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Evaluation of hypoglycemic and toxicological effects of leaf extracts of *Morinda lucida* in hyperglycemic albino rats

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The aqueous and 50% methanolic extracts of leaf of *Morinda lucida* were investigated for their phytochemical constituents, hypoglycemic and toxicological effects in alloxan-induced hyperglycemia in rats. In addition, the possible acute toxicological effects of the extracts were also studied for seven days.The results revealed the presence of alkaloids, cardenolides, flavonoids, glycosides, saponins and steroids in the aqueous extract while 50% methanolic extract contained anthraquinones in addition to those found in aqueous extract. Phenolics and tannins were not detected in both extracts while anthraquinones was not detected in aqueous extract. The extracts significantly (P<0.05) lowered the blood glucose level in the alloxan-induced hyperglycemia in rats. However, the aqueous extract was more effective than the 50% methanolic extract, though they may possibly be toxic at the doses used because marker enzymes (ALP, AST and ALT) were secreted into the sera of these extracts-treated rats. The acute intra-peritoneal toxicity study of the extracts at the limit doses of 500-1500 mg/kg body weight revealed toxicity of the extracts, most especially at relatively high concentrations for seven days.

Keywords: Leaf extracts, *Morinda lucida,* blood glucose level and toxicological effects.

INTRODUCTION

Hyperglycemia is a serious health condition that can also be referred to as diabetes mellitus or 'Sugar diabetes' as it is often called by lay people. Chronic disorder of carbohydrate, lipid and protein metabolism, characterised by persistent elevation of fasting blood glucose level above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action may lead to hyperglycemia (Murray and Pizzorno, 1997, Odutuga *et al*., 2010). It is associated with increased risk of heart disease, stroke, kidney disease, retinopathy, neuropathy, ulceration and gangrene of extremities (Rotshteyn and Zito, 2004). Thus, diabetes mellitus and its associated complications have significant impact on health, quality of life and life expectancy of its sufferers. The global epidemic of diabetes mellitus is worse or greater in developing than the developed countries (Rotshteyn and Zito, 2004).

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In response to this global health challenge, the World Health Organisation (WHO) expert committee on diabetes mellitus recommended further evaluation of the methods of managing the disease because of high mortality and morbidity arising from its complications and draw backs associated with the use of conventional antidiabetic drugs (Adeneye, *et al*., 2006). There is a broad therapeutic fight against diabetes, but unfortunately, there is no particular cure for it; the disease has only been managed.

In order to meet up with this challenge, there is urgent need to double the herbal therapeutic efforts aimed at selecting remedies for diabetes mellitus. In pursuit of this goal, several medicinal plants are being investigated for their antidiabetic efficacies. Of the several indigenous plants used in the local treatment of diabetes mellitus include the extracts of *Sarsaparilla* (Augusti and Mathew, 1973), *Beta vulgaris* (Yoshikawa *et al*., 1996), *Aloe barbadensis* (Al-Awadi and Gumaa, 1997; Grover *et al*., 2001) and *Morinda lucida*.

Morinda lucida benth (Rubiaceae) is a tropical West Africa rainforest tree also called Brimestone tree while

among the Yoruba natives, it is called 'Oruwo' (Adesida and Adesogan, 1972; *O*dutuga *et al*., 2010). Different parts of the plant are attributed with diverse therapeutic benefits. For instance, in Southern Cameroon, cold decoction of the plant's leaves is used for the treatment of fever (Adesida and Adesogan, 1972). However, in most parts of West Africa, the bitter water decoction of the stem bark of the plant, root and leaf are used as bitter tonic and as stringent for dysentery, abdominal colic and intestinal worm infestation (Adesida and Adesogan, 1972, *O*dutuga *et al*., 2010). The Europeans sometimes use the decoction of the plant root or stem to make "bitters" (Adesida and Adesogan, 1972). 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 malaria patients usually for a few days (*O*dutuga *et al*., 2010).

In the present study, the aqueous and 50% methanolic leaf extracts of *Morinda lucida* were investigated for their hypoglycemic activity and acute toxicological effects on some important organs of albino rats.

