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Histological evaluation of the antidyslipidemic effects of aqueous root extract of *Morinda lucida*

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Abstract:

BACKGROUND: Dyslipideamia is involved in the development of cardiovascular complications in diabetes, which is one of the major causes of morbidity and mortality in Nigeria

AIM AND OBJECTIVE: The present study was undertaken to investigate the antidyslipidemic effect of aqueous root extract of *Morinda lucida* in cholesterol-induced dyslipidemia in rats.

MATERIALS AND METHODS: The study comprises of five groups, Group 1 for control and 2, 3, 4 and 5. They were administered different doses of drug and extract by oral gavages using an orogastric tube at a once-daily dose for 14 days. At the end of 14 days, liver and abdominal aorta was harvested from rats in each group for histological study using the method.

RESULTS: It was observed that Group 3 showed intracytoplasmic perinuclear fat vacuoles in the liver among other evidences of tissue damage while the abdominal aorta showed luminal obstruction, infiltrates of chronic inflammatory cells, and intimal ulceration.

CONCLUSION: These effects were ameliorated after treatment with both atorvastatin and *M. lucida* extract, but there was more improved histology in the group treated with *M. lucida* extract.

Keywords:

Antilipidemia, atorvastatin, *Morinda lucida*

Introduction

Morinda lucida (L.) (*Rubiaceae*), Brimstone tree is a tropical West Africa rainforest plant (Adeneye and Agbaje, 2008). It is a medium size tree of about 15m tall with scaly gray bark, short-crooked branches, and shining foliage. *M. lucida* is one of the medicinal plants commonly use in Nigeria for the management and treatment of many ailments (Adeneye and Agbaje, 2008). These ailments include malaria (Makinde and Obih, 1985), cancer (Sowemimo *et al.*, 2007), hyperglycemia, and diabetics (Daziel, 1973). The leaf and stems have been reported to possess cytotoxic and genotoxic (Akinboro and Bakare, 2007; Ajaiyeoba *et al.*, 2006), hepatoprotective (Oduola *et al.*, 2010), and antispermatogenic (Raji *et al.*, 2005) activities. They have been used also in the treatment of bacterial infections (Adoni, 2005). Scientists have reported an associated increase in the consumption of foods high in salt, fat, and cholesterol as well a decrease in the level of activity and exercise. These changes have been said to be associated with the development of obesity, diabetes, hypertension, and dyslipidemia as well as cardiovascular diseases. The elevation of plasma cholesterol, triglycerides or both, or a low level of high density lipoprotein is referred to as dyslipidemia which contributes to the development of atherosclerosis, a precursor for ischemic heart disease (Franssen *et al.*, 2011). Dyslipidemia is involved in the development of cardiovascular complications in diabetes which are the major causes of morbidity

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and mortality (Reasner, 2008). Many researchers have reported that aqueous extract of *M. lucida* lowered lipid levels in patients with hyperlipidemia. The principal cause of morbidity and mortality worldwide is coronary heart disease (CHD) (Ludman *et al.*, 2009); a direct link between CHD and high cholesterol blood level has been established by many researchers. Studies showed that atorvastatin is very effective in the treatment of hyperlipidemia and prevention of atherosclerosis (Bjornsson *et al.*, 2012).

This is a histological study aimed at evaluating the antidyslipidemic effects of aqueous root extract of *M. lucida* in rats.

Materials and Methods

Plant material

The fresh roots of *M. lucida* were collected from a natural source, within the premises of Idia College in Oredo Local Government Area, Benin City, Edo State, Nigeria. Identification of the plant was done by a renowned herbal curator, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. Immediately after collection, the roots were rinsed with water to remove soil particles and chopped into smaller pieces and then air dried. It was then pulverized into a smooth fine powder. The powdered sample was weighed and kept for further analysis.

