

Antiplasmodial Activity of Methanolic Leaf Extract of *Anogeissus leiocarpus* and its Effect on Heart and Liver of Mice Infected with *Plasmodium berghei*

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Abstract

Background: The emergence of malaria parasite resistance to antimalarial drugs, especially to those that can be afforded by the population that live in malaria endemic areas has necessitated the need for discovery and development of alternative medicine. **Objective:** This work assessed the efficacy of the methanolic leaf extract of *Anogeissus leiocarpus* against malaria parasite and its effect on the liver and heart of mice infected with *Plasmodium berghei*.

Methods: Sixty mice used for this study were grouped into six groups. Group one not infected with malaria parasite (normal control), group two was infected with the parasite but not treated with drugs (negative control), group three was infected and treated with 16 mg/kg body weight (mg/kgbdwt) of Artemether (positive control), the fourth and fifth groups were also infected and treated with 100 and 200 mg/kgbdwt of *A. leiocarpus* respectively, while group six was not infected but was given 200 mg/kgbdwt of *A. leiocarpus* (extract control). The parasite was assessed for five days. The animals were sacrificed on the fifth day. The homogenates of liver and heart were prepared and used to test for liver and heart function.

Results: The parasite clearance was higher in the group treated with 200 mg/kgbdwt of methanolic leaf extract of *A. leiocarpus* when compared with the group treated with 100 mg/kgbdwt. The ALT, AST and ALP activities were higher in negative control than in all other groups studied but it was higher in the group treated with 200 mg/kgbdwt of *A. leiocarpus* than in the positive control and the group treated with 100 mg/kgbdwt of *A. leiocarpus*.

Conclusion: This study shows that the antiplasmodial activity of *A. leiocarpus* was higher at the dosage of 200 mg/kgbdwt and the level of liver enzymes is a function of the dosage.

Keywords: *Plasmodium berghei*; *Anogeissus leiocarpus*; Alanine aminotransferase; Aspartate aminotransferase; Alkaline phosphatase; Methanolic leaf extract

Introduction

Early treatment of malaria with appropriate drugs is very important in the eradication of malaria infection in the tropics [1]. Despite the fact that WHO has made Artemisinin Combination Therapy (ACT) drugs as first line treatment in all the malaria endemic areas, but because of the resistance of malaria parasites to almost all conventional drugs including artemisinin in some part of the world such as Thailand and some part of Asian countries, then, it becomes imperative now that each country should be able to study and choose the drugs of first and second line for the treatment of malaria in their countries and this should be based on the efficacy of the medicines against malaria parasites in every locality [2], and there is a serious need for continuous global monitoring and reporting of drugs efficacy and parasite resistance.

With the present episode of development of drug resistant malaria parasite to the artemisinin which was believed to be the hope of effective treatment of malaria infection, there is a need for the development of new drugs and therefore UNESCO had encouraged all localities to look inwardly for the effective the treatment of malaria locally [3,4]. The use of medicinal herbs has been a common method of treating malaria among the people living in malaria endemic areas [5,6]. This practice has also been part of Nigeria culture especially in the rural areas where they do not have access to the good hospitals and most people living in these areas are peasant farmers who cannot afford to buy antimalarial drugs which are highly potent [7,8]. At present, most people who live in urban areas in Nigeria are sometimes prefer to use the medicinal

plants for the treatment of malaria as a result of emergency of resistance of malaria parasites to antimalarial drugs. Some of the medicinal plants used for treatment of malaria in Nigeria include, *Terminalia avicennioides*, *Anogeissus leiocarpus*, *Morinda lucida*, *Citrus medica*, *Azadirachta indica* [7].

A. leiocarpus is one of the herbs commonly used for treatment of malaria in Africa, including Nigeria. The efficacy of *A. leiocarpus* in the treatment of gonorrhoea, antihelminthic, diabetes, hypertension, body pain and asthma has been reported [9,10]. Many works have been done on the efficacy of its methanolic extract of stem bark against malaria parasite [1,10]. Despite the fact some people are using the leaf extract of *A. leiocarpus* for the treatment of malaria locally, there is dearth information about the importance of methanolic leaf extract of *A. leiocarpus* in the treatment of malaria, therefore there is need to study the efficacy of methanolic leaf extract of *A. leiocarpus* and its effect on some vital organs needs to be assessed.

