Hypoglycaemic and hypolipidaemic effects of crude extracts and chromatographic fractions of *Morinda morindoides* root bark in diabetic rats

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Abstract

Hypoglycaemic and hypolipidaemic effects of different extracts and fractions of root bark from the plant *Morinda morindoides* (Baker) Milne-Redh of the family Rubiaceae were evaluated in alloxan-induced diabetic rats. The aqueous and methanolic extracts were administered to 48 rats orally at a dose of 400 mg·kg⁻¹ for 21 days. Fractions (hydromethanol, hexane, chloroform and ethyl acetate) from bio-activity guided fractionation and chromatographic sub fractions (CsF) A-F from accelerated gradient chromatography were also evaluated in 45 rats for the hypoglycaemic activity at doses of 400 mg·kg⁻¹, 200 mg·kg⁻¹ and 100 mg·kg⁻¹ of solvent fractions and (CsF) A-F, respectively. Glibenclamide was used as positive control. Polyoxyethylene sorbitan monooleate and distilled water administered to rats were used as negative controls. The dose of 400 mg·kg⁻¹ of aqueous and methanolic extracts and 100 mg·kg⁻¹ of chloroform CsF B of *Morinda morindoides* caused (62.8%, 56% and 74%, respectively) reductions in blood glucose level (BGL). The aqueous extract caused significant (*P* < 0.05) decreases in the values of serum cholesterol (133.48 ± 1.1) mg·dl⁻¹, low density lipoprotein (66.38 ± 2.5) mg·dl⁻¹ and significant (*P* < 0.05) increase in the value of high density lipoprotein (51.03 ± 3.0) mg·dl⁻¹ when compared to the control. These results confirm the folkloric claim of the hypoglycaemic and hypolipidaemic activities of *Morinda morindoides* root bark.

Antidiabetic, lipid lowering, medicinal plants

Diabetes mellitus which causes hyperglycaemia is a disease caused by insufficient insulin production by the pancreatic β cells or inability of the body to utilize insulin due to the peripheral tissue resistance. This leads to a serious damage of many parts of the body’s systems, especially nerves and blood vessels (WHO 2010).

The chemical agents used in the treatment of diabetes mellitus type 2 are expensive and could lead to adverse side effects. Development of alternative strategies for the prevention and treatment of diabetes is therefore necessary especially in countries with poor economy (Fertig et al. 1995).

*Morinda morindoides* (Baker) Milne-Redh of the family Rubiaceae has medicinal application in some African countries, especially in Nigeria, where it is used for the treatment of different diseases. It is called brimstone tree in English and “oju ologbo” in Yoruba (Southwest, Nigeria). *M. morindoides* is used in the form of infusion against malaria (Tona et al. 2001), diarrhoea, haemorrhoids, gonorrhoea, amoebiasis and rheumatism (Kambu 1990; Tona et al. 1999; Cimanga et al. 2006). The extract from root bark is used for the treatment of diabetes mellitus in South Western part of Nigeria.

Though many studies have been carried out on the leaf extract of this plant, less work has been done on the root bark extract in the treatment of diabetes mellitus as claimed by the traditional healers in Southwest Nigeria. This study is therefore aimed at investigating the hypoglycaemic and hypolipidaemic properties of aqueous and methanol extracts, and the bioactive solvent chromatographic sub fractions of *M. morindoides* root bark in alloxan-induced diabetic rats.

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Materials and Methods

Plant material and preparation of extract

Fresh root bark of *Morinda morindoides* was identified at the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan, and Botany Department, College of Natural Sciences, University of Agriculture, Abeokuta, Nigeria where a specimen was deposited.

Five hundred grams of the root bark were air-dried, pulverized and soaked in 2 l of distilled water for 48 h, filtered and freeze-dried. Then, two kilograms of the air-dried and pulverized root bark were soaked in 5 l of methanol for 72 h and concentrated at 55 °C in water bath. The samples were stored at -4 °C until the experiment.

Two grams of the freeze-dried solid *M. morindoides* were dissolved in 20 ml of distilled water (100 mg·ml⁻¹). Two g of the solid residue from concentrated methanol filtrate were dissolved in 20 ml of Tween-80 (100 mg·ml⁻¹). These solutions were used for administration to the rats.