Drugs and chemicals

Cholesterol powder (Kelong Chemicals, Xindu Mula, India), atorvastatin tablets (Evans Pharmaceuticals, Lagos, Nigeria), and general purpose chloroform (Sigma-Aldrich chemicals).

Animal model

Thirty Albino rats of weight between 170 g and 310 g of either sex were used and kept in the laboratory animal house of the Faculty of Pharmacy, University of Benin. The animals were maintained and kept in cages in a room with a 12-h light and dark cycle for 14 days to acclimatize. The animals were allowed free access to water and food in the form of pellets from Top Feeds Ltd, Nigeria.

Extraction of the plant material

Five-hundred grams of the powdered plant material *(M. lucida)* was macerated in 2500 ml of distilled water with continuous stirring for 72 h. The mixture was then filtered using a clean white plain cloth to obtain a debris-free solution which was then filtered using a funnel and cotton plugs. The resulting solution was then placed in the oven for concentration and a solid residue was obtained, weighed (42.19 g) and the percentage yield was calculated (8.43%). Appropriate concentrations of the extract were made in distilled water.

Phytochemical screening

Qualitative chemicals tests were performed to assess the presence of the various phytoconstituents of the aqueous root extract of the *M. lucida*. Phytochemical screening was done using standard procedures as documented by Trease and Evans (2009). Preparation of the cholesterol solution was done by titrating 100 mg of cholesterol powder with 50 ml of vegetable oil (2 mg/ml) obtained locally.

The experimental rats were divided into % groups of 6 rats each as follows:

Group 1 – control group received 2 ml of distilled water; Group 2 – extract only group received 200 mg/kg of the extract only; Group 3 – cholesterol (0.5 ml of 2 mg/ml) only;

Group 4 – cholesterol (0.5 ml of 2 mg/ml) then atorvastatin (5 mg/kg); and Group 5 – cholesterol (0.5 ml of 2 mg/ml) then 200 mg/kg of the extract.

All doses were administered by oral gavage using an orogastric tube at a once-daily dose for 14 days according to the method used by Nnodim *et al.* (2009). Twenty-four hours after the administration of the last dose, the animals were anesthetized using chloroform and then sacrificed after which the liver and the abdominal aorta were removed from animals in all the groups for histological studies.

Results

Phytochemical screening of the aqueous root extract of *Morinda Lucida*

The result of the preliminary phytochemical screening is shown in Table 1. This showed that aqueous root extract of *M. lucida* contained carbohydrates, flavonoids, saponins, alkaloids, glycosides, and steroids. Phenols and tannins were not present.

Histological profile of rats' liver and abdominal aorta

Histological profile of the liver of animals is shown in Figures 1-5 while that of the abdominal aorta is shown in Figures 6-11.

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

Secondary metabolites	Result
Carbohydrates	+
Saponins	+
Phenols	–
Tannins	–
Alkaloids	+
Steroids	+
Flavonoids	+

+ - Present, – - Absent

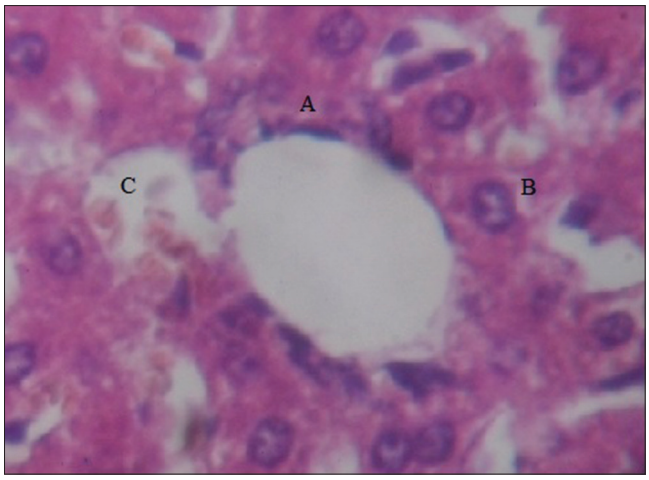

Figure 1: Rat liver composed of central vein A, hepatocytes B, and sinusoids C (H and E, ×400)

