



Effect of Aqueous Extract of *Lannea acida* and Chloroquine on Lipid Profile Levels in the Liver and Kidneys of Rats Infected with *Plasmodium berghei*

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Abstract

Keywords:

Lannea acida, chloroquine, Plasmodium berghei, parasite clearance, lipid profiles

Lannea acida belongs to the genus in the family of Anacardiaceae. It is a deciduous tree and is used in traditional medicine of several ailment and various infections. The present study investigates the antiplasmodial effect of the aqueous bark extract of *L. acida* on rats infected with *Plasmodium berghei* in order to ascertain its lipid profile levels. The aqueous extract of *Lannea acida* was administered orally to healthy and malaria infected mice at 200, 250 and 300 mg/kg body weight daily for 6 days compared with chloroquine. The rate of parasite clearance was higher in the group treated with aqueous extract of *Lannea acida* the group treated with chloroquine. Liver and kidney triglyceride, total cholesterol and HDL – cholesterol were significantly ($p=0.05$) higher in groups treated with chloroquine while HDL – cholesterol level was higher in groups treated with aqueous extract of *Lannea acida* than the group treated with chloroquine. This study shows that the aqueous extract of *Lannea acida* has a high potential for antiplasmodial activity and capable of increasing cholesterol level in malaria – infected organisms.

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INTRODUCTION

Malaria remains a serious global health burden, with an annual incidence of 247 million cases and nearly one million deaths, most of which afflict children living in Africa (WHO, 2008). Of the four human malaria parasite species, *Plasmodium falciparum* is reported to cause the highest morbidity and mortality. Young children with naïve immune systems (McGregor, 1960) and pregnant women with potentially compromised immune system are particularly vulnerable to this disease and so are considered to be the highest risk populations for malaria-related deaths. The development of drug resistance to the potent and affordable drug, absence of licensed vaccines and lack of conserved vector control strategy have left many developing countries without sustainable option for malaria control hence,

chemotherapy remains only the prompt and effective option.

Traditional plants play important roles in medical system and plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents. Since many drugs e.g. quinine and artemisinin were isolated from plants and because of the increased resistance of many pathogens e.g. malaria parasites, towards established drugs, investigations of the chemical compounds in plants is necessary (Phillipson, 1991).

It is believed strongly that if herbs used to treat malaria by our ancestors in Africa hundred years ago were not effective, malaria would have destroyed Africa. More so, Missionaries that came to Africa

would not have met a single person on the continent of Africa (Elujoba, 2005). In view of the problems associated with anti-malarial drug resistance, new drugs or drug combinations are urgently required today for treatment of malaria. Preferably, new drugs should have novel modes of action or be chemically different from the drugs in current use (Phillipson and Wright, 1991). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies (WHO, 2002; Zirihi et al., 2005), mainly from plants. Many medicinal plants with antimalarial properties have been used by the traditional healers. The efficacy and safety of many of these medicinal plants have been reported (Bala and Adamu, 2008).

Lannea acida (syn. *Odina acida* or Awerekagun in Yoruba language) belongs to the family of Anacardiaceae. *Lannea acida* is a 10 m high tree with a diameter of 35 cm. Its fruits are yellow and red drupes containing a thin pulp around a stone. It is a deciduous tree and is used in traditional medicine of several ailment and various infections. Also, this plant *Lannea acida* was suspected to have an antiplasmodial activity which is a potent cure of various plasmodium species.

MATERIALS AND METHODS

Fresh bark of *Lannea acida* was obtained locally from an open forest at Ugbe, Akoko North-East in Ondo State, Nigeria. It was chopped into smaller pieces and put into a clean pot filled with water and then boiled at optimum temprature for hours to a point whereby it was concentrated. Thirty-six white male rats of 4 weeks old, obtained from the central animal house of the University College Hospital, IMRAT Ibadan were used for this experiment. They were kept in a standard rat cage and fed with guinea grower feed for 2 weeks so as to acclimatize them before the experiment. Four mice passaged with the parasite *Plasmodium berghei* used for this study was procured from the central animal house of the University College Hospital, IMRAT Ibadan. They were kept in a separate cage until they were used for the experiment.