Bio-activity guided fractionation

One hundred grams of the crude methanol extract of the root bark *M. morindoides* were suspended in 1 : 3 ratio with methanol-water mixture (hydromethanol), -n-Hexane, -chloroform, and -ethyl acetate. This dilution was done in a separatory funnel at each stage. The organic fractions (phases) were collected and the solvent was removed by concentrating the fraction in water bath at 55 °C. This procedure was carried out for all n-Hexane, chloroform, ethyl acetate and MeOH. The bioactive solvent-fractions were subjected to purification by further fractionation using a standard procedure of accelerated gradient chromatography (Svoronos and Sarlo 1993) and the chromatographic sub-fractions were pooled into six (A-F) according to their TLC. All fractions and reference glibenclamide were suspended in Tween-80 and used for oral administration to the rats.

Animals

Ninety three, 8–10 week-old Wistar rats of both sexes, weighing 150–200 g were provided with rodent feed from Vital feeds Limited, Ibadan, Nigeria and supplied with water *ad libitum*. The animals were acclimatized to laboratory conditions for two weeks before the experiment with controlled lighting of 12 : 12 h of light : dark cycles, temperature of 26 ± 2 °C and relative humidity of 55%.

Induction of diabetes mellitus

The fasting blood glucose levels were measured at intervals of 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after a single dose of the extract and glibenclamide in rats. Diabetes mellitus was induced by a single injection of 5% alloxan monohydrate (Sigma-Aldrich, Belgium) at a dose rate of 125 mg·kg⁻¹ intraperitoneally (Trivedi et al. 2004). Diabetes mellitus was confirmed after 48 h. Only rats with blood glucose level of 150 mg·dl⁻¹ and more were included in the study. The control group of rats was injected with normal saline intraperitoneally. The third day to the alloxan injection was taken as Day 0. The rats were fasted for 12 h before the experiment but were allowed unrestricted access to water. Polyoxyethylene sorbitan monooleate (Tween 80, Sigma-Aldrich, Belgium) was administered orally as delivery vehicle for the plant extract, glibenclamide (Nigerian-German Chemical PLC) and the negative control group.

Effect of multiple administration of aqueous extract of *M. morindoides* on blood glucose level and lipids - Experiment 1

The animals were divided into 4 groups of 6 rats in one group. Group 1 included diabetic rats administered 400 mg·kg⁻¹ of aqueous extract of *M. morindoides*; Group 2 included diabetic rats administered 10 mg·kg⁻¹ of glibenclamide; Group 3 included diabetic rats given distilled water at 10 ml·kg⁻¹ and Group 4 included control rats (non-diabetic) administered 10 ml·kg⁻¹ of distilled water orally. All administration was done once daily for 21 days and the blood glucose level was measured at Day 0 and at Day 21.

The serum cholesterol, triglycerides, the high density lipoprotein (HDL) and low density lipoprotein (LDL) were also measured at Day 21 from blood collected from the retro-orbital plexus of ether-anesthetized rats using standard procedures (Bucolo and David 1973; Allain et al. 1974).

The effect of single dose of the methanol extract of *M. morindoides* in rats - Experiment 2

Rats used for this experiment were as described above except for Group 1 that included diabetic rats administered 400 mg·kg⁻¹ methanol extract of *M. morindoides* and Group 4 of rats administered 5 ml·kg⁻¹ Tween-80 serving as control; all 4 groups were treated only once. The fasting blood glucose levels were measured at intervals of 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after the administration. The percentage change in BGL was calculated using following equation:

\[ \% \text{ change in BGL} = \frac{Vx - Vo}{Vo} \times 100 \]

where

- Vo is the BG values at 0 h,
- Vx are the BG values at 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h, respectively (Fuentes et al. 2004).
Hypoglycaemic effect of the chromatographic fractions of *M. morindoides* - Experiment 3

In this experiment, 9 groups of 5 rats per group were used with Groups 1–8 consisting of diabetic rats and Group 9 consisting of non-diabetic rats. Groups 1–6 were given 100 mg·kg\(^{-1}\) of the chromatographic sub-fractions (CsF) A–F. Rats in Group 7 were treated with 10 mg·kg\(^{-1}\) glibenclamide and Groups 8–9 rats were given Tween-80 at a dose of 5 ml·kg\(^{-1}\) body weight. The fasting blood glucose levels were measured at intervals of 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after the administration.

