The lipid lowering effect of the aqueous root extract of *Morinda lucida* in albino rats fed on a high cholesterol diet

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**Background:** *Morinda lucinda* (Benth) Rubiaceae has been reported in folk medicine to be useful for the treatment of diabetes mellitus (DM) and hyperlipidemia. Previous studies have identified the hypoglycemic effect of this herb, but data on its lipid lowering effect is lacking.

**Objective:** To evaluate the effect of *Morinda lucinda* on hyperlipidemia.

**Methods:** *Morinda lucinda* roots were extracted via cold maceration using distilled water. It was administered to rats (n=6 per group) on a high cholesterol diet for 14 days (40 mg/rat/day). Five groups of rats were orally given the extract (100, 200 and 400 mg/kg), atorvastatin (5mg/kg), or distilled water (2ml/kg) for 14 days. Positive and negative control groups received cholesterol & distilled water and only distilled water respectively. After the 14th day, blood samples were withdrawn and separated plasma was tested for HDL, LDL, triglycerides and total cholesterol.

**Results:** Treatment with *Morinda lucinda* extract at all doses significantly reduced the levels of LDL (p <0.0001), while increasing the HDL levels (p<0.05) in the hyperlipidemic rats from 12.5 mg/dL to 24.2, 23.93, 26.67 mg/dL for the 3 doses respectively. Similarly, administered extract at all doses significantly reduced the total cholesterol (p<0.0001) and triglycerides (p = 0.001, p = 0.0005, and p<0.0001 for 100, 200 and 400 mg/kg respectively) in comparison with the positive control. The effect of the extract on total cholesterol and LDL were most prominent and was as effective as atorvastatin.

**Conclusions:** The effect of the extract on total cholesterol and LDL were most prominent and was as effective as atorvastatin. Our results suggest *Morinda lucinda* as a useful remedy for lowering lipid level in patients with hyperlipidemia.

**Keywords:** Triglycerides, High density lipoprotein, Low density lipoprotein, Total cholesterol

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1. Introduction

*Morinda lucida* (Benth), Rubiaceae is a medium-sized tree, 15m in height and has a characteristic yellow wood, from which it derives its common name "Brimstone tree. The name Morinda is derived from the Latin word ‘morus’ meaning ‘mulberry’ from the appearance of the fruits. Local names in Nigeria includes: Oruwo (South-West); Eze ogu (South East); Mangwara or Waawan kurmii (North).

In a study carried out on the ethanol and dichloromethane extract of the plant, the roots and leaves of *Morinda lucida* were found to contain volatile
Different parts of the plant are associated with diverse therapeutic benefits. The leaves are used in the preparation of fever teas, which are used as antimalarial, febrifuge and as an analgesic agent. All parts of the plant are used as a laxative (Iwu, 2014). In folk medicine in Nigeria, the plant is macerated in palm wine or water and its decoction used in the management of diabetes mellitus vis-a-vis hyperlipidemia (Adeneye and Aghaje, 2008).

Hyperlipidemia is a raised serum or plasma levels of one or more of the following; total cholesterol (TChol), Low density Lipoproteins (LDL-C), Triglycerides (TGs), or a combination of any or all of the above. Usually the condition may be associated with a decrease in High density Lipoprotein (HDL). It can be classified either as familial also called "primary"- caused by specific genetic abnormalities, or as secondary also called "acquired", when resulting from another underlying disorder that leads to alterations in plasma lipid and/or lipoprotein metabolism, such as diabetes mellitus DM, drugs (beta blockers, estrogens, diuretics), hypothyroidism, or renal failure (Chait and Brunzell, 1990).

Another classification is done based on the exact type(s) of lipids that is/are elevated, hence one could have hypercholesterolemia, hypertriglyceridemia or hyperlipoproteinemia (in which total cholesterol, triglycerides or lipoproteins are increased) respectively (Chait and Brunzell, 1990).

It is well established that individuals may have anomalies in their lipid levels. This also applies to individuals with DM, the common cause of secondary hyperlipidemia. In DM there is either total lack or insufficient insulin or there is tissue receptor resistance to insulin (Type 1 and Type 2). Lipoprotein lipase activated by insulin is an enzyme responsible for splitting the LDLs and chylomicrons into HDLs. In the absence of insulin this enzyme is not activated and LDLs and chylomicrons accumulate in the blood stream thus increasing the risk of hyperlipidemia in poorly managed DM.