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Methodology

Experimental animals and malaria parasite

Adults Swiss albino mice used for this study were obtained from the Animal unit at Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria. The animals were kept in well aerated wired cages, fed with standard mouse feed and were allowed to drink water freely. The mice were allowed to acclimatize to the new environment before they were infected with *P. berghei* which was donated by the laboratory of Prof. Ademowo O.G, of the same institute. The parasites were maintained in animals by serial passage of blood collected from a patent donor mouse to a naïve recipient.

Plant materials and their extract

The leaf of *Anogeissus leiocarpus* (locally called ayin) was collected in Akungba-Akoko, Ondo State, Nigeria, and was identified by Dr. A.O. Obembe, Plant science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo state, Nigeria. The Herbarium specimen with voucher number UIH22318 *Anogeissus leiocarpus* was deposited at the Herbarium unit of the University of Ibadan, Ibadan, Nigeria. The leaf of the plant was washed thoroughly; air dried and was later ground into powder. 100 g of powdered leaf was weighed into 70% methanol (2.7 L) and was left for 72 hours. The extract was filtered and evaporated to dryness with a rotary evaporator.

In-vivo Antimalarial assay

Mice weighing between 15-23 g were distributed into six groups. Each group comprised of five mice. The first and sixth groups were not infected with the parasite and were used for normal and extract control respectively, while the other four groups were infected intraperitoneally with an aliquot of 0.2 ml of standard inoculums (1×10^7 *Plasmodium berghei* strain NK 65 parasitized erythrocytes). Among the four infected groups, group 2 was not treated with antimalarial (negative control), group 3 was treated with 16.0 mg/kg body weight of artemether (positive control) while the fourth and fifth groups were infected and treated with 100 mg/kg body weight and 200 mg/kg body weight of methanolic leaf extract of *Anogeissus leiocarpus* respectively. All the treatments were administered once daily by gavages using intubator for four consecutive days. Blood was taken daily from the tail vein of the mice before treatment for the assessment of parasitaemia. The animals were sacrificed by decapitation twenty four hours after the last treatment and the thoracic and abdominal regions were opened to expose the heart and liver which were kept in the deep freezer at -20°C until analysis was done. The protocol was according to the guidelines of National Institute of Health (NIH) publication 85-23, [11], for laboratory animal care and use. The heart and liver of the animals were excised and homogenized in ice cold normal saline (1:4 w/v), centrifuged at 5,000 rpm for 5 minutes to obtain supernatant which was stored in the deep freezer at -20°C until analysis was done.

Biochemical assays

Liver and heart homogenates were used to assay for the liver and heart dysfunction using the spectrophotometric method with Randox test kits. Para-Nitrophenyl Phosphate (PNPP) was used to determine the ALP activity as described by Cathala et al. [12]. ALT and AST activities were determined by pyruvate and oxaloacetate respectively as described by Christen and Metzler [13].

Parasitological study

Thick blood film was prepared from blood collected from each

mouse for five days, and slides were screened for malaria parasite using Giemsa's stain. The number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density on the basis of 8000-leucocytes/ μl of blood as described by [14].

Statistical analysis

The differences among groups were analyzed by the one-way Analysis Of Variance (ANOVA). The SPSS 15.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. The results were expressed as mean \pm Standard Deviation (SD). The level of significance was estimated at $P < 0.05$

Results

Table 1 shows the percentage parasite increase and suppression in each group as compared with the initial parasite density before the treatment. The total parasite density before the treatment was considered to be 100% and this was used to determine the parasite suppression/increase on daily basis. In the negative control, the parasite growth rate increased by 7% in day 1 and finally by 25% in day 5 which was the day the animal was sacrificed. In the positive control, there was an increase in the growth rate of the parasite from 100% to 105% in day 1 and it was drastically reduced by 91% in day 5. There was an increase in parasitaemia by 7% and 6% in the groups treated with 100 mg/kg bwt and 200 mg/kg bwt of methanolic leaf extract of *A.leiocarpus* respectively in day 1 and these were finally suppressed by 85% and 90% in the two groups respectively in day 5 (Table 1).