Statistical analysis

Results were expressed as mean ± SEM. Analysis of the data was done using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. \(P\) value < 0.05 was considered significant in all cases.

**Results**

Effect of multiple administration of aqueous extract of *M. morindoides* on BGL and lipids

After 21 days of treatment, there was a significant \((P < 0.05)\) reduction in blood glucose level of the rats treated with 400 mg·kg\(^{-1}\) aqueous extract of *M. morindoides* (62.9% reduction in BGL). The group of rats treated with 10 mg·kg\(^{-1}\) of glibenclamide showed a significant \((P < 0.05)\) decrease in the blood glucose level (63.2% reduction) after 21 days of treatment compared to the control rats with 269.50 ± 10.6 mg·dl\(^{-1}\) at day 0 to 324.53 ± 22.3 mg·dl\(^{-1}\) at day 21 (20.1% increase in BGL) (Table 1).

The extract also caused significant \((P < 0.05)\) decreases in serum cholesterol (133.48 ± 1.1) mg·dl\(^{-1}\) and serum low density lipoproteins (66.38 ± 2.5) mg·dl\(^{-1}\) and significant \((P < 0.05)\) increase in serum high-density lipoprotein value (51.03 ± 3.0) mg·dl\(^{-1}\). Glibenclamide (10 mg·kg\(^{-1}\)) caused a significant \((P < 0.05)\) reduction in the serum cholesterol (134.13 ± 1.5) mg·dl\(^{-1}\), low-density lipoprotein (68.70 ± 3.5) mg·dl\(^{-1}\) and significant \((P < 0.05)\) increase in the serum high-density lipoproteins (47.32 ± 2.2) mg·dl\(^{-1}\) after 21 days post treatment when compared to the values from rats in the untreated diabetic control group (Table 1).

Effect of single dose methanolic extract of *M. morindoides*

Methanolic extracts of *Morinda morindoides* at a single dose of 400 mg·kg\(^{-1}\) body weight produced a significant \((P < 0.05)\) reductions in the blood glucose level of the rats at 1 h, 2 h, 4 h, 6 h, 8 h and 24 h post treatments (Table 2). The percentage hourly reductions in blood glucose level were from 2.5% in 30 min to 17.7%, 33.1%, 45.9%, 40.4% and 41.8% in 1 h, 2 h, 4 h, 6 h, 8 h and 24 h, respectively. The extract produced a significant reduction \((P < 0.05)\) of BGL (56%) at the 24th h after treatment when compared to the untreated diabetic control group (Table 2).

Group of rats dosed with 10 mg·kg\(^{-1}\) glibenclamide showed percentage hourly reduction of the BGL of 6.2%, 22.5%, 27.1%, 33.4%, 23.3%, 24.8% and 52.4% at 30

<table>
<thead>
<tr>
<th>Groups (n = 6)</th>
<th>Blood glucose (mg·dl(^{-1}))</th>
<th>Blood glucose % Change in blood glucose level (%)</th>
<th>Cholesterol (mg·dl(^{-1}))</th>
<th>TRIG (mg·dl(^{-1}))</th>
<th>HDL (mg·dl(^{-1}))</th>
<th>LDL (mg·dl(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
</tr>
<tr>
<td>Group 1</td>
<td>277.87 ± 17.5</td>
<td>103.00 ± 20.0*</td>
<td>-62.9</td>
<td>133.48 ± 1.1*</td>
<td>80.00 ± 4.6</td>
<td>51.03 ± 3.0*</td>
</tr>
<tr>
<td>Group 2</td>
<td>268.63 ± 14.8</td>
<td>98.85 ± 2.9*</td>
<td>-63.2</td>
<td>134.13 ± 1.5*</td>
<td>86.45 ± 4.5</td>
<td>47.32 ± 2.2*</td>
</tr>
<tr>
<td>Group 3</td>
<td>269.50 ± 10.6</td>
<td>324.53 ± 22.3</td>
<td>+ 20.1</td>
<td>159.62 ± 2.6</td>
<td>91.87 ± 4.8</td>
<td>25.70 ± 0.9</td>
</tr>
<tr>
<td>Group 4</td>
<td>113.18 ± 2.3</td>
<td>108.00 ± 3.1</td>
<td>-4.6</td>
<td>128.82 ± 4.1*</td>
<td>83.90 ± 4.3</td>
<td>40.80 ± 2.5*</td>
</tr>
</tbody>
</table>