This research was done basically to investigate the claim that the hyperlipidemia usually associated with hyperglycaemia can be corrected alongside the hyperglycaemia using extracts of *Morinda lucida*. The objective of this study therefore was to examine the antihyperlipidemic effect of *Morinda lucida*.

2. Materials and Methods

2.1 Plant collection and preparation

The fresh roots of *Morinda lucida* were collected in Benin City, Edo state, Nigeria in July, 2014. Identification of the plant was done by Mr Sunny Nweke a herbarium curator, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria and a voucher specimen was deposited in the departmental herbarium. A voucher specimen (FH1107459) also exists at the Forest Research Institute of Nigeria, Ibadan, Nigeria.

Immediately after collection, the roots were washed with water to remove soil particles, after which they were chopped into smaller pieces and then air dried. It was then pulverized into a smooth fine powder using an Impact mill. The powdered sample was weighed and kept for further analysis.

2.2 Plant Extraction

The powdered plant material (500 g) was macerated in 2500 ml of distilled water with continuous stirring for 72 h. The mixture was initially filtered using a clean white plain cloth to obtain a debris-free solution which was then further filtered using a funnel and cotton plugs. The resulting solution was then placed in the oven (< 40°C) for drying and a solid residue was obtained and weighed (42.19 g) and the percentage yield was calculated (8.44%). Appropriate concentrations of the extract were made in distilled water for the experiment.

2.3 Drugs and chemicals

Cholesterol powder (Kelong Chemicals (Xindu Mula, India), Atorvastatin tablets (Evans Pharmaceuticals, Lagos, Nigeria), Chloroform (Sigma Aldrich UK), Total cholesterol kit, HDL kit and Triglycerides kit (Randox Laboratories, UK).

2.4 Study Animals

Wister albino rats (170-310 g) of either sex were used. Animals were kept at the laboratory animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were maintained and kept in cages in a room with a 12-hour light and dark cycle for two (2) weeks to acclimatize. The animals were allowed free access to water and food (pelleted feed; Top Feed, Nigeria).

2.5 Phytochemical screening

Qualitative chemical tests were performed to assess the presence of the various phytochemical constituents of the *Morinda lucida* aqueous root extract (Trease and Evans, 1989).

2.6 Experimental protocol

**Formula of high cholesterol diet and dosing of animals**

The high cholesterol diet was prepared by suspending 200 mg of Cholesterol powder in 1 ml Olive oil to give a suspension of 40 mg in 0.2 ml. This was administered orally via an orogastric tube daily for 14 days to selected groups of animals as described below.

The albino rats were divided into 8 groups of 6 rats each and treated as follows:

- **Group 1-** Control group (2 ml/kg) of distilled water only;
- **Group 2-** Hyperlipidemic group; Cholesterol (40 mg/0.2 ml/rat) only;
- **Group 3-** 100 mg/kg of the extract only;
- **Group 4-** 200 mg/kg of the extract only;
- **Group 5-** 300 mg/kg of the extract only;
- **Group 6-** 400 mg/kg of the extract only;
- **Group 7-** 500 mg/kg of the extract only;
- **Group 8-** 1000 mg/kg of the extract only.

Group 4- 200 mg/kg of the extract only;
Group 5- Cholesterol (40 mg/rat) and 100 mg/kg extract;
Group 6- Cholesterol (40 mg/0.2 ml/rat) and 200 mg/kg extract;
Group 7- Cholesterol (40 mg/0.2 ml/rat) and 400 mg/kg extract; and
Group 8- Cholesterol (40 mg/0.2 ml/rat) and Atorvastatin 5 mg/kg.

All doses were administered by the oral route using an orogastric tube once daily for 14 days (Nnodim et al, 2011) - this constituted sub-acute treatment.

**Determination of lipid profile parameters.**

Twenty-four hours after administration of the last dose, the animals were sacrificed under chloroform anesthesia and then blood samples were collected via the abdominal aorta. The samples collected were transferred into lithium heparinized tubes. The tubes were centrifuged at 4000 rpm for 3 min. Clear plasma sample was obtained and transferred carefully with the aid of a micropipette (500 microliters) into small test tubes for estimation. Plasma samples were stored in -20°C refrigerator prior to analysis.

