ANTI-HYPERLIPIDEMIC EFFECT OF *Morinda lucida* PLANT EXTRACTS ON *Helicobacter pylori* INDUCED DYSLIPIDEMIC MICE.

Owolabi S. L., [1] Mamora, O.T., [1] Olanrewaju, G.O.[2]

1. Microbiology unit, Department of Science Laboratory Technology, Gateway (ICT) Polytechnic Saapade, Ogun State, Nigeria.

2. Environmental Biology unit, Department of Science Laboratory Technology, Gateway (ICT) Polytechnic Saapade, Ogun State, Nigeria.

ABSTRACT

Raised cholesterol increases the risks of heart diseases and stroke. This study was conducted to determine the effect of extract of different parts of *Morinda lucida* on the Total Cholesterol Level (TCL) of *Helicobacter pylori* induced hyperlipidemic mice. An ethanolic extraction of various part (root, stem bark and leaves) of *M. lucida* was carried out. Twenty-five (25) healthy young adult male and female albino mice were procured and divided into five equal groups. All the mice were inoculated with *H.* pylori strain to induce dyslipidemia. Afterward, the different groups were subjected to different anti-hyperlipidemic treatments. Group 1 served as the positive control (distill water treatment) while Group 2 served as the negative control (Astorvastatin treatment). The other three groups were treated with the root, stem bark and leaf extracts of *M. lucida* respectively. The treatments were administered intraperitoneally. Blood samples were collected through tail tipping method and cholesterol level was determined using a multi-parameter diagnostic system. *M. lucida* treatments produced a significantly better ($p < 0.05$) reduction in weight than treatment with Astorvastatin and distill water. Similarly, the stem bark extract of *M. lucida* had a higher anti-hyperlipidemic effect than all other extracts of the plants. The leaf extract and Astorvastatin had a similar anti-hyperlipidemic effect on the mice. The result from this study has established that *H. pylori* increase the risk of TCL and treatment with *M. lucida* plant extracts are more effective than Astorvastatin on *H. pylori* dyslipidemic mice.

Keywords: TCL, *H.Pylori*, Hyperlipidemic, Dyslipidemic, Astorvastatin, *Morinda Lucida*, *Lipoproteins Mice*

Introduction

Cholesterol is a fatty substance carried around the body, in the blood, by the high density (HDL) and low density (LDL) lipoproteins. The body produces most cholesterol naturally, while some are derived from foods (AHA, 2018). The lower the density of the lipoproteins, the more fats it contains. High density lipoprotein (HDL cholesterol), also called 'good cholesterol' helps to keep cholesterol from building up in the arteries. Low density lipoprotein (LDL cholesterol) is the main source of cholesterol build-up and blockage in the heart arteries. Kizer (2010) reported that heart disease caused an approximation of 25% global deaths in 2008 and coronary heart disease is the most common type of heart disease involved in such death. *Helicobacter pylori* colonizes the stomach of at least half the world's population and is a key constituent of the human microbiome. Infection is usually acquired early in life and, when left untreated, persists throughout the life of the host (Kim *et al.*, 2016). Over the past few decades, a large amount of epidemiologic and clinical data regarding associations with non-gastric systemic diseases and *H. pylori* infection have been reported, including cardiovascular disease and its risk factors (Grupta, 2012). A number of epidemiologic studies reports a significant correlation of cardiovascular disease or its risk factors with *H. pylori* infection (Kim *et al.*, 2011). However, the results of several other studies failed to confirm the association (Oduola, 2010).