Group 1 – *Morinda morindoides*, Group 2 – glibenclamide, Group 3 – untreated diabetic, Group 4 – non-diabetic

*Superscripted figures are significant at \(P < 0.05\), value in % implies decrease in blood glucose level (BGL)

TRIG – triglycerides, HDL – high density lipoprotein, LDL – low density lipoprotein
Hypoglycaemic effect of solvent fractions of *M. morindoides*

Administration of the 400 and 200 mg.kg⁻¹ chloroform fractions had the highest hypoglycaemic activity with percentage BGL change of 0.3% and 2.5%, respectively, at 30 min and 272

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>MEOH 400 mg·kg⁻¹</th>
<th>MEOH 200 mg·kg⁻¹</th>
<th>Hexane 400 mg·kg⁻¹</th>
<th>Hexane 200 mg·kg⁻¹</th>
<th>Chloroform 400 mg·kg⁻¹</th>
<th>Chloroform 200 mg·kg⁻¹</th>
<th>Ethylacetate 400 mg·kg⁻¹</th>
<th>Ethylacetate 200 mg·kg⁻¹</th>
<th>Glibenclamide</th>
<th>Non Diabetic</th>
<th>Diabetic Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>449.3 ± 47.6</td>
<td>477.0 ± 53.1</td>
<td>402.1 ± 56.6</td>
<td>567.0 ± 13.3</td>
<td>350.6 ± 75.3</td>
<td>606.6 ± 3.6</td>
<td>539.6 ± 23.0</td>
<td>541.4 ± 19.0</td>
<td>438.6 ± 5.8</td>
<td>121.40 ± 5.6</td>
<td>540.0 ± 12.5</td>
</tr>
<tr>
<td>30 min</td>
<td>445.3 ± 44.9</td>
<td>486.0 ± 50.5</td>
<td>466.6 ± 52.6</td>
<td>578.5 ± 9.9</td>
<td>349.2 ± 74.8</td>
<td>591.8 ± 6.1</td>
<td>537.5 ± 32.0</td>
<td>538.9 ± 26.4</td>
<td>408.0 ± 5.1</td>
<td>123.6 ± 5</td>
<td>570.0 ± 16.5</td>
</tr>
<tr>
<td>1</td>
<td>443.2 ± 49.2</td>
<td>481.3 ± 52.9</td>
<td>468.7 ± 59.6</td>
<td>566.6 ± 13.3</td>
<td>306.2 ± 70.2</td>
<td>524.6 ± 2.8</td>
<td>523.1 ± 32.2</td>
<td>532.8 ± 26.7</td>
<td>334.8 ± 55.9</td>
<td>122.6 ± 5</td>
<td>584.0 ± 10.1</td>
</tr>
<tr>
<td>2</td>
<td>424.1 ± 45.2</td>
<td>480.2 ± 53.7</td>
<td>491.4 ± 57.3</td>
<td>579.0 ± 10.2</td>
<td>263.4 ± 50.9</td>
<td>477.2 ± 9.1</td>
<td>513.7 ± 34.7</td>
<td>533.2 ± 33.2</td>
<td>327.0 ± 64.4</td>
<td>118.2 ± 3.6</td>
<td>567.2 ± 8.0</td>
</tr>
<tr>
<td>4</td>
<td>433.3 ± 53.6</td>
<td>480.2 ± 42.3</td>
<td>539.3 ± 32.4</td>
<td>595.8 ± 10.4</td>
<td>235.1 ± 41.5</td>
<td>442.9 ± 14.4</td>
<td>507.6 ± 37.5</td>
<td>515.5 ± 34.5</td>
<td>300.6 ± 5.6</td>
<td>114.8 ± 4.1</td>
<td>580.2 ± 3.3</td>
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<tr>
<td>6</td>
<td>488.9 ± 35.9</td>
<td>484.2 ± 51.3</td>
<td>521.6 ± 27.9</td>
<td>600.1 ± 8.1</td>
<td>272.5 ± 42.2</td>
<td>488.7 ± 5.8</td>
<td>583.2 ± 9.1</td>
<td>579.6 ± 17.7</td>
<td>327.0 ± 64.2</td>
<td>111.2 ± 4.8</td>
<td>599.8 ± 6.8</td>
</tr>
</tbody>
</table>

Table 3. Acute effect of solvents fraction of *Morinda morindoides* root bark extract on blood glucose level (mean + standard error of mean)

*Superscripted figures are significant at P < 0.05; values in parenthesis represent % change in blood glucose level (BGL), minus values in parenthesis imply reduction in BGL while plus values imply increase in BGL.