MATERIALS AND METHODS

Plant material

Morinda lucida leaves were obtained at Ikeji-Arakeji Village, Osun State, Nigeria. The plant was identified at the Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria.

Animals

The albino rats (*Rattus norvegicus*) used for this study were obtained from the Animal House, Department of Biochemistry, College of Natural Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria.

Assay Kits

Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotranferase (ALT) were products of Randox Laboratories Limited, UK.

Chemicals and Reagents

Alloxan monohydrate was a product of Mekphar Chemical Pharmaceutical Joint-Stock Company, Chiminh City, Vietnam. All other reagents used were of analytical grade and obtained from BDH, London.

Preparation of extracts

Morinda lucida leaves were air-dried for 2 weeks and milled to powdered form using milling Machine. The powder (500 g each) was soaked in 1 litre of aqueous and 50% methanol respectively for 24 hours and then filtered with Muslin cloth. The filterate obtained from both extracts were completely dried into powder over a boiling water-bath. The residue obtained from the aqueous and methanolic leaf extracts were stored separately in water- and air-proof containers and kept in the refrigerator until when needed for analysis.

Phytochemical Screening

Qualitative phytochemical analysis of aqueous and 50% methanolic leaf extracts was carried out with the method described by Odebiyi and Sofowora (1978).

Induction of diabetes in rats

A single intra-peritoneal (ip) injection of 1 ml of 150 mg/kg body weight of Alloxan monohydrate dissolved in 0.1M freshly prepared cold Citrate buffer of pH of 3.0 was injected into each rat (Pari and Venkateswaran, 2002). Stable hyperglycemia was confirmed on the 5^{th} day using glucometer. Rats with fasting blood glucose level greater than 200 mg/dl were considered hyperglycemic and used for this study.

Experimental design for hyperglycemic activity of the leaf extracts of *Morinda lucida*

Fourty albino rats (*Rattus norvegicus*) weighing between 140-145 g each were used for the experiments.

Twenty rats were divided into four groups (A-D) of five rats each for the aqueous extract. Group A consists of control rats, which are not hyperglycemic but given 1 ml of distilled water while group B consist untreated alloxan-induced hyperglycemic rats. Groups C and D consist of the alloxan-induced hyperglycemic rats treated with 120 and 240 mg/kg body weight of aqueous respectively.

The remaining twenty rats were divided into four groups (A-D) of five rats each for 50% methanolic extract. Groups A and B were treated as in aqueous extract while groups C and D were also treated with 120 and 240 mg/kg body weight of 50% methanolic extract respectively.

Preparation of serum

Rats in each group were starved overnight but had

Table 1 Phytochemical constituents of leaf aqueous and methanolic extracts of *Morinda lucida*

Phytochemical constituents	Extracts of leaf of *Morinda lucida*	
	Aqueous	**Methanolic**
Alkaloids	+	+
Anthraquinones	-	+
Cardenolides	+	+
Flavonoids	+	+
Glycosides	+	+
Phenolics	-	-
Saponins	+	+
Steroids	+	+
Tannins	-	-

+ = present
- = Absent

access to water *ad libitum,* weighed and sacrificed by cervical dislocation while under mild anesthesia. Blood (2 ml) was collected from each rat by cardiac puncture into plain tube. The blood was left undisturbed for 5 minutes and then centrifuged at 3500 rpm (Beckman GS-6R, Germany) for 10 minutes at 4^0C. The supernatant (serum) was collected and used for measuring fasting blood glucose level and enzymes (AST, ALP and ALT) activities.

Preparation of tissue homogenate

Rats were anesthetised and their liver and kidney were quickly excised, rinsed in isotonic sterile saline and blotted dry on a tissue paper before weighing. A portion of each liver and kidney was cut out, weighed and chopped into small pieces and then homogenised with pre-cooled pestle and mortar in a bowl of ice cubes. Each tissue homogenate was then placed in a separate plastic containing ice-cold isotonic sterile saline and stored at -4^0C for further analysis.

Enzyme assay

The procedure described by Reitman and Frankel (1957) was employed for the assay of aspartate aminotransferase (EF 2.6.1.1) and alanine aminotranferase (EC 2.6.1.2) activities while the procedure described in the assay kit of alkaline phosphatase was used.