Animals

A total of 36 rats aged six weeks, obtained from IMRAT, Ibadan, were used for this work.

Experimental Design

The animals were evenly grouped according to their body weight into 6 groups. The animals in groups 1,3 and 5 were not infected. All the animals in the remaining three groups were infected with the parasite. The passaged animals were sacrificed and their blood was collected into a blood bag containing anticoagulant. Infection was achieved by intraperitoneal injection of 0.2 ml of the blood into the animals in groups 2,4 and 6.

Group 1: Positive control (P-CTR): the animals in this group were not infected with the parasite, *Plasmodium berghei*.

Group 2: Negative control (N-CTR): the animals in this group were infected with the parasite, *Plasmodium berghei* and not treated with neither the aqueous extract of *Lannea acida* nor chloroquine.

Group 3: The animals in this group were not infected with the parasite, *Plasmodium berghei* but treated with the aqueous extract of *Lannea acida*.

Group 4: The animals in this group were infected with the parasite, *Plasmodium berghei* and then treated with the aqueous extract of *Lannea acida*.

Group 5: The animals in this group were not infected with the parasite, *Plasmodium berghei* but treated with chloroquine.

Group 6: The animals in this group were infected with the parasite, *plasmodium berghei* and then treated with chloroquine.

Acute toxicity test

The safety of the extract was assessed by determining its median lethal dose (LD₅₀) using the OECD Guideline 423 (2001). Three rats were injected with the extract of *L. acida* bark (200, 250, 300 mg/kg) intraperitoneally. The rats were acclimatized and fasted overnight. Signs of toxicity such as death, changes in physical appearance, behavioral change, and organ damage were recorded for 24 hours.

Total cholesterol

Standard (10 µl) and samples (10 µl) were pipetted into labelled test tubes. Reagent (10 µl) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 15 minutes at room temperature. The absorbance was then taken at 500nm against the blank. (Kayamori et al., 1999). Cholesterol concentration was calculated as follows:

$$\frac{\text{A sample}}{\text{of standard}} \times \text{concentration of standard. Triglyceride}$$

Standard (10 µl) and samples (10 µl) were pipetted into labelled test tubes. Reagent (10 µl) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 15 minutes at room temperature. The absorbance was then taken at 500 nm against the blank. (Bucolo and David, 1973).

$$\frac{\text{A sample}}{\text{standard}} \times \text{Concentration of A standard}$$

HDL- cholesterol

Precipitation buffer (100 µl) was mixed with 100 µl of serum sample in labelled micro-centrifuge tubes. After 10 minutes incubation at room temperature, the mixtures were centrifuged at 5000 rpm for 10 minutes. The supernatants were transferred into labelled clean tubes and these were the HDL fractions. The precipitates were the LDL/VLDL fractions. Two to twenty microlitres of the HDL or LDL/VLDL fractions were adjusted to the volume of 50 µl/well with cholesterol reaction buffer. Fifty microlitres of the reaction mixture was added to each well containing the cholesterol standard or test samples and was vigorously mixed. The reaction mixture was incubated for 60 minutes at 37°C. The absorbance was measured at 570 nm in a micro-plate reader.

The LDL or HDL concentration was calculated as follows;

$$\frac{\text{A sample}}{\text{A standard}} \times \text{X concentration of standard} \times \text{dilution Factor}$$

TOTAL PROTEIN ASSAY.

Cupric ions interact with proteins to give an intense violet-blue complex colour with Cu²⁺ in alkaline medium. The intensity of the colour was proportional to the amount of proteins present in sample.

Equal volume (0.2 ml) of distilled water, serum and protein standard were pipetted into different test tubes respectively. Then 1 ml of protein reagent was added to all the test tubes and the solutions were thoroughly mixed and incubated at 25°C for 30 min (Weichselbaum, 1974). The absorbance was then read at 546 nm against a reagent blank and total proteins were determined using the following formula:

$$\frac{\text{Absorbance of test standard}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Absorbance of standard 1

The protein reagent contained 100 mM NaOH, 16 mM Na-K-tartrate, 15 mM potassium iodide, 6 mM CuSO₄. Protein reagent blank contained 100 mM NaOH and 16 mM Na-K-tartrate. Protein standard solution was 6 mg/ml.

