International Research Journal of Biochemistry and Bioinformatics (ISSN-2250-9941) Vol. 2(1) pp.016-021 January, 2012
Available online http://www.interesjournals.org/IRJBB

Full length Research Paper

Phytopharmacological evaluation of *Morinda morindoides* for anti-hyperglycemic activity in normal rabbits

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Accepted 09 January, 2012

This study aimed to evaluate anti-hyperglycemic activity of aqueous and 70% ethanol extracts of *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae) in normoglycemic rabbits by oral glucose tolerance test. Blood samples were collected from the marginal vein (0 h) and then drugs (extracts and **glibenclamide) were administered to each group of rabbit by oral route. One hour (1 h) later, glucose (4 g/kg, p.o.) was administered in all groups. Blood samples were collected at 1 h, 2 h, 4 h and 6 h after administration of drugs. Blood was centrifugated at 3500 rpm during 5 min and the serum was analyzed to determine glucose, triglycerides and cholesterols levels. Aqueous and ethanol extracts (500 and 800 mg/kg) of *M. morindoides* had no effect on basal glycemia. Both extracts of *M. morindoides* significantly lowered the subsequent hyperglycaemia induced with glucose (10 mg/kg) administered *per os*. Moreover, the pretreatment of normal rabbits with *M. morindoides* aqueous and ethanol extracts normalized faster the glucose levels (3 h after glucose administration) compared to the hyperglycemic control group (5 h after glucose administration). *M. morindoides* have a considerable anti-hyperglycemic activity which justifies the use of this plant in traditional herbal medicine practice for the treatment of diabetes mellitus.**

Keywords: *Morinda morindoides,* anti-hyperglycemic, Oral Glucose Tolerance Test

INTRODUCTION

Diabetes, which is one of the most severe metabolic disorders in humans, is characterized by hyperglycemia as a result of a relative or an absolute lack of insulin or insulin activity on target tissue, or both (Lebovitz and Banerji, 2004). In the year 2000, 150 million people world-wide had diabetes and this is expected to reach 366 millions by 2030 (Nakasone et al., 2009).

Diabetic disorders are classified into two major groups: type 1 or insulin-dependent diabetes mellitus (IDDM), and type 2 or non-insulin-dependent diabetes (NIDDM) (Leslie and Pozzilli, 1994). Among the cases diagnosed, 95% are type 2 diabetes (Newell, 2004). This disease (type 1 and type diabetes) is characterized by polyuria, glycosuria and hyperglycemia (N'guessan et al., 2009).

Before 1922, diabetes therapy rested essentially on dietary measures including the use of traditional anti-hyperglycemic plants (Bailey and Day, 1989). Present therapy for diabetes mellitus relies on an arsenal of drugs developed since the introduction of insulin (Bailey and Day, 1989). Although many traditional plants were described for the treatment of diabetes mellitus (Gray and Flatt, 1997), only few could receive scientific or medical scrutiny. The World Health Organization has

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recommended, accordingly, that traditional plant treatments for diabetes warrant further evaluation (World Health Organization Expert Committee on Diabetes Mellitus, 1980). Such studies might reveal effective dietary adjuncts for the treatment of the disease or the discovery of natural products to develop new antidiabetic drugs.

Numerous medicinal plants such as *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae) and their formulations are being used for therapy of diabetes in ethnomedicinal practices (N'guessan et al., 2009). *M. morindoides* is found in the borders of tropical forests. In the Democratic Republic of Congo, *M. morindoides* has long been used in villages and towns for the treatment of some parasitic diseases and diabetes mellitus. The active constituents of this plant and its antiprotozoal, anti-malarial, anti-rheumatic, anti-oxidative, anti-amoebic and antidiarrheal activities have already been studied (Newinger, 2000; Cimanga et al., 2006; Méité et al., 2010). Although, the leaves of *M. morindoides* are used for the treatment of diabetes mellitus by traditional medical practitioners in some countries like, Democratic Republic of Congo and Ivory Coast, scientific informations relating to its anti-hyperglycemic properties are not sufficiently available.

Hence in the present study, an attempt has been made to investigate the hypoglycemic and anti-hyperglycemic effects of the leaves of *M. morindoides* in experimental animal model.