Acute toxicity studies

Fourty rats were also used for the experiment. For the aqueous extract, twenty rats were divided into four groups (A-D) of five rats each. Groups A-D were administered 0, 500, 1000 and 1500 mg/kg body weight of aqueous leaf extract of *Morinda lucida.* Similarly, this was repeated for 50% methanolic leaf extract of *Morinda lucida*. The acute toxicological effects of the two extracts with these concentrations on rats were studied for seven days.

Statistical analysis

Data were expressed as means ± S.D of five determinations. Data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Of the phytochemicals investigated, alkaloids, cardenolides, flavonoids, glycosides, saponins and steroids are present in the aqueous extract while 50% methanolic extract contained anthraquinones in addition. Phenolics and tannins were not detected in both extracts (Table 1).

The aqueous and 50% methanolic extracts significantly ($P<0.05$) reduced the blood glucose level of the hyperglycemic treated rats compares with the untreated group. The hypoglycemic activities of the extracts are concentration dependent. This is evident in the blood glucose level of the hyperglycemic animals (group D) treated with 240 mg/kg body weight of both extracts, which were significantly ($P<0.05$) reduced compared with those hyperglycemic animals (group C) treated with 120 mg/kg body weight of the extracts. However, the aqueous extract showed more hypoglycemic effects compared with the 50% methanolic extract (Table 2).

There was a significant ($P<0.05$) decrease in the final body weights of both extracts-treated and untreated hyperglycemic rats (groups B, C and D) compared with the control (group A). However, the final body weights of

Table 2 Hypoglycemic activities of leaf extracts of *Morinda lucida* on rats

Animal groups	Blood glucose level (mg/dl)			
	Aqueous extract		Methanolic extract	
	Initial	Final	Initial	Final
A	118.00 ± 1.01	118.00 ± 1.30[a]	118.00 ± 1.01	118.00 ± 1.30[a]
B	220.00 ± 2.70	236.00 ± 3.30[d]	220.00 ± 2.70	236.00 ± 3.30[d]
C	235.00 ± 0.78	134.00 ± 2.40[c]	244.00 ± 2.60	169.50 ± 1.10[c]
D	239.00 ± 2.90	76.00 ± 1.80[b]	248.00 ± 1.60	78.00 ± 1.20[b]

Values are mean of five determinations ± SD

Table 3 Effects of leaf extracts of *Morinda lucida* on weight of rats

Animal groups	Body weight (g)			
	Aqueous extract		Methanolic extract	
	Initial	Final	Initial	Final
A	140.50 ± 3.24	149.00 ± 3.26[a]	140.50 ± 3.24	149.00 ± 3.26[a]
B	140.50 ± 2.90	125.80 ± 2.60[d]	140.50 ± 2.90	125.80 ± 2.60[d]
C	141.70 ± 2.70	149.50 ± 1.20[b]	142.05 ± 1.15	148.35 ± 2.90[b]
D	143.00 ± 1.40	147.30 ± 3.50[c]	139.85 ± 1.80	144.90 ± 2.00[c]

Values are mean of five determinations ± SD

Table 4 Effects of leaf extracts of *Morinda lucida* on alkaline phosphatase activity

Animal groups	Alkaline phosphatase activity (nm/min./mg/protein)					
	Aqueous extract			Methanolic extract		
	Serum	Liver	Kidney	Serum	Liver	Kidney
A	515.20 ± 0.10[a]	991.63 ± 0.60[a]	655.04 ± 0.30[a]	515.20 ± 0.10[a]	991.63 ± 0.60[a]	655.04 ± 0.30[a]
B	581.97 ± 0.70[e]	396.96 ± 0.10[d]	506.92 ± 0.70[e]	581.97 ± 0.70[d]	396.96 ± 0.10[d]	506.92 ± 0.70[d]
C	520.07 ± 0.50[b]	496.01 ± 0.70[c]	634.34 ± 0.60[c]	517.98 ± 0.20[b]	976.64 ± 0.90[b]	648.11 ± 0.20[b]
D	527.35 ± 0.50[c]	615.11 ± 0.30[b]	644.21 ± 0.30[b]	519.25 ± 0.60[c]	842.49 ± 0.50[c]	633.70 ± 0.10[c]

Values are mean of five determinations ± SD

the extracts-treated hyperglycemic rats (C and D) were significantly ($P<0.05$) increased compared with the untreated hyperglycemic rats (Group B). Furthermore, there was a significant reduction in the final body weights of the hyperglycemic rats treated with 50% methanolic extract compared with those treated with the aqueous extract (Table 3).