Statistical Analysis

All the values of biochemical estimation were expressed as mean ± standard error of the mean (SEM) using graph pad prism 5.0.

RESULTS

The effects of different concentrations of aqueous extract of *Lannea acida* on the lipid profiles of kidney and liver of rats infected with *Plasmodium berghei* are shown in Figures 1-4 below. Relative to the water control group (Group 1), infection with *Plasmodium berghei* and treatment with both the extract of *Lannea acida* and chloroquine led to a significant reduction in the levels of triacylglycerols in both the kidney and liver of rats, as shown in the different treatment groups.

However, there was no significant difference in the levels of the triglyceride in Groups B, C, D, E and F. Also, there was significant reduction in the kidney and liver total cholesterol and HDL cholesterol levels in all the test groups compared to water control group, but there was no significant difference within the test groups (Groups B-F).

Figure 4 shows a significant increase in the levels of the liver total protein concentration in the groups infected and treated with *Lannea acida* extract and chloroquine. However, a significant reduction observed in the kidney levels of the total protein in the test groups relative to the water control group.

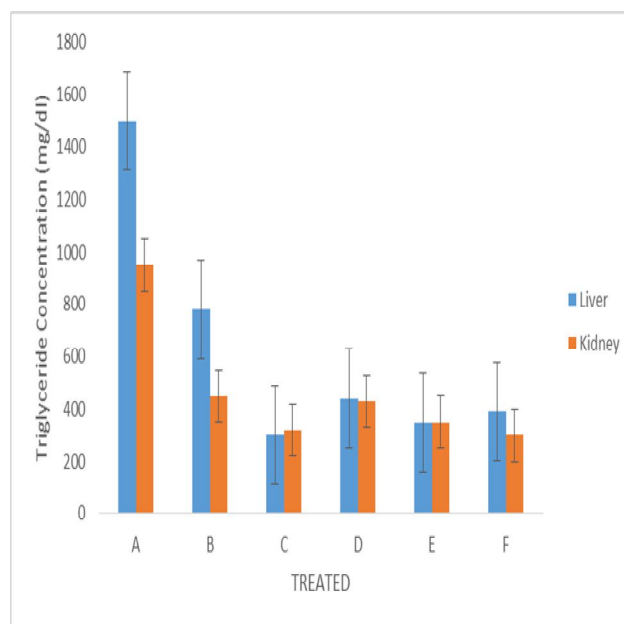


Figure 1. The effects of the extract on the activity of triglycerides in the liver and kidney

Keys:

- A = No infection or treatment
- B = Infected but not treated (negative control)
- C = Treated with the extract 300 mg/kg but not infected
- D = Infected and treated with the extract of 300 mg/kg
- E = Not infected but treated with chloroquine
- F = Infected and treated with chloroquine

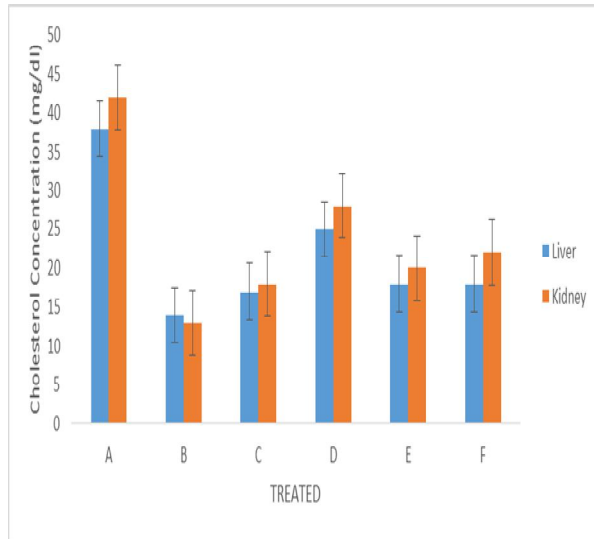


Figure 2. The effects of the extract of *L. acida* on the activity of the liver and kidney cholesterol

Keys:

