Research Article

Hypoglycaemic and Antioxidant Activities of the Stem Bark of *Morinda Lucida* Benth in Streptozotocin – Induced Diabetic Rats

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ABSTRACT: *Introduction:* This work was conducted in order to investigate the hypoglycaemic and antioxidant activity of the stem bark of *Morinda lucida* Benth (Rubiaceae), which has traditionally been used in Ghana for the management of diabetes mellitus. *Methods:* The powdered stem bark was successively Soxhlet-extracted with petroleum ether and 70% v/v ethanol, respectively, and were then evaporated to dryness to produce crude petroleum ether (PML) and ethanolic (EML) extracts. The hypoglycaemic activity of PML and EML were determined in streptozotocin-induced diabetic rats. Five groups of six diabetic rats received oral administrations of 100, 200 and 400 mg per kg body weight of PML and EML once daily for 19 days. Glibenclamide (5 mg per kg body weight) was used as positive control while distilled deionised water (2 ml) acted as the diabetic control. Blood glucose levels were monitored initially for 6 hours and subsequently over the full 20 days. *Results:* All extracts exhibited statistically significant (*P* < 0.001) hypoglycaemic and substantial antioxidant activities throughout the study period. While EML demonstrated the strongest hypoglycaemic activity 6 hours after administration, PML exhibited the highest activity 24 hours after administration and throughout the rest of the study period. Again, both extracts showed antioxidant activities, however EML was more effective. *Conclusion:* The results of our investigation indicate that the stem bark of *M. lucida* possesses hypoglycaemic constituents that are well distributed in both petroleum ether and 70% v/v ethanol extracts. In addition, both extracts also showed considerable antioxidant activity. These results give credibility towards the traditional use of the stem bark of *M. lucida* as an antidiabetic agent.

KEYWORDS: *Morinda lucida*, diabetes mellitus, hypoglycaemic activity, antioxidant activity.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder in which the pancreas produces little or no insulin in order to meet the body’s needs, or where the insulin produced is ineffective.[1] It is the result of a complex interaction of several factors, including genetic and environmental components, which lead to hyperglycaemia. Chronic hyperglycaemia is associated with long term damage, dysfunction and failure of various organs that include the eyes, kidneys, nerves, heart, blood vessels, liver and pancreas.[2,3] These complications have been linked to the production of reactive oxygen species which lead to oxidative stress on body tissue.[4-7] Antioxidants are protective agents that inactivate reactive oxygen species, thereby delaying or preventing oxidative damage.[8]

Diabetes is now a global health problem affecting the populations of both developed and developing nations, including locations where medicines are not easily accessible. This has necessitated the search for new drugs from various sources to either augment or replace existing ones. The last few decades have seen an unprecedented upsurge in the search for hypoglycaemic agents from plant sources. Hypoglycaemic medicinal plants which possess antioxidant agents may be more beneficial in diabetes by acting to lower oxidative stress and minimise diabetic complications.[9]
Morinda lucida Benth (Rubiaceae) is a small to medium-sized tree that can be found in areas stretching from Senegal to Sudan and extending southward to Angola and Zambia. 

In West Africa, the leaves, stem bark and roots are used as infusions or decoctions, either alone or in combination with other plants. They are used to treat numerous conditions such as chest pains, dysentery, fever, haemorrhoids, constipation, diabetes, stroke, malaria, anaemia and threatened miscarriage. 

The plant contains anthraquinones, iridoids, triterpenoids and volatile oil. Extracts from M. lucida have shown some biological activities, comprising antimalarial, trypanocidal, antimicrobial, purgative, anti-inflammatory and analgesic activities. Meanwhile, the leaf and root extracts exhibit anti-diabetic activities. Within Ghana, the stem bark is commonly used by herbalists in an attempt to manage diabetes.

The purpose of the present study was to validate the hypoglycaemic claims of the stem bark of M. lucida in streptozotocin (STZ)-induced diabetic rats, to examine its antioxidant potential and the hypoglycaemic property of stigmasterol isolated from the petroleum ether extract of the plant.

MATERIALS AND METHODS

Plant material
M. lucida stem barks were collected (March, 2008) from the Kwame Nkrumah University of Science and Technology (KNUST) campus. The species was verified by Mr. Amponsah of the Department of Botany, University of Ghana, Legon and a Voucher specimen KNUS/ HMI/2010/S-006 has been deposited at the herbarium of the Faculty of Pharmacy and Pharmaceutical Science, KNUST.