The plasma concentration of the total cholesterol, high density lipoproteins and triglycerides were measured by standard procedures using commercial analytical kit from Randox Laboratory Limited, UK. The total cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Distilled water (blank), cholesterol standard, and sample (5 ml each) were pipetted into 3 different cuvettes. Reagent (500 µL) was added to each the cuvettes containing the sample. The contents in the cuvettes were mixed and incubated for 5min at 37°C. The cuvettes were then inserted into the UV spectrophotometer to read the absorbance of the blank, standard and the sample (Allain et al, 1982; Roeschlau et al, 1974). The concentration of cholesterol in each of these samples was calculated from the absorbance. Similar procedure and formula was used to determine the concentration of plasma triglycerides and High density lipoproteins using their appropriate reagents.

The Low density lipoproteins was calculated using a formula:

\[
LDL = TC - (TG/5 + HDL)
\]

Where: LDL- Low density lipoproteins
HDL- High density lipoproteins
TC- Total cholesterol
TG- Triglyceride.

**2.7 Statistical analysis.**

Data are presented as the mean ± standard error of the mean (S.E.M). Comparisons were made where appropriate by One way ANOVA (GraphPad Prism Software, UK, version 2.05a) with Tukey post hoc test.

**2.8 Ethical approval.**

Ethical approval was obtained from the ethical committee of the Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria. Approval letter dated October 10th, 2014, and animals were handled according to the standard protocols for the use of laboratory animals (National Institute of Health, USA, 2002).

**3. Results**

**3.1 Phytochemical screening of the aqueous root extract of Morinda lucida.**

The result of the preliminary phytochemical screening is shown in Table 1, it revealed the presence of carbohydrates, saponins, alkaloids, glycosides and steroids. Phenols and tannins were absent.

**Table 1: Phytochemical screening of the aqueous root extract of Morinda lucida**

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

*Present; - Absent

**3.2 The effect of different doses of Morinda lucinda on the total cholesterol**

These results are shown in Figure 1. Administration of ML to normal rats had no significant effect on their total cholesterol (p>0.05) when compared to the control group. The significant increase (p<0.05) in the total cholesterol observed in the hyperlipidemic group in comparison with the control was however noted to be significantly reduced (p<0.05, p<0.0001 and p<0.05) for 100, 200 and 400 mg/kg doses respectively in comparison with the hyperlipidemic group (cholesterol only group) after 14 days treatment with the aqueous root extract of ML. The effect of the extract was similar to that of atorvastatin at a dose of 5mg/kg in which the total cholesterol was also significantly reduced (p <0.05) when compared to the Cholesterol-only group.

It was however noted that this effect was non-dose dependent as the 200 mg/kg dose gave the highest reduction.
3.3 The effect of different doses of Morinda lucida on the triglycerides

A significant increase (p<0.05) in the triglycerides was observed in the hyperlipidemic group in comparison with the control animals. Interestingly, treatment with the aqueous root of Morinda lucida at all doses (100, 200 and 400 mg/kg) produced a significant decrease (p = 0.005, p = 0.001 and p< 0.0001) respectively in the plasma triglyceride levels when compared to the hyperlipidemic group. In this case the decrease may be said to be dose dependent because of the graded difference between values of plasma triglycerides obtained for the 3 different doses used as was observed in the previous result on total cholesterol the extract also seems not to have any significant effect on the triglycerides of normal rats. This comparison was done with the control group administered with distilled water (Figure 2).

Figure 1: The effect of Morinda lucida on the total cholesterol of normal and high cholesterol fed rats.

Figure 2: The effect of Morinda lucida on the triglycerides of normal and high cholesterol fed rats.

Values are expressed as Mean ± S.E.M for the animals in each group.
n= 6; *p<0.05 and #p<0.05 significantly higher and lower than the control group respectively and *p < 0.0001 significantly lower than the cholesterol only group.