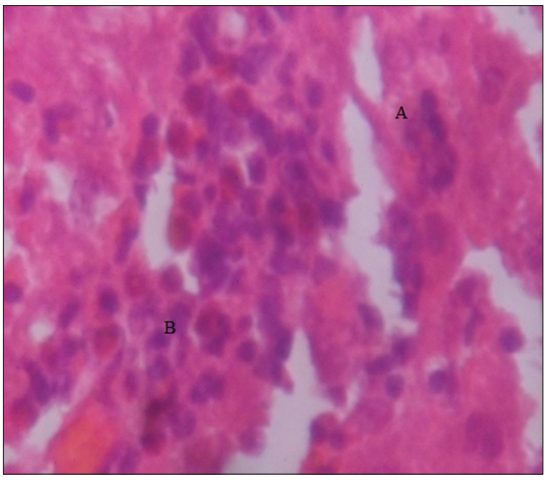

Figure 2: Rat liver treated with extract only (*Morinda lucida*) for 14 days showing mild periportal lymphocytosis A and mild portal congestion B (H and E, ×400)

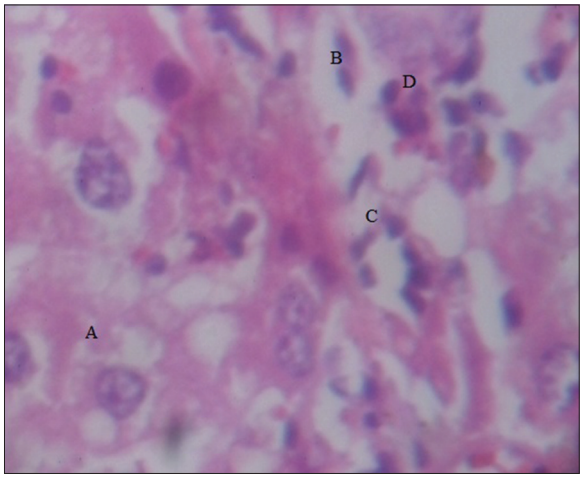

Figure 3: Rat liver treated with cholesterol only showing mild fat vacuolation A, moderate congestion B, moderate Kupffer cell activation C, and lymphocytosis D (H and E, ×400)

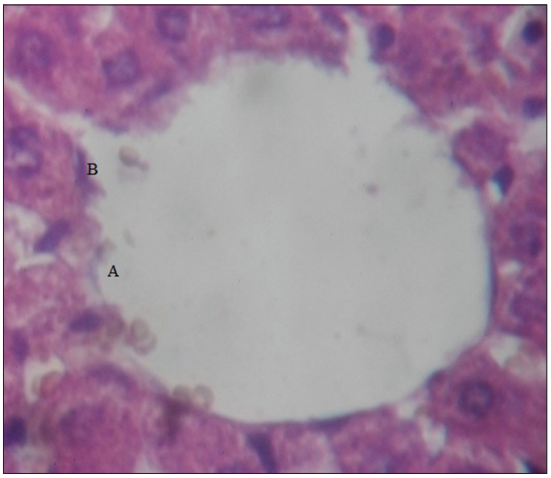

Figure 4: Rat liver treated with atorvastatin and cholesterol showing unremarkable hepatocytes A and mild sinusoidal congestion B (H and E, ×400)

Histological study of the liver

The result in Figure 1 showed a section of liver of rats in the control group showing normal arrangement of hepatocytes. The result from Group 2, the extract only group, as in Figure 2 also showed mild periportal lymphocytosis indicating the presence of some lymphocytes and some congestion within the cytoplasm. The result from Group 3, the cholesterol only group, as in Figure 3 showed Kupffer cell activation and inflammatory cells including lymphocytes. There were intracytoplasmic perinuclear fat vacuoles, and all these put together make up the features of nonalcoholic steatohepatitis meaning functional disorder due to damage to the liver's hepatocytes.