Extraction of plant material
The harvested stem bark was cut into smaller pieces and oven-dried for 24 hours at 40 °C. The dried bark was milled into coarse powder using a hammer mill, and the powdered material (600 g) was Soxhlet-extracted sequentially with petroleum ether and 70% ethanol to produce petroleum ether (PML) and ethanolic (EML) extracts respectively. The extracts were rotary-evaporated to solvent-free dark brown semisolid masses under reduced pressure (yields: PML = 0.9% w/w and EML = 4.6% w/w respectively). The extracts were stored in a desiccator at room temperature until further use. Phytochemical screening of the powdered stem bark of M. lucida, using standard methods described by Sofowora and Harborne, revealed the presence of reducing sugars, tannins, anthraquinones, flavonoids and sterols. Initial chromatographic fractionation of the petroleum ether extract with petroleum ether and ethyl acetate mixtures over silica gel yielded a terpenoid constituent which was identified by its 1HNMR spectrum as well as co-thin layer chromatography (co-tlc) with an authentic sample (stigmasterol).

Experimental Animals
Healthy male Sprague-Dawley rats aged approximately 4 months and weighing 285-310 g were used in the study. The animals were acquired from the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, and housed under standard conditions in the animal house of the Faculty of Pharmacy and Pharmaceutical Sciences, and supplied with standard pellets as basal diet and drinking water ad libitum.

Induction of Diabetes Mellitus
Streptozotocin (STZ) was used to induce diabetes in all experimental rats. The compound was freshly prepared in 0.1 M citrate buffer (pH 4.5), and after an overnight fast animals were injected intraperitoneally with a single dose of 80 mg STZ per kg body weight. After injection, rats were given free access to feed and water. Diabetes was monitored by determining blood glucose levels of the rats, 48 hours after injection of STZ. Rats with blood glucose levels greater than 10.0 mM were selected for further experimentation.

Determination of hypoglycaemic activity
PML and EML extracts were suspended in 2% tragacanth solution and were administered orally using a catheter. Rats were randomly divided into eight groups of five animals and each group then treated with the respective test drug at the given dose once daily for 19 days. Animals in Groups A, B and C were administered EML at dosages of 100, 200 and 400 mg/kg body weight, respectively. Animals in Groups D, E and F were given PML at dosages of 100, 200 and 400 mg/kg body weight, respectively. Group G rats were administered glibenclamide (GB), a standard anti-diabetic drug, at a dose 5 mg/kg body weight (positive control group for this study). The negative control animals (Group H) received 2 ml of distilled water only.

This protocol was followed to evaluate the activity of stigmasterol which was isolated from the hypoglycaemic active PML. It was suspended in 2% tragacanth solution and administered orally to two groups of five diabetic rats, at dosages of 50 and 100 mg per kg body weight. GB (5 mg/kg body weight) and 2 ml of distilled water were administered to the positive and negative control groups respectively. The blood glucose levels were monitored hourly for 6 hours.

Blood glucose measurements
Blood glucose levels were measured using Accu-Chek® Glucometer (Roche Diagnostics GmbH, Mannheim...
Germany). Initial measurements were conducted hourly over the first six hours after drug administration in order to observe the immediate effects, and then subsequently measured at 24 hours for the next 19 days for monitoring long term effects of each extract. Blood samples were collected from the tail vein. Throughout the study period, rats received unrestricted access to standard diet and water.

**Determination of Total Phenolic Content of extracts**

The total phenolic content of each extract was determined by Folin-Ciocalteu's reagent and as described by Slinkard and Singleton,[35] with tannic acid as standard. Aliquots (1 ml) of standard solution of tannic acid (0.50, 0.75, 1.00, 1.50 mg/ml) and each extract added to 1 ml distilled deionised water and 1 ml Folin-Ciocalteu's reagent in a test tube. After mixing, the samples were allowed to stand for 5 minutes at 25 °C in an incubator. A 2% solution of sodium bicarbonate (1 ml) was then added, and the mixture thoroughly mixed and incubated for 2 hours at 25 °C. Tests tubes were centrifuged at 3000 g for 10 minutes and the absorbances of the supernatants determined at 760 nm. Three replicates were prepared for each concentration of tannic acid and extracts. Distilled water (1 ml) was added to 1 ml Folin-Ciocalteu's reagent and processed in the same way as the test drugs and used as a blank sample. Total phenolic contents were expressed as Tannic acid equivalent (TAE) per dry weight of extract (mg/g).