The activities of ALP in the serum of the untreated and extracts-treated hyperglycemic rats were significantly ($P<0.05$) increased compared with the control. Also, the activities of ALP in the liver and kidney of the untreated and extracts-treated hyperglycemic rats were significantly ($P<0.05$) reduced compared with the control (Table 4).

A significant increase ($P<0.05$) in the activities of AST in the serum of extracts-treated rats (groups C and D) was observed compared with the hyperglycemic - untreated group (group B) and control (group A). However, there was a significant reduction in the activities of AST in the liver and kidney of hyperglycemic - untreated and extracts-treated rats compared with the control (Table 5).

Furthermore, there was a significant increase in the

Table 5 Effects of leaf extracts of *Morinda lucida* on aspartate aminotransferase activity

Animal groups	Aspartate aminotransferase activity (nm/min./mg/protein)					
	Aqueous extract			Methanolic extract		
	Serum	Liver	Kidney	Serum	Liver	Kidney
A	48.47 ± 0.40^{a}	50.47 ± 0.30^{a}	41.70 ± 0.50^{a}	48.47 ± 0.40^{a}	50.47 ± 0.30^{a}	41.70 ± 0.50^{a}
B	61.03 ± 0.00^{e}	38.23 ± 0.60^{d}	33.03 ± 0.40^{e}	61.03 ± 0.00^{d}	38.23 ± 0.60^{d}	33.03 ± 0.40^{c}
C	48.83 ± 0.80^{b}	46.50 ± 0.40^{b}	40.87 ± 0.10^{b}	49.27 ± 0.30^{b}	40.30 ± 0.10^{c}	41.27 ± 0.10^{b}
D	49.53 ± 0.40^{c}	45.43 ± 0.60^{c}	39.80 ± 0.60^{c}	49.33 ± 0.70^{c}	47.97 ± 0.80^{b}	41.60 ± 0.80^{a}

Values are mean of five determinations ± SD

Table 6 Effects of leaf extracts of *Morinda lucida* on alanine aminotransferase activity

Animal groups	Alanine aminotransferase activity (nm/min./mg/protein)					
	Aqueous extract			Methanolic extract		
	Serum	Liver	Kidney	Serum	Liver	Kidney
A	10.97 ± 0.20^{a}	71.87 ± 0.40^{a}	42.47 ± 0.20^{a}	10.97 ± 0.20^{a}	71.87 ± 0.40^{a}	42.47 ± 0.20^{a}
B	15.27 ± 0.80^{b}	81.97 ± 0.40^{e}	56.13 ± 0.60^{c}	15.27 ± 0.80^{c}	81.97 ± 0.40^{e}	56.13 ± 0.60^{d}
C	16.00 ± 0.60^{c}	78.70 ± 0.40^{c}	45.87 ± 0.60^{b}	16.03 ± 0.20^{d}	59.60 ± 0.70^{b}	34.17 ± 0.60^{b}
D	16.23 ± 0.10^{d}	81.70 ± 0.00^{d}	79.37 ± 0.00^{e}	17.10 ± 0.10^{e}	68.07 ± 0.00^{c}	79.37 ± 0.00^{e}

Values are mean of five determinations ± SD

activities of ALT in the serum, liver and kidney of the untreated and extracts-treated hyperglycemic rats except in the kidney of those rats treated with 120 mg/kg body weights of 50% methanolic extract when compared with the control (Table 6).

The results from acute toxicological studies revealed that the control rats were very healthy and active while those administered with 500, 1000 and 1500 mg/kg body weight of the aqueous extract were weak and lost appetite but no mortality was recorded throughout the experimental period.