The result from Group 4, the atorvastatin + cholesterol group, as shown in Figure 4 showed mild sinusoidal congestion with the hepatocytes appearing normal. This shows that atorvastatin exerted a protective effect on the hepatocytes from the toxic effects of the cholesterol.

For Group 5, the extract + cholesterol group, as shown in Figure 5, the result showed normal hepatocytes with the exception of the activation of Kupffer cells which are macrophages that are activated in cases of early injury to the liver and which could have been caused by the administration of cholesterol. The Kupffer cell activation as observed was milder compared to that observed in the cholesterol only group indicating also that the extract may have ameliorated the toxic effects of cholesterol administration on the liver of the rats.

Histological study of the abdominal aorta

The abdominal aorta of the Group 3 rats showed luminal obstruction, infiltrates of chronic inflammatory cells, and intimal ulceration as shown in Figure 8. Hypertrophy of the tunica media was also observed, signaling an early initiation step of atherosclerosis. The media is the middle

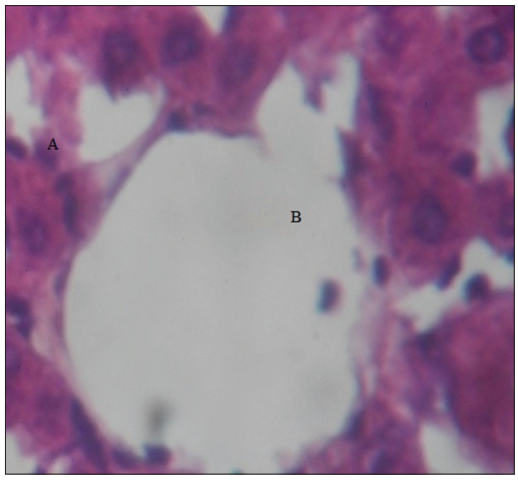

Figure 5: Rat liver treated with extract and cholesterol showing unremarkable hepatocytes A and moderate Kupffer cell activation B (H and E, ×400)

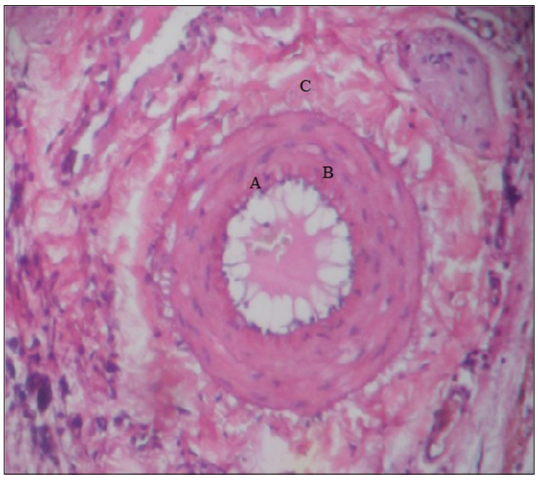

Figure 6: Rat abdominal aorta composed of tunica intima A, tunica media B, and tunica adventitia C (H and E, ×100)

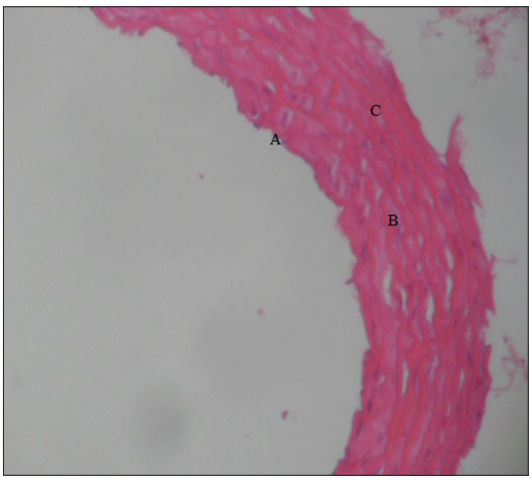

Figure 7: Rat abdominal aorta treated with extract only for 14 days showing unremarkable intima A, media B, and adventitia C (H and E, ×100)