MATERIAL AND METHODS

Plant material

The leaves of *M. morindoides* were collected from Daloa (central west region of Ivory Coast) in June 2006. The plant was identified and authenticated by Pr AKE ASSI, of the Department of Botany, University of Cocody. A voucher specimen (N° 17710) of the plant was deposited in the herbarium of the National Floristic Center of the University of Cocody-Abidjan.

Animals

Rabbits (*Oryctolagus cuniculus)* of 12-16 weeks old weighing 1.5-2 kg and bred at the Department of Biosciences, University of Cocody-Abidjan, Ivory Coast, were used for the experiments. The animals were kept in standard cages with good ventilation, free access to food and water. Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences, University of Cocody-Abidjan. These guidelines were in accordance with the internationally accepted principles for care and use of laboratory animals.

After randomization into various groups and before initiation of experiments, the rabbits were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and 12 h dark/light cycle. Animals described as fasting were deprived of food and water for 16 h *ad libitum*.

Preparation of extracts

Leaves of *M. morindoides* were air dried at room temperature for 3 weeks to a constant weight and grounded to powder. Then, the dried material was pulverized and extracted with 70% ethanol. 100 g were mixed with 2 L of water or 70 % ethanol and agitated with a magnetic agitator (IKAMAG RCT) for 48 h at room temperature. After cotton and paper (Watmann 3 mm filters) filtration, evaporation of the solvent was achieved with rotatory evaporator (Büchi 461 water bath) at 40 ºC and 20 g of each extract (water and 70% ethanol) were obtained after evaporation (yield 20%).

Phytochemical analysis

The freshly prepared extract was screened for the presence or absence of secondary metabolites such as tannin, alkaloids, saponins, flavonoids, sterol, quinines and phenolic compounds. They were identified by characteristic color change using standard procedures (Harborne, 1976).

Experimental Protocol

Oral Glucose Tolerance Test

Rabbits were divided into seven groups containing six animals in each group. All animals fasted for 18 h before treatment. All treatments were done by the oral route. Group I was kept as vehicle control which received distilled water (10 ml p.o.), group II received distilled water (10 ml p.o.), group III received glibenclamide (10 mg/kg b.w.), group IV received aqueous extract of *M. morindoides* (500 mg/kg b.w.), group V received aqueous extract of *M. morindoides* (800 mg/kg b.w.), VI and VII received ethanol extract of *M. morindoides* (500 mg/kg and 800 mg/kg) respectively. Sixty (60) minutes after drug administration, the rabbits of group II, III, IV, V, VI, VII were loaded with glucose (4 g/kg, p.o.). Blood samples were collected from the marginal vein, in sterile tubes just prior to drug administration and 1 h, 2 h, 4 h, 6 h after drug administration. The serum was separated at 3500 rpm for 5 min at 37 °C using a centrifuge (Jouan) and blood glucose level was determined by glucose

Table 1. Effect of aqueous and ethanol extract of *M. morindoides* on blood glucose levels of hyperglycemic rabbits

Blood glucose levels (mg/mL)					
Groups	**0 h**	**1 h**	**2 h**	**4 h**	**6 h**
Normal Control	0.95±0.06	0.95±0.07 (0%)	1.01±0.09 (+6.31%)	1.03±0.09 (+8.42%)	0.90±0.03 (-5.26%)
Glibenclamide (10 mg/kg)	0.89±0.05	0.61±0.10* (-31.46%)	1.42±0.19* (+59.55%)	0.98±0.45 (+10.11%)	0.84±0.07 (-5.62%)
Hyperglycemic control	0.87±0.07	0.94±0.12 (+8.04%)	1.75±0.23*** (+101.15%)	1.68±0.25*** (+93.10%)	1.05±0.03 (+20.69%)
EAMM (500 mg/kg)	0.98±0.08	1.11±0.01 (+13.26%)	1.34±0.12** (+36.73%)	1.01±0.09 (+3.06%)	1.05±0.11 (+7.14%)
EAMM (800 mg/kg)	0.93±0.05	1.11±0.08 (+19.35%)	1.40±0.11*** (+50.54%)	0.98±0.14 (+5.38%)	0.99±0.01 (+6.45%)
EEMM (500 mg/kg)	0.95±0.09	1.04±0.09 (+9.47%)	1.28±0.13** (+34.74%)	0.82±0.10 (-13.68%)	0.91±0.06 (-4.21%)
EEMM (800 mg/kg)	0,94±0,08	0.99±0.03 (+5.32%)	1.25±0.09** (+32.98%)	1.02±0.13 (+8.51%)	0.99±0.03 (+5.32%)

Values are mean percent blood glucose levels (± S.E.M.) of six animals. Significant difference as compared to 0 h level: *p < 0.05, **p < 0.01, ***p < 0.001
AEMM: Aqueous extract of *M. morindoides*; EEMM: Ethanol extract of *M. morindoides*

oxydase method using available commercial kits (Chronolab System S.L., Spain) (Siest et al., 1981).