Figure 1 shows the effect of treatment of mice infected with *P.berghei* with 100 mg/kg bwt and 200 mg/kg bwt of methanolic leaf extract of *A. leiocarpus* on the liver and heart ALT level. The ALT activity in the liver was significantly lower in the normal control when compared with other groups. There was a significant increase in the Liver and heart ALT activities in the negative control and the group treated with 200 mg/kg bwt of methanolic leaf extract of *A. leiocarpus* when compared with other groups. The heart ALT activity was significantly lower in the group treated with 100 mg/kg bwt of methanolic leaf extract of *A.leiocarpus* than the groups treated with 200 mg/kg bwt of *A.leiocarpus*, negative control, positive control and extract control. This study showed that ALT activities in the liver were higher in all the groups studied when compared with the ALT activities in the Heart (Figure 1).

The ALP activity in the liver was significantly higher than the ALP activity in the heart in all the groups studied. The ALP activity in the liver and heart was significantly lower in the normal control than in the other groups studied. There was a significant increase ($P < 0.05$) in the level of ALP in the liver and heart in the negative control and group treated with 200 mg/kg bwt of methanolic leaf extract of *A. leiocarpus* than in all other groups studied, while the liver ALP activity was highest in the group treated with 200 mg/kg bwt of methanolic leaf extract of *A.leiocarpus*. The group treated with 100 mg/kg bwt of methanolic leaf extract of *A.leiocarpus* had the lowest ALP activity among the groups treated with methanolic leaf extract of *A. leiocarpus* (Figure 2).

Figure 3 shows that the AST activity in the liver and heart was significantly reduced in the normal control than in all other groups. The AST level in the liver was significantly higher ($P < 0.05$) in the negative and positive control and in the infected group which was treated with 200 mg/kg bwt of methanolic leaf extract of *A.leiocarpus* than in the normal control and the infected group treated with 100 mg/kg bwt of *A. leiocarpus*. The AST level and heart was significantly higher ($P < 0.05$) in the negative control and group treated with 200 mg/kg bwt

of *A.leiocarpus* than in the normal and positive controls and also in the group treated with 100 mg/kgbdwt of *A. leiocarpus*. The group treated with 100 mg/kgbdwt of methanolic leaf extract of *A. leiocarpus* had the lowest AST activity in both liver and heart among the infected and treated groups (Figure 3).

Discussion

Treatment of malaria infection with medicinal plants is now becoming more popular as a result of development of resistant by the malaria parasite to almost all conventional antimalarial drugs that are available [15], and most people living in malaria endemic areas cannot afford those antimalarial drugs that are still potent, therefore, a large population living in these areas have resulted into making effective use of medicinal plants for the treatment of malaria [16]. *A.leiocarpus* is one of the medicinal herbs that is commonly use for treatment of malaria infection in Africa. Some studies have reported the efficacy of the methanolic stem bark extract of this plant, and it has been confirmed that it has antimalarial activity [7,5]. There is also a need to study the antiplasmodial activity of methanolic leaf extract of *A. leiocarpus* since both leaf and stem bark of this plant have been considered to be potent for the treatment of malaria infection locally. Therefore, this

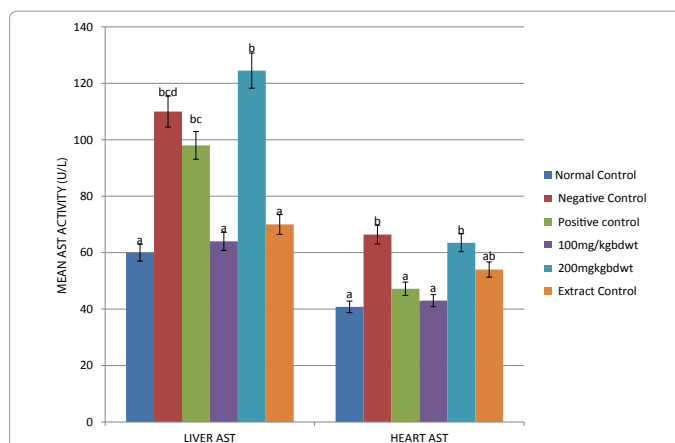


Figure 3: Effect of methanolic leaf extract of *A. leiocarpus* on mean AST activities in liver and heart of mice infected with *P. berghei*. Means carrying different letters are statistically different at $p < 0.05$