Morinda lucida, belonging to the family *Rubiaceae,* is a tropical rainforest tree with the English name 'Brimstone tree'. It is also known as Sangogo or Bondoukou alongua (in Cote d'Ivoire), Twi, Kon kroma or Ewe amake (in

Ghana), Ewe amake or Atak ake (in Togo) and Oruwo or Ruwo amongst the Yoruba tribe (South-west Nigeria) and Huka or Eze-ogu amongst the Igbo speaking tribe of South-east Nigeria (Adeneye, 2013). The major constituents of *M. lucida* extract are the various types of alkaloids, anthraquinones and anthraquinols. In Southwest Nigeria, fresh leaves of the plant are macerated in fresh palm wine and the filtrate taken orally for blood sugar control in suspected diabetic patients. The leaves have also been reported to possess strong hypoglycemia, trypanocidal and aortic vasorelaxant activities (Adeneye, 2013). The major constituents of *M. lucida* extracts were found to be essential oils, anthraquinones and anthraquinols. Oruwalol, oruwal, ursolic acid, and oleanolic acid were also isolated from this plant. The aqueous extracts of different parts of this plant were found to be effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Plant sample collection and preparation

2kg each of fresh *Morinda lucida* root, bark and leaves were collected from an open farmland in Isolo, Lagos State, Nigeria in the month of February, 2018. The harvested plant materials were processed for voucher referencing as described by Adeneye and Agbaje (2008) and identified at the Biology unit of Gateway ICT Polytechnic, Ogun State. The fresh leaves, bark and root were separately rinsed with water and dried under room temperature for three weeks. Afterwards, the dried samples were grinded and bagged for further work.

Aqueous extraction of plant material

50g each of the different parts of the grinded plant sample were soaked in 100 ml of ethanol. These were continually agitated using an electric shaker for 72 hours in the laboratory, after which filtration was done on the samples. The filtrates were then transferred into different crucibles and placed in a water bath at 78^{o}C until solid residue was left behind for each parts. The residues obtained were stored in the refrigerator maintained at -4^{o}C until required for experimentation.

Experimental animals

Twenty five (25) healthy young adult male and female white mice (weighing 18-25g) used in this study were obtained from the Pharmacological department of Olabisi Onabanjo University Teaching Hospital, Ogun State, Nigeria. The mice were housed in plastic cages and handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes for Health, 1985). The mice were fed with Mice Livestock feeds obtained from Animal Care, Sagamu- Ogun State; and the feeding regime was as described by Sowemimo *et al.* (2007). The mice were maintained at an ambient temperature between 23-26^{o}C.

Induction of mice with *Helicobacter pylori*

H. pylori clinical isolate was obtained from the medical microbiology department of Olabisi Onabanjo University Teaching Hospital, Ogun State, Nigeria. Different colonies from the obtained cultures of the *H. pylori* were picked and homogenized in a 0.9ml of normal saline. 0.2ml of the homogenized samples was then injected into all the mice through the intraperitoneal route. Total cholesterol level in the mice were measured four days after *H. pylori* inoculation; total cholesterol level equal to or above 200mg/dl were considered hyperlipidemic.

Body weight measurement

Body weights of all mice were measured, on the 1st and 8th day after establishing cholesterol induction using digital mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 1st and 8th day in reference to the initial weight per group was calculated.

Experimental design and intraperitoneal treatment of hyperlipidemic mice:

Group I (positive control): *H. pylori* induced mice treated with treated with distilled water

Group II (negative control): *H. pylori* induced mice treated with *Atorvastatin.*

Group III: *H. pylori* induced mice treated with treated with *Morinda lucida* root extracts.

Group IV: *H. pylori* induced mice treated with treated with *Morinda lucida* bark extracts.

Group V: *H. pylori* induced mice treated with treated with *Morinda lucida* leaf extracts.

All the treatments were administered via the intraperitoneal route and each group consist of five (5) mice.

Total Cholesterol measurement

Blood samples of mice were collected by tail tipping method and Total cholesterol was determined by using a Multiparameter Diagnostic System® (Biochemical System International, Arezzo, Italy). The diagnostic system was calibrated and validated at the beginning of, midway into and at the end of the experiment.