Assay for serum triglycerides and total cholesterol

The serum was also analyzed for triglycerides by the enzymatic method using commercially available reagent kit (Chronolab System S.L., Spain). This method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (Buccolo, 1973).

The cholesterol level was determined with a commercially available reagent kit (Cypress Diagnostics, Belgium) by the enzymatic method (Meiattini, 1978).

Determination of sugar in plants extracts

Water-soluble carbohydrates were determined by the sulphuric acid method, according to Dubois et al. (1956) and total reducing sugars were quantified using the dinitrosalicilic acid method described by Bernfeld (1956).

Statistical analysis

Data obtained are presented as means ± standard error of mean (S.E.M.) for the number of animals in each group (n = 6). The differences between the data obtained from 'test' animal groups and the data obtained from untreated animal groups, were subjected to one way analysis of variance (ANOVA; 95% confidence interval), followed by Dunnett's test. Values with $p < 0.05$ compared with the control group were considered as being significantly different.

RESULTS

Phytochemical screening

The results found the presence of alkaloids, polyphenols, flavonoids, catechic tanins, gallic tannins, quinons, sapononins, sterols and polyterpens in all the extracts of *M. morindoides*.

Sugar content

The results show various water soluble carbohydrates contents in the extracts of 4.4% and 8.8% respectively for the aqueous extract *of M. morindoides* and the ethanol extract of *M. morindoides*. The total reducing sugar contents are 1.4% and 3% respectively for the aqueous extract of *M. morindoides* and the ethanol extract of *M. morindoides*.

Effects of extracts on blood glucose level of normoglycemic rabbits

The effects of glibenclamide and the different extracts indeed the aqueous extract of *M. morindoides* (500 and 800 mg/kg), the ethanol extract of *M. morindoides* (500 and 800 mg/kg) on the fasting normoglycemic rats (before glucose administration) are shown in table 1. The

Table 2. Effect of aqueous and ethanol extract of M. morindoides on cholesterol level of hyperglycemic rabbits

cholesterol levels (mg/mL)					
Groups	**0 h**	**1 h**	**2 h**	**4 h**	**6 h**
Normal Control	1.40±1.02	1.46±0.79 (+4.28%)	1.34±0.81 (-4.28%)	1.41±0.85 (+0.71%)	1.43±0.92 (+2.14%)
Glibenclamide (10 mg/kg)	1.32±0.16	1.56±0.39 (+18,18%)	1.58±0.09 (+19.70%)	1.44±0.30 (+9.10%)	1.22±0.22 (-7,57%)
Hyperglycemic control	0.99±0.27	1.00±0.22 (+1.01%)	0.97±0.24 (-2.02%)	1.06±0.10 (+7.07%)	1.03±0.17 (+4.04%)
EAMM (500 mg/kg)	1.86±0.70	1.26±0.67 (-32.26%)	1.49±0.65 (-19.90%)	1.27±0.49 (-31.72%)	1.54±0.66 (-17.20%)
EAMM (800 mg/kg)	1.40±0.22	1.33±0.29 (-5%)	1.14±0.32 (-18.57%)	1.10±0.11 (-21.43%)	1.15±0.22 (-17.86%)
EEMM (500 mg/kg)	1.27±0.18	1.02±0.22 (-19.68%)	1.19±0.29 (-6.30%)	0.92±0.27 (-27.56%)	1.06±0.25 (-16.53%)
EEMM (800 mg/kg)	0,95±0.09	0.95±0.11 (0%)	0.92±0.33 (-3.16%)	0.72±0.31 (-24.21%)	0.90±0.14 (-5.26%)

Values are mean percent blood cholesterol levels (± S.E.M.) of five animals. There was no significant changes in cholesterol levels as compared to 0 h level.
AEMM: Aqueous extract of *M. morindoides*; EEMM: Ethanol extract of *M. morindoides*

results indicate a non-significant elevation ($p>0.05$) of blood glucose level varying from 2.3% to 19.35% at 1 h in all groups treated with tested extracts. On the other hand, there was a significant decrease (-31.46%) of blood glucose level ($p<0.05$) in the group treated with glibenclamide (10 mg/kg) 1 h after drugs administration to fasted rabbits.