Table 2. Acute effect of methanol extract of *Morinda morindoides* roots bark on blood sugar level in diabetic rats (mean + standard error of mean)

<table>
<thead>
<tr>
<th>Blood glucose level (mg·dl⁻¹)</th>
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<tbody>
<tr>
<td>Groups (n = 6)</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Group 1</td>
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<td></td>
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<td>Group 2</td>
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<td>Group 3</td>
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<td>Group 4</td>
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</table>


*Superscripted figures are significant at P < 0.05, values in parenthesis represent % change in blood glucose level (BGL), minus values in parenthesis imply reduction in BGL while plus values imply increase in BGL.
Hypoglycaemic effect of the chromatographic fractions of *M. morindoides*

For the A-F (6) chloroform chromatographic sub-fractions of *M. morindoides* at 100 mg·kg⁻¹, sub-fraction B showed the highest activity of 472.8 ± 57 mg·dl⁻¹ blood sugar reduction at 0 h to 218.9 ± 65 mg·dl⁻¹ at 4 h and then to 121.2 ± 13.4 mg·dl⁻¹, 24 h post administration (Table 4). This implies a 53.7% and 74.4% reduction of blood glucose, respectively (Table 3). These values are higher than that obtained for the glibenclamide group with blood glucose level from (438.6 ± 5.8) mg·dl⁻¹ at 0h to (300.6 ± 5.6) mg·dl⁻¹ at 4 h and to (210.2 ± 31.8) mg·dl⁻¹ at 24 h post administration implying (31.5% and 52.1%, respectively) (Table 4).

**Discussion**

In this study, the hypoglycaemic and hypolipidaemic activities of the crude extract, solvent fractions, and chromatographic sub-fractions of *M. morindoides* were evaluated in alloxan-induced diabetic rats. The 21-day treatment of the aqueous extract of *M. morindoides* caused a significant decrease in the blood glucose level of hyperglycaemic rats, and in serum triglyceride, cholesterol, low density lipoprotein (LDL), and in the HDL level. These effects were comparable to those obtained with the standard antidiabetic agent, glibenclamide. It was also observed that the methanol extract of *M. morindoides* caused a significant reduction in blood glucose levels compared to glibenclamide. The 21-day treatment caused a significant decrease in the blood glucose level of hyperglycaemic rats, and in serum triglyceride, cholesterol, low density lipoprotein (LDL), and in the HDL level. These effects were comparable to those obtained with the standard antidiabetic agent, glibenclamide.
the hypoglycaemic effect of the chloroform fraction *M. morindoides* was higher than that of glibenclamide. This may suggest that the extract of *M. morindoides* is anti-diabetic as WHO (2010) associated the treatment of diabetes with the lowering of blood glucose and the concentrations of other known risk factors that damage blood vessels and nervous tissues. Plants such as *Laportea ovalifolia* have been reported to be anti-diabetic as they cause the reduction of blood glucose level and serum lipids of diabetic animals (Momoh et al. 2006). The hypoglycaemic and hypolipidaemic actions of *M. morindoides* may be similar to those of insulin because insulin is hypoglycaemic and lowers lipid levels (Ahmed et al. 2001). The hypoglycaemic activity of *M. morindoides* may be attributable to its flavonoids content and flavonoid o-glycosides (Cimanga et al. 1995; Harisolo et al. 2009). Flavonoids are reported to potentiate the increase of pancreatic secretion of insulin from β-cells and to increase the peripheral utilization of glucose. Vessal et al. (2003), reported that quercetin, a flavonoid with antioxidant properties brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozocin-induced diabetic rats. This study suggests the hypoglycaemic and hypolipidaemic potential of *Morinda morindoides*.

References


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