RESULTS

Table 1: Average body weight of each groups of mice

		Body weight (g)		
	Group	Before Induction	After induction	After treatment
1	*H. pylori* induced mice treated with treated with 0.1 ml distilled water	18.79^{c} ± 0.01	21.31^{b} ± 0.03	23.62^{a*} ± 0.03
2	*H. pylori* induced mice treated with 0.1ml	18.80 ± 0.02	22.78^{a} ± 0.03	21.81^{b**} ± 0.05

	Astorvastatin.			
3	*H. pylori* induced mice treated with treated with 0.1ml *Morinda lucida* root extracts.	18.81^{b} ± 0.00	21.74^{a} ± 0.01	19.63^{b***} ± 0.03
4	*H. pylori* induced mice treated with treated with 0.1ml *Morinda lucida* bark extracts.	18.80^{b} ± 0.04 b	22.32^{a} ± 0.02 c	19.21^{b***} ± 0.01 a
5	*H. pylori* induced mice treated with treated with 0.1ml *Morinda lucida* leaf extracts.	18.79^{c} ± 0.02	21.94^{a} ± 0.03	20.18^{b***} ± 0.02

- Values with different letters are significantly different across the column at α = 0.05
- Value with ***are significantly different across the rows at α = 0.05

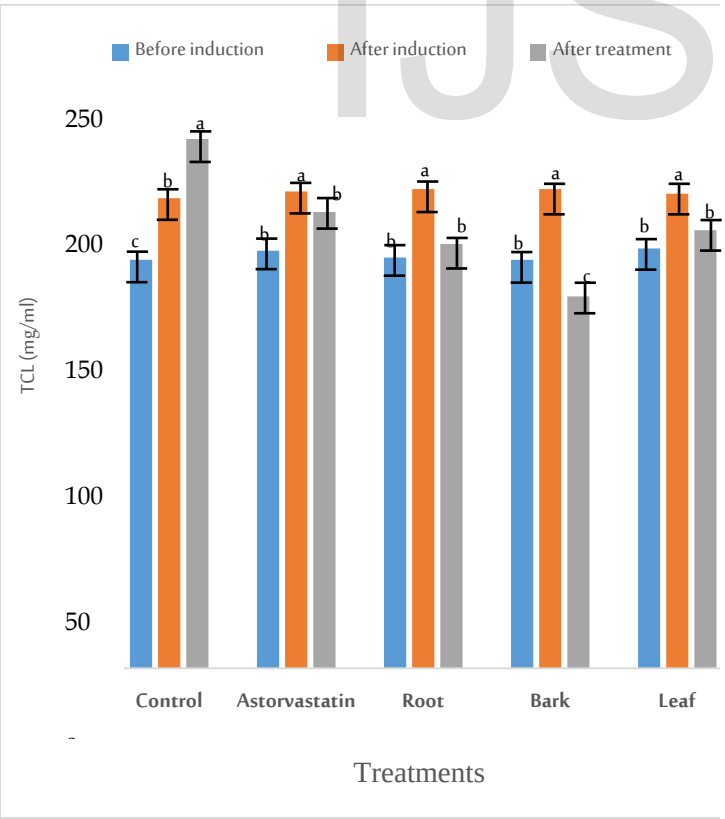
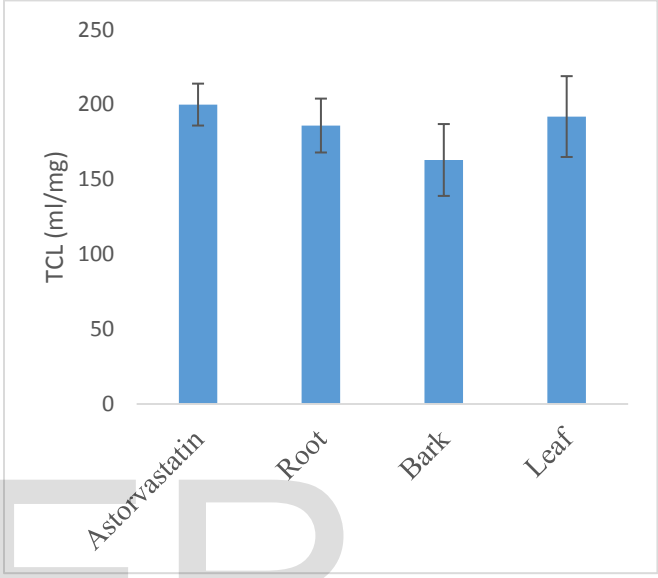


Figure 1: Total Cholesterol Level (TCL) in mice before induction with *H. pylori*, after induction and after treatment

Plotted means on the same horizontal axis represented with different letters are significantly different at α= 0.05.