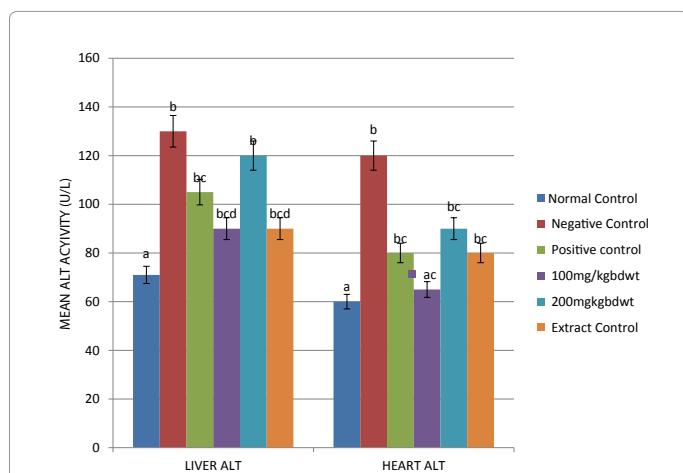


Figure1: Effect of methanolic leaf extract of *A. leiocarpus* on mean ALT activities in liver and heart of mice infected with *P. berghei*. Means carrying different letters are statistically different at $p < 0.05$

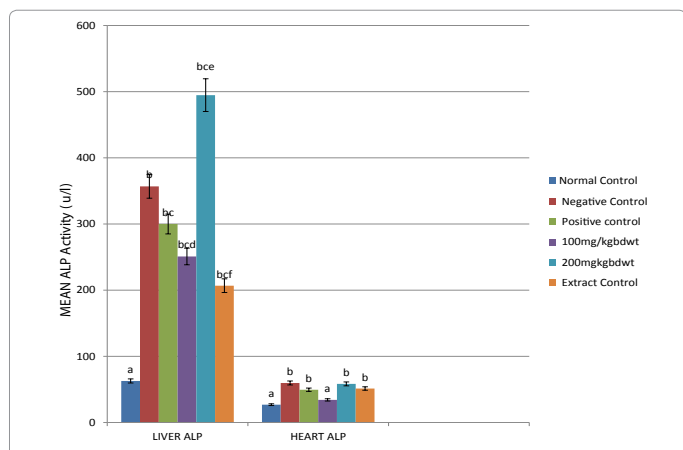


Figure 2: Effect of methanolic leaf extract of *A. leiocarpus* on mean ALP activities in liver and heart of mice infected with *P. berghei*. Means carrying different letters are statistically different at $p < 0.05$

DAY	Negative control	Positive Control of <i>A. leiocarpus</i>	MP+100 mg/ Kgbdwt of <i>A.leiocarpus</i>	MP+200 mg/Kg bdwt
1	107 ± 0.3 ^a	105 ± 0.9 ^a	107 ± 1.2 ^a	06 ± 1.7 ^a
2	112 ± 0.6 ^a	66 ± 1.9 ^b	83 ± 0.9 ^b	66 ± 1.7 ^b
3	117 ± 0.6 ^a	31 ± 1.7 ^b	44 ± 1.1 ^b	35 ± 2.0 ^b
4	119 ± 0.5 ^a	14 ± 1.0 ^b	25 ± 1.3 ^b	16 ± 3.0 ^b
5	125 ± 0.4 ^a	09 ± 0.3 ^b	15 ± 0.1 ^b	10 ± 0.3 ^b

Table 1: Effect of Methanolic Leaf Extract of *A. Leiocarpus* on Parasitaemia Level. The parasitaemia are in percentages (%). The parasitaemia are compared with day 0 which is regarded as 100%. Means carrying different letters are statistically different at $p < 0.05$

work studied the efficacy of methanolic leaf extract of *A. leiocarpus* in the treatment of malaria infection and its effect on the liver and heart function in mice infected with *P. berghei*. The result of this study shows a drastic reduction in the parasitaemia level in day 5 in both groups treated with 100 mg/kgbdwt and 200 mg/kgbdwt of *A.leiocarpus* when compared with day 0. The rate of clearance was higher in the group treated with 200 mg/kgbdwt of *A.leiocarpus* when compared with the group treated with 100 mg/kgbdwt of *A.leiocarpus*. This result concurs with the previous study where the methanolic stem bark of *A. leiocarpus* was used for treatment of malaria [7]. The parasite growth inhibition in positive control was almost similar with the group treated with 200 mg/kgbdwt of *A.leiocarpus* which is an indication that efficacy of *A. leiocarpus* at that concentration can be compared with the arthemeter (positive control).