Effects of extracts on blood glucose level of hyperglycemic rabbits (Glucose tolerance test)

The anti-hyperglycemic effect of extracts on the blood sugar levels of hyperglycemic rats is shown in table 1. The results show that there is not significant variation of the blood glucose levels at 1, 2, 4 and 6 h intervals in the normal control group.

However in hyperglycemic control group, the administration of glucose solution (4 g/kg, p.o) caused a significant ($P < 0.001$) elevation in blood glucose level at 2 h (+101.15 %) and 4 h (+93.10%) while the blood glucose level at 6 h was similar to its initial value (0.87±0.07 mg/mL) at 0 h.

After glucose administration to the group treated with 500 mg/kg and 800 mg/kg of aqueous extract of *M. morindoides*, there was a significant increase ($P < 0.01$-0.001) of the blood glucose level only at 2 h (+36.73% and +50.54%). At 4 and 6 h, there were no significant differences of blood glucose level compared to the initial value ($P > 0.05$). In the group treated with glibenclamide (10 mg/kg), there was also a significant increase ($P > 0.05$) of the blood glucose level at 2 h and thereafter, the blood glucose level was quite similar ($P > 0.05$) with its initial value at 4 and 6 h.

After glucose administration to the group treated with 500 mg/kg and 800 mg/kg of ethanol extract of *M. morindoides*, there was a significant increase ($P < 0.01$) of the blood glucose level only at 2 h (+34.74% and +32.98%). At 4 and 6 h, there were no significant differences of blood glucose level compared to the initial value. The lowering of blood glucose level is faster in the groups treated with the aqueous and ethanol extracts of *M. morindoides* (500 mg/kg and 800 mg/kg) than the hyperglycemic control group.

Cholesterol level

As in the control group, serum cholesterol levels were not significantly ($P > 0.05$) modified by glibenclamide, *M. morindoides* (aqueous and ethanol extracts) in normoglycemic and hyperglycemic rabbits (Table 2).

Triglycerides levels

As in the control group, they were no significant variation ($P > 0.05$) in the groups treated with glibenclamide (10 mg/kg) and *M. morindoides* (aqueous and ethanol extracts) of the serum triglyceride level (table 3).

Table 3. Effect of aqueous and ethanol extract of M. morindoides on triglycerides level of hyperglycemic rabbits

Triglycerides levels (mg/mL)					
Groups	**0 h**	**1 h**	**2 h**	**4 h**	**6 h**
Normal Control	1.90 ± 0.58	1.74 ± 0.69 (-12.12%)	1.84 ± 0.79 (-7.07%)	1.91 ± 0.64 (-3.53%)	1.85 ± 0.60 (-6.56%)
Glibenclamide (10 mg/kg)	1.63± 0.49	1.54 ± 0.37 (-5.52%)	1.38± 0.15 (-15.34%)	1.53± 0.27 (-6.13%)	1.31 ± 0.11 (-19.63%)
Hyperglycemic control	1.97 ± 0.34	1.50 ± 0.33 (-23.86%)	1.44 ± 0.17 (-26.9%)	1.34 ± 0.19 (-31.98%)	1.53 ± 0.16 (-22.33%)
EAMM (500 mg/kg)	2.08 ± 0.30	2.29 ± 0.42 (+10.10%)	1.55 ± 0.40 (-25.48%)	2.12 ± 0.46 (+1.92%)	1.91 ± 0.51 (-8.17%)
EAMM (800 mg/kg)	1.59 ± 0.23	2.06 ± 0.28 (+29.56%)	1.54 ± 0.21 (-3.14%)	1.34 ± 0.22 (-15.72%)	1.71 ± 0.07 (+7.55%)
EEMM (500 mg/kg)	2.16 ± 0.04	2.41 ± 0.33 (+11.57%)	1.95 ± 0.28 (-9.72%)	1.91 ± 0.38 (-11.57%)	1.73 ± 0.23 (-19.91%)
EEMM (800 mg/kg)	1.37 ± 0.12	2.01 ± 0.28 (+46.71%)	1.42 ± 0.20 (+3.65%)	1.43 ± 0.40 (+4.38%)	1.53 ± 0.49 (+11.68%)