**Total Antioxidant Capacity Assay**

This assay is based on the reduction of Mo (IV) to Mo (V) and subsequent formation of a green phosphate-Mo (V) complex at acidic pH, as described by Prieto et al.[36] Ascorbic acid or the extracts (1 ml) at different concentrations (0.50-1.50 mg/ml) were added to 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) and 1 ml of 2% Na2CO3. Samples were shaken, incubated at 95 °C for 90 minutes and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was then determined at 695 nm after the mixture had cooled to room temperature. The procedure was repeated using distilled deionised water (1 ml) as a blank. The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) per dry weight of extract (mg/g).

**Free Radical Scavenging Activity (FRSA)**

The free radical scavenging activity (FRSA) was determined using the method described by Blois.[37] One millilitre of methanolic DPPH solution (20 mg/L) was added to either 3 ml of extract solution, or 3 ml pure methanol (for the blank sample). Absorbances were measured at 517 nm following a 30 minute incubation period. The reference compound n-propyl gallate (1-30 ug/ml) was also incorporated into the protocol. Percentages of DPPH inhibition by samples were calculated according to the following formula:

\[
\text{% inhibition} = \frac{A - A_i}{A} \times 100
\]

Where \( A \) is absorbance of the control, and \( A_i \) the absorbance of sample.

The IC₅₀ values (the concentration in mg/ml at 50% inhibition) were determined from curves plotting percentage inhibition with logarithmic sample concentration.

**Reducing Power**

Extract reducing power was determined according to the method described by Oyaizu[38] using n-propyl gallate as reference. One millilitre of increasing concentrations (0.50, 0.75, 1.00, 1.50 mg/ml) of extract or n-propyl gallate was added to 1 ml of water and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After incubation at 50 °C for 20 minutes, trichloroacetic acid (2.5 ml) was added and samples centrifuged at 3000 g for 10 minutes. A 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl₃, and the absorbances of the resulting mixtures were measured at 700 nm. Distilled water (1 ml) was added to 2.5 ml sodium phosphate buffer and 2.5 ml potassium ferricyanide in a test tube as the blank. The reducing power was expressed as n-propyl gallate equivalent (NPGE) per dry weight of extract (mg/g).

**Linoleic Acid Autoxidation**

This protocol was performed according to the method of Mitsuda et al.[39] Extracts (0.1-3 mg/ml) suspended in absolute alcohol were directly compared with n-propyl gallate (0.1-3 mg/ml) in absolute alcohol as a reference antioxidant. Equal volumes (2 ml) of the extract and and 2.5% linoleic acid (in absolute ethanol), were mixed with 4 ml of 0.05 M phosphate buffer (pH 7) and 1.9 ml of distilled water in test tubes sealed with screw caps and incubated in the dark at 40 °C for 7 days. Then, 2 ml of the sample solutions were added to 1 ml of 20% aqueous trichloroacetic acid solution and 1 ml of 0.6% aqueous thiobarbituric acid solution. Samples were placed in boiling water bath for 10 minutes and then allowed to cool to room temperature and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 490 nm.

The % inhibition of linoleic acid autoxidation was calculated using the following equation:

\[
\text{% Inhibition} = \frac{(C_o - C_f) - (D - D_i)}{(C_o - C_f)} \times 100
\]

where \( C_o \) (full reaction mixture) is the degree of lipid peroxidation in the absence of antioxidant, \( C_f \) is the
underlying lipid peroxidation before the initiation of lipid peroxidation, \( D \) is any absorbance produced by the extract, and \( D_j \) is the absorbance produced by the extract alone.

**Statistical analysis**
Results were analysed by one-way ANOVA followed by Bonferroni’s multiple comparison test to establish significance (\( P < 0.001 \)) between groups. All data provided represents the means ± S.E.M.