While advocating for the treatment of malaria infection with the medicinal plants, there is a need to study the concentration at which this plant can function most and also to know the cytotoxicity effect of these plants on some ectopic organs in the body. The malfunction of liver can be detected by studying the level of liver enzymes. Therefore, this work also studied the effect of methanolic leaf extract of *A.leiocarpus* on heart and liver function by considering the activities of liver enzymes in all the groups studied. The liver enzymes activities in the treated groups were compare with normal and negative control. This study shows that there was an increase in the activities of both liver and heart ALT, AST and ALP in the negative and positive controls as well as in the groups treated with 100 mg/kgbdwt and 200 mg/kgbdwt of *A. leiocarpus* when compared with the normal control, though the activities was

not significantly higher in the heart of the group treated with 100 mg/kgbdwt of *A. leiocarpus*. The observed increase in the liver and heart enzymes activities of ALT, AST and ALP in the negative and positive controls and in the group treated with 200 mg/kgbdwt of *A. leiocarpus* when compared with positive control and group treated with 100 mg/kgbdwt of *A. leiocarpus* is an indication that *P. berghei* infection and treatment with high dosage of *A. leiocarpus* may be responsible for liver dysfunction noticed in this study. The elevation in the enzymes activities in the negative control could be as a result of leakage from hepatic cell that were damaged by the immune response or due to activation induced by the parasite during the hepatic stage of the parasite life cycle [17]. While the increase in those enzymes in the treated groups might be as a result of accumulation of free radical generated by the extract used to treat the animals, which may also be responsible for the destruction of the parasite [18,19]. The upsurge in the enzymes activities in the positive control and group treated with 200 mg/kgbdwt of *A. leiocarpus* when compared with the group treated with 100 mg/kgbdwt of *A. leiocarpus* is a reflection of the rate of parasite clearance which was higher in these two groups as compared with the group treated with 100 mg/kgbdwt of *A. leiocarpus*. This shows that the level of toxicity may be responsible for the high rate of parasite clearance by the drug which may be indirectly responsible for a certain level of hepatic impairment as the drug metabolism takes place in the liver. The level of free radicals in the body determines the rate of parasite clearance in the body and this may be determined by the concentration of the antimalarial drug [20]. The deleterious effects of free radicals produced by antimalarial drugs might be responsible for the increase in the ALT, AST and ALP activities observed in this study. Considering the reduction in the level of the indices used to assess liver dysfunction (ALT, AST and ALP) in the group treated with 100 mg/kgbdwt of *A. leiocarpus* as compared with the negative control showed that *P. berghei* infection caused an increase in these enzymes which were reduced drastically by treatment with a 100 mg/kgbdwt of *A. leiocarpus*. The destruction of *P.berghei* at this concentration reduced the liver infection by the parasites and thus ameliorated its negative effect on the liver.

The significant increase in ALT, AST and ALP activities in the negative control and group infected with *P.berghei* and treated with 200 mg/kgbdwt of *A. leiocarpus* when compared with the extract control (Group not infected with *P.berghei* but treated with 200 mg/kgbdwt of *A. leiocarpus*) shows clearly that *P. berghei* infection has a serious influence on the level of the liver dysfunction. The increase in the activities of ALT, AST and ALP in the extract control group as compared with the normal control (Figures 1-3) also showed that the methanolic leaf extract of *A. leiocarpus* at 200 mg/kgbdwt could also contribute to dysfunction of some ectopic organs in the body. Therefore, care should be taken when a higher concentration more than 200 mg/kgbdwt of *A. leiocarpus* is being used for the treatment of malaria infection or other infections in the body.

The significant increase in the ALT, AST and ALP activities in the infected group treated with 200 mg/kgbdwt of *A. leiocarpus* when compared with the group treated with 100 mg/kgbdwt of *A. leiocarpus* showed that the level of liver damage by the treatment with *A. leiocarpus* may be dose related.

This study also showed that the ALT, AST and ALP activities were significantly higher in the negative groups as compared with the normal control. This supports the previous study which showed that liver enzymes increase in the body with the presence of malaria parasite [21,22].

This study showed that methanolic leaf extract of *A. leiocarpus* has

antiplasmodial activity that is equivalent to arthemeter at dosage of 200 mg/kgbdwt, but treatment at this dosage requires serious monitoring because of the very high activities of ALT, AST and ALP at this dosage. It was also observed that methanolic leaf extract of *A. leiocarpus* ameliorated the level of ALT, AST and ALP when treated at 100 mg/kgbdwt

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