Although there was no mortality recorded with the administration of 500 mg/kg body weight of 50% methanolic extract throughout the experimental period, but the animals were weak. However, the animals administered with 1000 mg/kg body weight of 50% methanolic extract started to die on the third day while all the rats administered with 1500 mg/kg body weight died within 4½ hours. The last rat of the group that was administered with 1500 mg/kg body weight of 50% methanolic extract died after four hours of administration (Table 7).

DISCUSSION

Scientists in the area of phytomedicine and toxicology are now interested in herbal medicine in developing countries (Obici, *et al*., 2008). Herbal treatments involve mainly the use of plant extracts and other plant products (Akerele, 1993; Saggu, *et al*., 2007), which contain bioactive substances that may have potentials to prevent or cause adverse effects (Bent and Ko, 2004). The results of phytochemical screening obtained from extracts of this study agree with those previously reported by Ogundare and Onifade, (2009) and Patience *et al*., (2010). One or more of these phytoconstituents present in the leaf extracts of *Morinda lucida* might be responsible singly or in combination for its observed hypoglycemic effects in the alloxan-induced hyperglycemia in rats. However, the higher potency demonstrated by aqueous extract as observed in this study may be due to the amount/quantity of phytochemicals responsible for the hypoglycemic activity in the aqueous extract since the same concentrations of the extracts were administered. Observations of hypoglycemic activities of these extracts agree with the previous work carried out with the stem bark of the same plant (Odutuga *et al*., 2010). The mechanism of action of the extracts may possibly be by increasing the effects of insulin, either by increasing the secretion of insulin from pancreas in the cells of islet of Langerhans or its release from bound insulin (Pari and Amarnah, 2004; Odutuga *et al*., 2010).

Table 7 Acute toxicity effects of leaf extracts of *Morinda lucida* on rats

Animal groups	Aqueous extract		Methanolic extract	
	Observation	Mortality	Observation	Mortality
A	The animals were healthy and active throughout the experimental period	No death throughout the experimental period of seven days	The animals were healthy and active throughout the experimental period	No death
B	The animals were weak for about one hour and became active again	No death throughout the experimental period of seven days	The animals were weak for about 12 hours	No death but lost appetite throughout the experimental period of seven days
C	The animals were weak and lost appetite for about six hours and became active again	No death throughout the experimental period of seven days	The animals were weak for about two days	They started to die on the third day
D	The animals were weak and lost appetite for about 24 hours and became active again	No death throughout the experimental period of seven days	The animals were immediately weak	They all died after four hours

The measurement of the activities of various enzymes in tissues and body fluids play a significant and well known aid in disease investigation and diagnosis (Malomo, 2000; Yakubu *et al*., 2003). ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Shahjahan *et al*., 2004) and often employed to assess the integrity of the plasma membrane (Akanji *et al*., 1993). The observed reduction in ALP activities in the liver and kidney following the administration of the extracts may limit or hinder the adequate transportation of the required ions on the molecules across the plasma membrane (Akanji *et al*., 1993). It may also lead to the less availability of the phosphate groups for the phosphorylation of ethanolamine and choline needed for the synthesis of major phospholipids like phosphatidylethanolamine and phosphatidylcholine. Furthermore, it can also hinder the synthesis of nuclear acids (Ramalingam and Vimaladevi, 2002). AST and ALT are also useful marker enzymes in assessing damage to organs. They are normally localised within the cells of the liver, kidney, and some other organs. These enzymes 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 (Oluba *et al*., 2008; Odutuga *et al*., 2010). Therefore, their presence in the serum of the extracts-treated animals may be given information on the tissue injury or dysfunction.

The study of acute toxicity revealed that the extracts are very toxic at relatively high concentrations. Methanolic extract may be more toxic than aqueous extract because mortality was recorded in the methanolic

extract-treated rats but not with the aqueous extract.

CONCLUSION

This study revealed that the leaf aqueous and methanolic extracts of *Morinda lucida* have hypoglycemic effects on alloxan-induced hyperglycemia in albino rats. However, the doses of the extracts used may be toxic. The methanolic extract may be more toxic compared with the aqueous extract. Care should therefore be taken in the use of the extracts.

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