N: Control group (2ml/kg) of distilled water only.
C: Hyperlipidemic group, cholesterol only.
ML100: 100mg/kg of the extract only.
ML200: 200mg/kg of the extract only.
CML100: Cholesterol and 100 mg/kg extract.
CML200: Cholesterol and 200 mg/kg extract.
CML400: Cholesterol and 400 mg/kg extract.
CA5: Cholesterol and 5 mg/kg atorvastatin.
3.4 The effect of different doses of *Morinda lucida* on the high density lipoprotein

These results are shown in Figure 3. The aqueous root extract of *Morinda lucida* at 100, 200 and 400 mg/kg (p< 0.0001), (p< 0.05), (p< 0.01) significantly increased the plasma HDL levels respectively when compared to the Cholesterol-only group (the hyperlipidaemic group). This increase in HDLs was higher when compared to the standard drug (Atorvastatin 5mg/kg). The increase can be said not to be dose dependent because of the comparable values of HDLs observed in 100 200 and 400 mg/kg (mean values of 24.20, 23.93 and 26.67 mg/dL respectively).

3.5 The effect of different doses of *Morinda lucinda* on the low density lipoprotein

The result is presented in Figure 4. A careful look at the result shows a reduction of the LDL in normal rats in comparison with the control group administered distilled water. This reduction was seen again in rats placed on a high cholesterol diet. Both 100 and 200 mg/kg significantly (p<0.05) lowered the LDL of normal rats, while the 3 doses used (100, 200 and 400 mg/kg) produced significant reduction in the plasma LDL levels when compared to the hyperlipemic group (Cholesterol-only group) with p values <0.0001 for all doses tested, the reduction here is said to be non-dose dependent because the decrease in the values obtained were not graded.

The effect of different doses of *Morinda lucinda* on the Atherogenic index (AIP) of normal rats and rats administered cholesterol.

The result is presented in Table 2. As seen in the result, a significant (p<0.0001) increase in the AIP was observed in the hyperlipidemic group in comparison with the normal control animals, while in normal rats given ML, in comparison with the control, an increase was observed in the AIP, despite this, the values obtained were still within normal ranges. Simultaneous treatment with all doses of the extract to rats fed on the high cholesterol diet significantly (p<0.0001) lowered the AIP in comparison with rats fed only on the cholesterol. This reduction was dose dependent with the 400 mg/kg dose giving the best effect. Atorvastatin also produced a significant reduction (p<0.05), although the effect of the extract was more prominent.

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Table 2: The Atherogenic index of the various treatment groups

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Atherogenic index (AIP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (2ml/kg distilled water)</td>
<td>-0.020</td>
</tr>
<tr>
<td>Hyperlipidemic rats (cholesterol 40mg/rat)</td>
<td>0.640*</td>
</tr>
<tr>
<td>Normal rats + 100 ML</td>
<td>0.064*</td>
</tr>
<tr>
<td>Normal rats + 200 ML</td>
<td>0.053*</td>
</tr>
<tr>
<td>Cholesterol fed rats (40 mg/rat) + 100 ML</td>
<td>0.190*</td>
</tr>
<tr>
<td>Cholesterol fed rats (40 mg/rat) + 200 ML</td>
<td>0.160*</td>
</tr>
<tr>
<td>Cholesterol fed rats (40 mg/rat) + 400 ML</td>
<td>0.060*</td>
</tr>
<tr>
<td>Cholesterol fed rats (40 mg/rat) + 5 atorvastain</td>
<td>0.310*</td>
</tr>
</tbody>
</table>

n= 6 animals per group.  
*p<0.0001 significantly higher than the normal rats,  *p<0.0001 and  *p<0.05 significantly lower than the hyperlipidemic group 
ML: Aqueous extract of Morinda lucida
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4. Discussion

Hyperlipidemia was assumed confirmed in all the groups fed on the high cholesterol diet because of the significant difference in the total cholesterol, triglycerides, HDL and LDL values of the Distilled water group and the Cholesterol-only group.

An increase in HDLs indicates a lesser likelihood of atherosclerosis, while LDLs may sometimes be referred to as "bad fats" and an increase above the threshold value of this index indicates a higher likelihood of atherosclerosis (Sadur et al, 1984; Chait and Brunzell, 1990).