Values are mean percent blood triglycerides levels (± S.E.M.) of six animals. Significant difference as compared to 0 h level: *p < 0.05, **p < 0.01, ***p < 0.001
AEMM: Aqueous extract of *M. morindoides*; EEMM: Ethanol extract of *M. morindoides*

DISCUSSION

This work has been conducted in order to explore the anti-hyperglycemic activities of *M. morindoides* a plant which are claimed to possess antidiabetic activities. The results have indicated that there are no differences of the variation of measured parameters between male and female rabbits indicating that the metabolism of sugar is similar in both sexes.

Our results have indicated that before the glucose oral tolerance test, the blood sugar concentration is comprised between 0.86±0.05 mg/mL and 1.05±0.03 mg/mL. The normal blood sugar concentration is from 0.8 mg/mL to 1.2 mg/mL in a number of mammals. This blood sugar concentration is constant in normal individuals, and the regulation is very finely adjusted. By administering glucose, the concentration is increased rapidly within 60 minutes, where after it decreases to the normal, 4 h after glucose administration in the hyperglycemic control group.

Our study indicates that *M. morindoides* increases blood glucose in the first time in normal rabbits. This hyperglycemic activity of extracts could be correlated with their content in reduced sugar. In fact, the results have indicated that the different extracts contains sugars which probably enhance the glycemia of the experimental animals.

In the other hand, the data of Oral Glucose Tolerance Test (OGTT) revealed that the blood glucose levels of the hyperglycemic control group reached peak 60 min after the oral glucose load and gradually decreased to the preglucose load level after 6 h. While, the blood glucose level, after reaching its peak, decreased to the initial value (0 h) after 4 h in the group treated with *M. morindoides* and glibenclamide.

The data obtained clearly indicate that the oral administration of *M. morindoides* aqueous and ethanol extracts exhibited significant anti-hyperglycemic. On the other hand, *M. morindoides* did not produce any change in the blood cholesterol level, nor the blood triglycerides level of hyperglycemic rabbits. Moreover, both the extracts (aqueous and ethanol) of *M. morindoides* lowered blood glucose levels with similar efficacy indicating that water as so as ethanol can extract the anti-hyperglicemic components of *M. morindoides*. The possible mechanism by which *M. morindoides* brings its anti-hyperglycemic action may be by potentiating the insulin effect by stimulating insulin release from pancreatic β-cells or its release from the bound form (Mohamed and Ojewole, 2003). Beside this, it might involve extra-pancreatic action, which may include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Andrade-Cetto and Wiedenfield, 2004).

The phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, catechic tanins, gallic tannins, quinons, sapononins, sterols and polyterpens in the aqueous and ethanol extracts of *M. morindoides*. It is well documented that flavonoids and tannins stimulate secretion or possess an insulin like-effect (Grover et al., 2002). This anti-hyperglycemic effects of *M. morindoides*

could be attributed to flavonoids like kampferol and quercetol previously isolated in this plant by Cimanga et al. (2006) and which have been reported to stimulate insulin secretion (Andrade-Cetto and Wiedenfield, 2004).

CONCLUSION

From the present study, it is concluded that *M. morindoides* may be useful in treating diabetes mellitus. The 70% ethanol and aqueous extracts of *M. morindoides* have indicated high level of antidiabetic potencies. The extracts exhibited anti-hyperglycemic activity comparable to that of a standard antidiabetic drug, glibenclamide. This result provides a scientific support for the use of *M. morindoides* for the treatment of diabete mellitus in folk medicine. Further studies will be conducted to study the antidiabetic activity of this plant in an experimental model of diabetes induced with streptozotocin and in Zucker diabetic fatty rats.

ACKNOWLEDGEMENTS

The authors are thankful to Professor Dagui Monnet, Director of the Biochemical and Molecular Biology Laboratory (Pharmaceutical and Biological Sciences Department, University of Cocody-Abidjan) for providing all the facilities to carryout the studies.

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