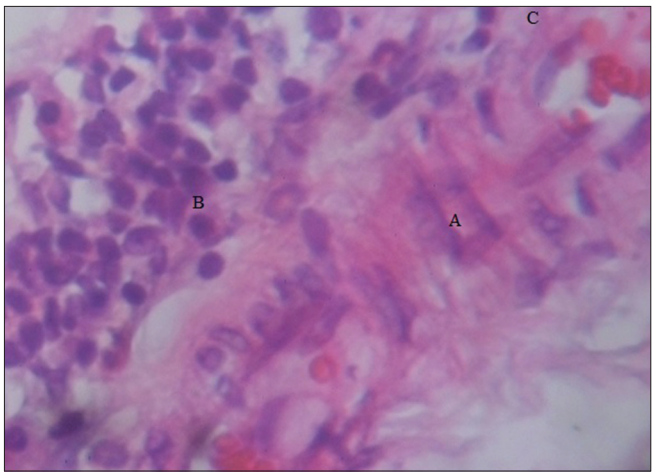

Figure 8: Rat abdominal aorta treated with cholesterol only for 14 days showing luminal obstruction A, infiltrates of chronic inflammatory cells B, and intimal ulceration C (H and E, ×400)

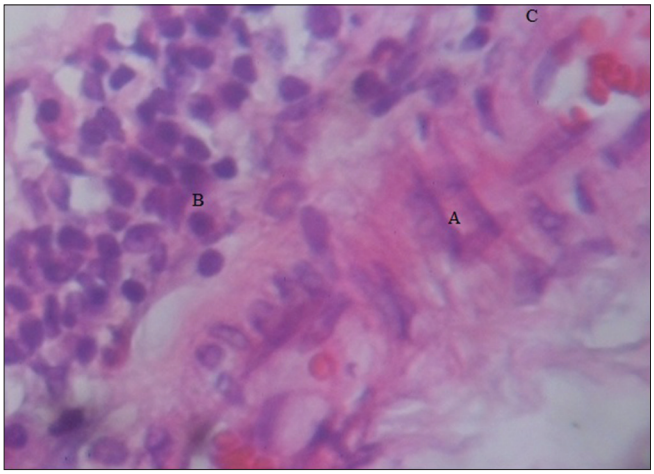

Figure 9: Same slide as Figure 8 showing intima ulceration C (H and E, ×400)

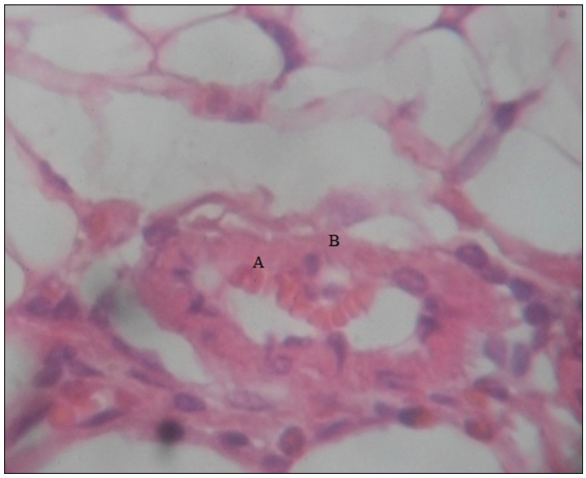

Figure 10: Rat abdominal aorta treated with atorvastatin and cholesterol for 14 days showing unremarkable intima A and patent lumen B (H and E, ×400)

layer of the 3 distinct layers of the blood vessels. The result also showed fibrosis which may inadvertently have led to the narrowing of the blood vessel. The result also showed stimulation of chronic inflammatory cells observed resulting from complex atherosclerosis. When