- A = No infection nor treatment
- B = Infected but not treated (negative control)
- C = Treated with the extract 300 mg/kg but not infected
- D = Infected and treated with the extract of 300 mg/kg
- E = Not infected but treated with chloroquine
- F = Infected and treated with chloroquine

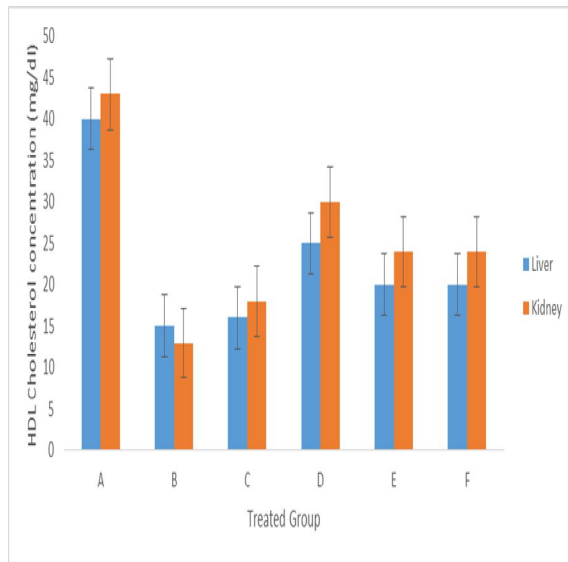


Figure 3. The effects of the extract of *L. acida* on the activity of HDL-cholesterol in the liver and kidney

Keys:

- A = No infection nor treatment
- B = Infected but not treated (negative control)
- C = Treated with the extract 300 mg/kg but not infected
- D = Infected and treated with the extract of 300 mg/kg
- E = Not infected but treated with chloroquine
- F = Infected and treated with chloroquine

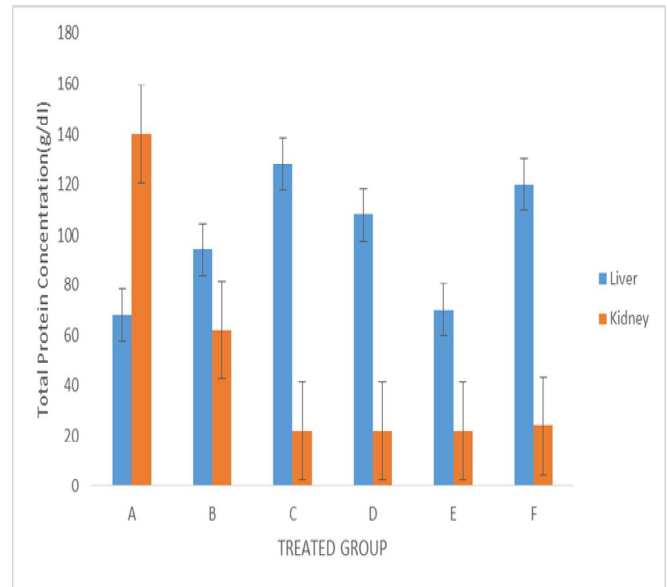


Fig 4, The effects of the extract on the activity of total protein in the liver and kidney.

Keys:

- A = No infection nor treatment
- B = Infected but not treated (negative control)
- C = Treated with the extract 300 mg/kg but not infected
- D = Infected and treated with the extract of 300 mg/kg
- E = Not infected but treated with chloroquine
- F = Infected and treated with chloroquine

DISCUSSION

The lipid profile test is a group of test that are often ordered together to determine risk of coronary heart disease. The test making up a lipid profile test has been shown to be good indicator of heart attack and of stroke caused by blockage of blood vessels (hardening of the arteries) when the lipid profile is found to be bad (Mackay and Mensah, 2004). It has been reported that malaria produces moderate changes in lipid profile in man, with typical rise in serum triglyceride concentration and decline in HDL concentration (Faucher *et al.*, 2002). Khrisna *et al* (2005) also reported increases in serum triglyceride, total cholesterol and LDL-cholesterol concentrations in malaria patients, though distinction was not made with regards to the severity of malaria. During intra-erythrocytic development, the human malaria parasite actively internalizes phospholipids from its erythrocyte membrane and the extracellular medium. The import of exogenous lipids is not due to endocytosis; but to energy dependent, trans-bilayer movement of phospholipids induced by the parasite in the erythrocyte surface (Haldar, 1992). These changes might be as a result of acute phase response to malaria infection. The acute phase response is associated with changes in lipid metabolism including a moderate increase in triglyceride, but a decrease in HDL and

LDL (Sibmooh *et al.*, 2004). Also, Nilsson-Ehle and Mohanty *et al.* reported that during malaria infection, the levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are decreased while triglycerides and cholesterol are moderately increased.

