5±15.6	169.5±12.1	178±17.2
Initial	145±8.3	127.1±4.6	125.4±12.5	126.9±5.0	118±10.1	236±22.1	294±23.6	248±119.6
Change (%)	4.0	-10.9	-2.7	-0.8	2.5	18.5	-73.5	-39.3

Results are means±SEM of 5 independent determinations

Table 4: Effect of aqueous and methanolic extracts of *M. lucida* stem bark on serum enzymes' activities in alloxan-induced diabetic rats

	ALP (mg dL^{-1})				AST (mg dL^{-1})				ALT (mg dL^{-1})			
Values	A	B	C	D	A	B	C	D	A	B	C	D
Final	15.1±1.3	15.8±5.7	15.2±1.4	13.8±6.2	48.5±14.1	58±6	31.8±3.8	40.3±1.3	11±3.2	16.3±4.7	15±3.6	15.6±2.2
Initial	15.2±1.4	15.6±5.6	15.8±6.2	15.2±7	46.5±4.4	51.4±2.2	53.3±2.7	51.8±1.2	9.8±3.1	12.7±3.3	12.7±3.1	12.6±2.2
Change (%)	-0.7	1.3	-3.9	-10.1	4.1	11.4	-67.6	-28.5	10.9	22.1	15.3	19.2

Results are means±SEM of 5 independent determinations

Table 5: Effect of aqueous and methanolic extracts of *M. lucida* stem bark on liver enzymes' activities in alloxan-induced diabetic rats

	ALP (mg dL^{-1})				AST (mg dL^{-1})				ALT (mg dL^{-1})			
Values												
Final	9.9±2.1	14.9±4.4	10±4.9	10.7±4.4	50.5±22.5	47.2±3.5	46.5±7.4	43.3±1.1	71.9±9.4	82±2.4	78.7±2.4	59.6±1.7
Initial	9.3±1.2	13.8±2.1	13±2.2	13.1±3	48.5±3.5	46.7±5.9	45.8±2.9	44.7±2.1	70.6±4.6	81.7±1.8	75.8±4.1	68.1±7
Change (%)	6.1	7.4	-30	-22.4	4.0	1.1	1.5	-3.2	1.8	0.4	3.7	-14.3

Results are means±SEM of 5 independent determinations

DISCUSSION

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial/heterogenous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes (Akhtar and Ali, 1984) but only a few have been scientifically evaluated. Therefore, we investigated the effect of aqueous and methanolic extracts of *M. lucida* on body weight, blood glucose and on serum and liver enzymes of experimental diabetic rats. Along with, hyperglycemia and abnormalities in serum lipids diabetes is usually associated with microvascular and macrovascular complications which are the major causes of morbidity and death in diabetic suspects (Nagappa *et al*., 2003).

It can be managed by exercise, diet and pharmaceutical drugs which are either too expensive or have undesirable side effects or contraindications (Adewole and Ojewole, 2006). The research for more effective and safer hypoglycemic agents therefore has continued to be an area of research interest. The World Health Organization has recommended and encourages the use of alternative therapy especially in countries where access to conventional treatment of diabetes is not adequate (WHO, 1980).

Observations from this study correlate with the reports from previous studies, in that, the blood glucose level significantly increased in alloxan untreated diabetic rats (Adewole and Ojewole, 2006). In the present study, the continuous treatment of alloxan-induced diabetic rats with both aqueous and methanolic extracts of *M. lucida* stem bark for 1 week (7 days) caused a significant decrease in blood glucose concentrations. The possible mechanisms responsible for this hypoglycemic action of *M. lucida* stem bark extracts may be by potentiating of insulin effect, either by increasing the pancreatic secretion of insulin from the cells of the islet of Langerhans or its release from the bound insulin (Pari and Amarnath, 2004).

Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) are released into the serum especially when there is damage to the hepatic membrane as a result of chemical assaults. Serum levels of these enzymes therefore are significant diagnostic tools in assessing the level of hepatic damage (Crook, 2006; Oluba *et al*., 2008). Data presented in this study on serum ALP and ALT activities were not significantly altered across the groups (normal; diabetic untreated and treated). Serum AST activity was however significantly reduced in diabetic groups treated with both aqueous and methanolic extracts compared to diabetic untreated and normal control rats. This clearly demonstrates that long term consumption of the extracts may not provoke some harmful effects on the animals. This observation is consistent with the findings of Ogbonnia.

Phytochemical screening results from both extracts revealed the presence of the same type of secondary metabolites, saponins, streroids, glycosides and tannins (Table 2). Further research on the quantification of the amount of each phytochemical in the extracts is suggested to be able to delineanate between the extracts

(aqueous and methanolic) in terms of composition. This will also enable us to ascertain which of the constituent metabolites is responsible for their hypoglycemic action. A number of research studies have however shown that saponins, flavopnoids and a host of other plant secondary metabolites possess hypoglycemic, hypotensive, anti-inflammatory and other pharmacological properties in various experimental animals (Ojewole, 2004, 2005; Akah and Okafor, 1992).

CONCLUSION

Conclusively, based on the results of this study, the consumption of *M. lucida* stem bark extract in various forms should be encouraged based on its rich nutritional profile and pharmacological properties.

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