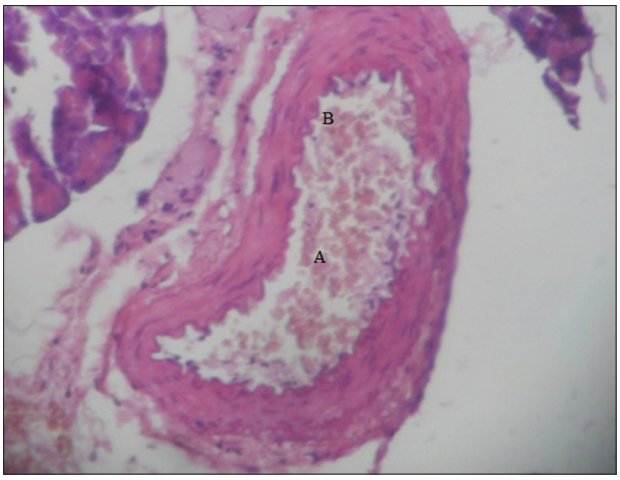

Figure 11: Rat abdominal aorta treated with extract and cholesterol showing unremarkable intima A and patent lumen B (H and E, ×100)

compared to the control group [Figure 6], there was a marked difference as the control group showed patent tunica media, tunica adventitia, and tunica intima.

For the cholesterol + atorvastatin group in Figure 10, there was amelioration of these effects seen in the cholesterol only, although there was difference between this group and the control group [Figure 6]; this difference was seen in the tunica intima as damage to this portion was mild and this damage may be due to the fact that cholesterol was administered alongside the atorvastatin.

Discussion

The present study was carried out to investigate the antidyslipidemia effect of *M. lucida.* Liver remains the key organ regulating homeostasis within the body and its disorders such as dyslipidemia are detrimental to the body functioning which may result to severe metabolic disorders or even death. Consumption of oxidized oil can lead to liver injury (Premila, 2010) which was in agreement with the findings in the present study, resulting in abnormal retention of fat in the liver and moderate congestion of blood vessels of the liver. Management of liver injury is of great concern in medicine; hence, in recent years, attention has been shifted to plants like *M. lucida* which contains products such as flavonoids and steroids which are known to have pharmacological properties including antioxidants and hepatoprotective activities (Defeuids *et al.*, 2003).

In this present study, liver sections of the rats treated with only cholesterol showed abnormal retention of fat in the liver and moderate congestion; these maybe indicative of hepatic tissue damage (Nithianantham *et al*., 2011). The damage caused by ingestion of cholesterol was later reverted by treatment with aqueous extract of *M. lucida* indicating protection of liver cells in cholesterol-induced hepatic damage. Atorvastatin, a drug known to reduce blood cholesterol level, conferred a kind of protection against the toxic effects of the cholesterol on the blood vessel. This was in agreement with the work of Argo *et al.* (2008), who reported that there was an improved hepatic inflammation in patients with nonalcoholic fatty liver disease in a short-term study, but long-term study showed inflammation in liver tissues; aggregation of lipid droplets was seen which suggests early progression of liver disease (Pourahmadi *et al*., 2015). Morris (1997) also reported that statins block the enzymes of synthesizing of lipids in liver, and the patients with active liver infection or severe hepatic disease should be taking statins with caution. A similar situation was observed for the Group 5 as a normal tunica intima was also observed here, and the results show a blood vessel that is histologically normal than that observed in the Group 4. Moreover, this may be an early indication that the aqueous root extract of *M. lucida* confers a more pronounced protective effect against atherosclerosis than the standard drug used.

Conclusion

The present study has demonstrated that aqueous extract of *M. lucida* stem bark can be used as a complementary medicine as it showed the same effects as an official drug, atorvastatin but may be time–dose dependent.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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