In the present study, *Morinda lucida* significantly increased and lowered the good and bad fats respectively. Previous researches have also suggested that HDL is more important than the other lipoproteins in influencing atherosclerosis; this finding needs to be interpreted since there is a close metabolic interrelation between lipoprotein species. Recent epidemiological studies have elucidated the importance of individual lipoproteins in predicting future clinical coronary heart disease. HDL appears to exert the greatest influence independently of other lipoproteins, with LDL having a weaker, though still significant, independent relation with coronary heart disease. This correlated negatively with HDL and positively with LDL, so probably HDL retards while LDL accelerates the development of clinical events (Salahuddin and Jalalpure, 2010). It is thus safe to say that *Morinda lucida* will be a good alternative to routine hypolipidemic agents such as atorvastatin. Apart from the alteration of the abnormal values likely to be seen in hyperlipidemia to acceptable/normal levels, treatment with ML also confers the advantage of retarding the development of coronary heart disease and its sequelae.
The extract also significantly reduced the total cholesterol and triglycerides although its effect on TC was not dose dependent, as a lower dose gave a better reduction. It is not clear why, although it is not out of place to see such results, possibly the active principle responsible for the lowering effect may be constant in the extract irrespective of the dose used. This was the observation, in all, what is important is the lowering effect observed at all doses.

Many laboratories measure the total cholesterol, and generally this is equivalent to the LDL level, and the lower the better. Low cholesterol and triglyceride levels also reduce diabetic complications in addition to lower cardiovascular risk (Salahuddin and Jalalpure, 2010).

The aqueous root extract of *Morinda lucida* is thus important in not just lowering the bad fats, and increasing HDL but in addition reduces cardiovascular risk and possibly diabetic complications (Owolabi and Omogbai, 2013).

AIP refers to the atherogenic index of plasma and is calculated in an attempt to predict cardiovascular risk. AIP is based on the ratio of the values of triglycerides to high-density lipoprotein (HDL) levels. When placed into the scope of AIP, triglycerides and HDL refers to the relationship of atherogenic lipids to protective lipids. The AIP has demonstrated cardiovascular risk in clinical trials. Usually values less than 0.11 indicate low cardiovascular risk, values between 0.11 and 0.21 points to an intermediate risk, while values greater than 0.21 indicates increased cardiovascular risk, hence increased tendency towards myocardial infarction, atherosclerosis and ultimately coronary deaths (Onat et al, 2010).

The highest dose of the extract gave an AIP far below the 0.11, thus pointing to the protective effect of the extract against cardiovascular risk. This positive effect (low cardiovascular risk) was observed in both the normal rats and rats fed on a high cholesterol diet treated with ML.

Previous studies have shown the antidiabetic effect of this plant (Olajide et al, 199; Odutuga et al, 2010; Kamanyi et al, 1994), so this present study which points to a lowering of the bad lipids and an increase in the good lipid coupled with an AIP far less than 0.11 will indeed be an advantage to normal, diabetic and hyperlipidemic patients. Indeed, even when low density lipoprotein cholesterol concentration is normal or slightly raised in type 2 diabetes (the major abnormalities being low HDL cholesterol and high triglyceride concentrations), the patients have a lot to benefit from the use of *Morinda lucida* as shown from this present study. Even in situations where the LDL particles become qualitatively different and more atherogenic than those in non-diabetic patients, the use of *Morinda lucida* still offers protection against various cardiovascular risks as proven from its low AIP (0.05-0.06). This is in contrast to what was seen in the group given only cholesterol, with an AIP of 0.64 which is far higher than the 0.21 documented in literature to indicate increased cardiovascular risk.

These effects observed on the lipid profile parameters by the extract, may be due to the presence of lipid altering secondary metabolites present in the plant. Secondary metabolites confirmed in the plant *Morinda lucida* include alkaloids, saponins and sterols.

From the foregoing and results obtained, it is safe to conclude that, the aqueous root extract of *Morinda lucida* possesses antihyperlipidemic effects, as evident by the significant reduction in the plasma levels of the total cholesterol, triglycerides and low density lipoproteins (LDLs) and significant increase in the high density lipoproteins (HDLs). *Morinda lucida* also significantly reduced the tendency towards cardiovascular risk.

As a way of continuation in this study, an attempt may be made to know which exact secondary plant metabolite or principle is responsible for these effects seen. And such metabolite if known may serve as a lead compound to the discovery of an orthodox antihyperlipidemic agent.

**Conflict of Interest declaration**

The authors declare no conflict of interest.

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**References**