**RESULTS**

**Hypoglycaemic activity during the first 6 hour monitoring period**
Blood glucose levels in the diabetic rats were monitored hourly for 6 hours after doses were administered. While blood glucose levels of the untreated diabetic rats steadily increased over the initial 6-hour period, there was a steady, general decline in the blood glucose levels of rats treated with PML, EML or GB (Figure 1). Specifically, blood glucose increased in the untreated diabetic rats (diabetic control) by 29.56 ± 4.48% after 6 hours, but decreased in diabetic rats treated with PML (100, 200 and 400 mg/kg body weight) by 30.74 ± 3.77, 32.87 ± 3.86 and 39.30 ± 7.42%, respectively, at 6 hours post-treatment. EML treatment (100, 200 and 400 mg/kg body weight) lowered blood glucose levels by 22.53 ± 3.40, 34.02 ± 6.36 and 44.58 ± 5.06%, respectively, within the same period. During 2-6 hours following drug administration, all doses of EML exerted a greater effect on reducing blood glucose than the PML doses. GB, the standard anti-diabetic drug that stimulates insulin release (5 mg/kg body weight) by 37.26 ± 6.39, 46.99 ± 5.04 and 53.02 ± 3.82%, respectively. GB (5 mg per kg body weight) lowered blood glucose by 78.89 ± 1.98%. This indicates that, over the 20 day study period, all of the PML doses used in this study lead to a stronger hypoglycaemic response than with the same doses of EML. In addition, the hypoglycaemic activity of PML at 400 mg per kg body weight was comparable to that of the standard anti-diabetic drug, GB.

**Stigmasterol**
Stigmasterol, obtained from the petroleum ether extract, was identified by comparing its \(^1H\)-NMR spectrum with that of the published data for this compound,\(^{[34]}\), and via co-TLC with an authentic sample. It showed the characteristic oxymethine proton multiplet at \( \delta_{H} \ 3.55 \) attributable to \( H-3 \), the broad vinylic proton doublet at \( \delta_{H} \ 5.35 \) attributable to \( H-6 \), the symmetrical olefinic proton doublet of doublets in a transoid relationship at \( \delta_{H} \ 5.05 \) and \( \delta_{H} \ 5.15 \) attributable to the \( H-22 \) and \( H-23 \), and the characteristic methyl groups between \( \delta_{H} \ \ 0.65 \)–1.30. This compound (stigmasterol) caused dose-dependent decreases in blood glucose levels in diabetic rats during the 6 hours study period (Fig 3). A dose of

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**Figure 1:** Time course of % changes in blood glucose levels of diabetic rats after oral administration of distilled water (control), glibenclamide and various doses of petroleum ether (PML) and 70% ethanol (EML) extracts. Values are mean ± SEM of % changes in blood sugar levels from 5 animals/group. The significance in % change in blood sugar levels for all pairs of groups after 6 hours were compared using One-Way analysis of variance (ANOVA) followed by Bonferroni’s Multiple Comparison Test.

**Figure 2:** Time course of % changes in blood glucose levels of diabetic rats after oral administration of distilled water (control), glibenclamide and various doses of petroleum ether (PML) and 70% ethanol (EML) extracts. Values are mean ± SEM of % changes in blood sugar levels from 5 animals/group. The % change in blood sugar levels for all pairs of groups after 20 days were compared using One-Way analysis of variance (ANOVA) followed by Bonferroni’s Multiple Comparison Test.
100 mg per kg body weight treatment led to a 40.5% reduction in blood glucose levels in the diabetic rats at the end of the 6-hour monitoring period. GB caused a 48.3% reduction while in the negative control group there was an average of 18.8% increase in blood glucose levels (Fig 3).

**Antioxidant activities of PML and EML of *M. lucida* extracts**

The results in the following section are shown in Table 1. The total phenol was assayed based on the reduction of phosphomolybdic salts to form a blue complex that can be detected quantitatively at 695 nm. The total phenolic content, expressed as tannic acid equivalent (TAE) per weight of extract (mg/g) for the EML extract was 1.000 ± 0.035, and 0.164 ± 0.051 for the PML counterpart.

Both PML and EML showed considerable antioxidant capacities. EML exhibited a higher total antioxidant capacity than PML. The total antioxidant capacity expressed as ascorbic acid equivalent (AAE) in mg/g dry weight of extract were 1.000 ± 0.054 for EML and 0.292 ± 0.013 for PML.

The reducing power were expressed as *n*-propyl gallate equivalent (NPGE) in mg/g dry weight of extract, and were calculated to be 0.108 ± 0.029 for PML and 0.179 ± 0.057 for EML. This suggests that the EML exerts a higher reducing power than the PML.

The free radical scavenging activity (FRSA) of the extracts, expressed as percentage inhibition of DPPH, exhibited dose-dependent activity for all the extracts. The absorbance decreased with increasing free radical scavenging activity. The IC<sub>50</sub> values of the *n*-propyl gallate, PML and EML were 0.1114 ± 0.0048, 7.426 ± 0.0066 and 0.4403 ± 0.0053 mg/ml respectively. Thus EML was found a better free radical scavenger than PML.