Figure 2: Comparison of Total Cholesterol Level (TCL) in mice after treatment



Plotted means on the same horizontal axis represented with different letters are significantly different at α= 0.05.

DISCUSSION

Table 1 shows an increase in the weights of the experimental mice, across all the groups, after inoculation with *H. pylori*. Hannah *et al.* (1997) reported a positive correlation between weight and Low density Lipoprotein (LDL) which is associated with high cholesterol level in the body system. It can therefore be inferred that *H.* pylori strain is a successful inducer of cholesterol in the body system. This is in line with the works of Kim *et al.* (2011), who reported a positive association between invasions of the body system by *H. pylori* and increased risk of cholesterol induced cardiovascular disease. However, all the treatments yielded a significant ($p< 0.05$) decrease in weight in the experimental mice. This was different with the positive control (distill water) where a successive significant increase ($p< 0.05$) was noticed in the weight of the mice throughout the experiment. Mice treated with *Morinda lucida* stem bark had the least weight after treatment, while those treated with Astorvastatin (negative control) had the least weight after treatment (Table 1). Similarly, there was no significant difference ($p< 0.05$) in the weights of mice treated with the different extract of parts of *M. lucida*.

Figure 1 showed the Total cholesterol level (TCL) of the experimental mice before cholesterol induction with *H.*

pylori, after induction and after subjecting the mice to different treatment. Similar to the observations on the mice weight, There was a significantly ($p < 0.05$) progressive increase in the cholesterol level of mice subjected to positive control (distill water) throughout the experimental procedures. In contrast to this, mice treated with Astorvastatin (negative control), *M. lucida* root and leaf had the significantly higher ($p < 0.05$) cholesterol level after induction with *H. pylori* while the cholesterol level before induction and after treatment were not significantly different ($p > 0.05$) from each other. However, treatment with *M. lucida* stem bark produced a significantly lesser ($p < 0.05$) cholesterol level than 'before and after induction'. This may be as reported by Daniele *et al.* (2013), that steroids and other plant phytochemicals demonstrate a sub-cellular localization. There is a further suggestion that plant phytochemicals tend to be more localized to the aerial parts which are stem and leaves. However, active photosynthesis and evapotranspiration going on the in leaf region makes these chemical compounds unstable.

Figure 2 compared the cholesterol level of the *H. pylori* induced mice across the various treatments. Hyperlipidemia mice treated with Astorvastatin retained the highest cholesterol level after treatment; which was not significantly different from the cholesterol level in mice treated with *M. lucida* leaf. This suggests that Astorvastatin and *M. lucida* had a similar anti-hyperlipidemia effect on the body system. Hyperlipidemia mice treated with *M. lucida* stem bark had the least cholesterol level after treatment. This was also significantly lower ($p < 0.05$) than the cholesterol level of hyperlipidemia mice treated with the roots. The more pronounced anti-hyperlipidemic effects of extracts of *M. lucida* over Astorvastatin may be linked to the works of Smith *et al.* (2003) who reported the antimicrobial activities of *M. lucida* against *H. pylori* bacteria. Therefore, *M. lucida* anti-hyperlipidemic action is probably by its bactericidal effect directly on *H. pylori,* unlike Astorvastatin which works directly on body fats and lipids. This study also discovered that at high concentration ($\geq$ 0.2 ml/ kg), *M. lucida* extracts was toxic to the mice as none of them survived treatments at such concentration.

CONCLUSION

The result from this study has established that *H. pylori* increase the risk of TCL and treatment with *M. lucida* plant extracts are more effective than Astorvastatin on *H. pylori* dyslipidemic mice. The study also revealed that the stem bark extract of *M. lucida* had more anti-hyperlipidemic effect and this is closely followed by the root extract. There was no major difference in the treatment with the leaves extract of *M. lucida* and Astorvastatin, a synthetic anti-hyperlipidemia drug.

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