It was observed that there was a significant difference between groups A (positive control) compared to the other groups in Figure 1. There was a little difference between group C (treated with extract but not infected) and group E (not infected but treated with chloroquine), also a significant difference between group D (infected and treated with extract) and group F (infected and treated with chloroquine). There was a significant difference between group A (positive control) and group B (negative control). The plant extract reduced the level of triglycerides in liver of groups treated with the extract (Group C&D) compared with positive group A and also with groups treated with chloroquine which also lower the triglycerides level, this corresponds to the result of Ethan *et al.* (2003). In triglycerides activities in kidney, there was also significant decrease in triglycerides level of groups treated with aqueous extract of *Lannea acida* compared to the positive group A. Also, there is slight decrease in triglyceride levels of group E and a decrease in triglyceride levels in group F.

Total cholesterol in liver, there was a significant difference between positive control group A and negative control group B showed elevated level of cholesterol. There is a significant distinction in group D showed cholesterol level decreased. Groups treated with chloroquine group E and F have elevated level of cholesterol is also apparent in negative control group B and group C. The reduction in serum total cholesterol of the positive control group is in agreement with the report of Obianime and Aprioku (2009); the best single indicator of the likelihood of developing atherosclerotic heart disease is not total plasma cholesterol but rather the ratio of plasma LDL-cholesterol to plasma HDL-cholesterol. This reduction also reveals that malaria uses cholesterol and phospholipids of its host, thus, resulting in decrease level of cholesterol in liver (Kittl *et al.*, 1992). It is evident that there was a significant difference between the control groups. Group C treated with extract but not infected have low level of cholesterol compared with other groups, and group E have a slight low level of cholesterol. Group F showed slight reduction in cholesterol level compared to group D. The reduction in cholesterol level in kidney of groups treated with aqueous extract (group C and D) shown that administration of the aqueous extract of *Lannea acida* reduces the cholesterol level which give an account that malaria uses host cholesterol and phospholipids (Kittl *et al.*, 1992; Goldstein *et al.*, 1976).

Figure 3 showed that there was a significant difference between positive group A and negative control group B in both HDL-cholesterol in liver and

kidney. There was also a significant difference ($p < 0.05$) between groups treated with plant extract (*Lannea acida*) group C and D compared with groups treated with chloroquine group E and F. Group F and E infected and non-infected but treated with chloroquine was slightly different from group C and D. HDL was significantly decreased in severe malaria according to Das *et al.* (1996). HDL-cholesterol was slightly higher in the kidney compared to its level in the liver in the treated group. This shows that administration of aqueous extract of *Lannea acida* was able to boost HDL-cholesterol in kidney, which is in agreement with the report of Khovidhunkit *et al.* (2000). In a malaria infected mice, the amount of the high density lipoprotein is always low, hence making the trophozoite of the malaria life cycle to grow faster. The ability of the extract to increase the high-density lipoprotein level of the mice shows that it has an antimalarial property that inhibits the further growth of the malaria parasite. This agrees with the report of Krishna *et al.* (2009).

From the result obtained in figure 4, there was significant difference between the positive control group and the infected group ($p < 0.05$), meanwhile the treated group with the extract significantly reduce the total protein in the kidney. *Lannea acida* treatment reduced the total protein built up in the kidney more than the chloroquine when the group D compared with group F.

CONCLUSION

This study showed that *L. acida* extract may have antiplasmodial and hypolipidemia effects. It is therefore recommended that more intensive research is carried out to identify and characterize the active principles in *L. acida* that could be used in drug development for the treatment of malarial parasites and lipid lowering agents in health care management.

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