Finally, the IC<sub>50</sub> values for lipid autoxidation inhibition were found to be 3.554 ± 0.0086 mg/ml for PML and 1.928 ± 0.0066 mg/ml for EML. The value determined for the reference compound, *n*-propyl gallate, was 0.9176 ± 0.0051 mg/ml.

**DISCUSSION**

The present study reveals the hypoglycaemic activity of the stem bark of *M. lucida* in streptozotocin-induced diabetic rats under both acute and chronic treatment regimes. Both the petroleum ether (PML) and 70% ethanol (EML) extracts were found to be active in this regard. They also demonstrated considerable antioxidant activity in the *in vitro* experiments.

Data on the blood glucose levels within the first 6 hours confirmed the acute (or immediate) hypoglycaemic effect following administration of PML, EML and GB. Measurements during the following days of treatment showed that PML, EML and GB continued to reduce blood glucose levels 24 hours after the first drug administration and across the entire study period of 20 days. In the acute treatment phase, 400 mg/kg body weight of EML demonstrated relatively stronger hypoglycaemic activity than an equivalent dose of PML. However, at this dosage, PML showed a more improved hypoglycaemic activity over the chronic treatment period. The differences in action of the two extracts may be attributable to the type of chemical constituents contained in each. PML, being a petroleum ether extract, contains mainly non-polar

![Figure 3: Time course of % changes in blood glucose levels of diabetic rats after oral administration of distilled water (control), glibenclamide and various doses of stigmasterol (STG). Values are mean ± SEM of % changes in blood sugar levels from 5 animals/group. The % change in blood sugar levels for all pairs of groups after 6 hours were compared using One-Way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test.](image)

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameter</th>
<th>Sample</th>
<th>EML</th>
<th>PML</th>
<th><em>n</em>-propyl gallate</th>
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<tbody>
<tr>
<td>Total phenolic</td>
<td>TAE (mg/g)</td>
<td>1.000 ± 0.035</td>
<td>0.164 ± 0.051</td>
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<tr>
<td>Antioxidant activity</td>
<td>AAE (mg/g)</td>
<td>1.000 ± 0.054</td>
<td>0.292 ± 0.013</td>
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<tr>
<td>Reducing power</td>
<td>NPG (mg/g)</td>
<td>0.179 ± 0.057</td>
<td>0.108 ± 0.029</td>
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<tr>
<td>FRSA</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/g)</td>
<td>0.4403 ± 0.0053</td>
<td>7.426 ± 0.0066</td>
<td>0.1114 ± 0.0048</td>
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<tr>
<td>Lipid autoxidation</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/g)</td>
<td>1.928 ± 0.0066</td>
<td>3.554 ± 0.0086</td>
<td>0.9176 ± 0.0051</td>
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*Values are the mean ± SEM where applicable. FRSA = free radical scavenging activity.*
constituents. EML is a 70% aqueous ethanol extract and contains largely polar constituents. Earlier findings on the hypoglycaemic activities of the methanolic leaf\cite{28} and the aqueous root\cite{29} extracts of M. lucida generally show a similar trend, which could be supportive of our findings in the present study.

Stigmasterol, a ubiquitous phytosterol, was isolated from the active petroleum ether extract of M. lucida. It also demonstrated hypoglycaemic activity in the STZ–induced diabetic rats. Notably, stigmasterol isolated from Parkia speciosa seeds showed no effect in alloxan-induced diabetic rats, although in a mixture with β-sitosterol, it showed appreciable activity.\cite{30}

Our work also demonstrates the antioxidant potential of the stem bark of M. lucida. This is in agreement with a previous report using methanolic stem bark extract.\cite{31} This may suggest that the polar constituents of the stem bark have a greater antioxidant potential than the non-polar constituents, and thus could justify the use of the traditional formulation as an aqueous decoction. M. lucida stem bark could thus provide additional therapeutic benefit for diabetic patients, due to its sequestration of the reactive oxygen species that are generated as a result of chronic hyperglycaemia and which lead to complications associated with diabetes mellitus.

**CONCLUSION**

This investigation has shown that M. lucida stem bark extracts (PML and EML) can elicit hypoglycaemic effects in diabetic animal model and also display antioxidant properties. The antioxidant potential could represent a protective mechanism against reactive oxygen species (ROS) associated with chronic hyperglycaemia and diabetic complications. Furthermore, stigmasterol which was isolated from the hyperglycaemic – active petroleum ether extract, showed significant hypoglycaemic activity, contrary to an earlier report. These findings may justify the traditional use of the stem bark of M. lucida in the management of diabetes mellitus.

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