

Potential antimalarial activity of Methyl Jasmonate and its effect on lipid profiles in *Plasmodium Berghei* infected mice

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Abstract

Background: The antimalarial activity and lipid profiles of Methyl Jasmonate (MJ) were investigated against established malaria infection *in vivo* using BALB/c mice.

Methods: Arteether (AE) and chloroquine (CQ) were used as reference drugs while ethanol was used as the vehicle for drug delivery for MJ.

Results: Mice treated with 10 and 25 mg/kg MJ showed a remarkable reduction in percentage parasitemia by 68.3% and 78.2% on day 10 (post treatment) respectively while 45.4% and 87.2% reduction in percentage parasitemia were observed in the group treated with 50 mg/kg on day 3 and 10 (post treatment) respectively. The highest mean survival time was observed in CQ followed by AE and MJ in dose-dependent manner. A progressive decrease in packed cell volume (PCV) was observed in infected untreated mice which led to the death of all the mice by day 9 (post treatment). Infected mice treated with MJ showed reduced level of HDL and LDL compared with infected untreated group. As the dose of MJ increased in infected mice cholesterol levels increased while there was reduction in triglyceride.

Conclusion: Overall there was marked decrease in parasitemia in *Plasmodium berghei* infected mice treated with graded doses of MJ but appears to have reduced antimalarial activity compared with CQ and AE.

Keywords: Antimalarial activity, Lipid profile, Methyl jasmonate, Mice, *Plasmodium berghei*.

DOI: <http://dx.doi.org/10.4314/ahs.v15i3.19>

Cite as: Oyinloye OE, Kosoko AM, Emikpe B, Falade CO, Ademowo OG. Potential antimalarial activity of Methyl Jasmonate and its effect on lipid profiles in *Plasmodium Berghei* infected mice. *Afri Health Sci.* 2015;15(3):841-50. doi: <http://dx.doi.org/10.4314/ahs.v15i3.19>

Introduction

Malaria is a major threat to public health and economic development in Africa .Current estimates indicate that at least one million children die of malaria each year in Africa alone.¹ Efforts at eradicating malaria have failed as parasite resistance to the most commonly used and affordable antimalarial drugs are developing rapidly.¹ There is need for discovery of new drugs for the treatment of *P. falciparum* malaria which should be efficacious against drug-resistant strains, provide cure within

a reasonable time (ideally three days or less) safe , suitable for children and pregnant women, have appropriate formulations for oral use and above all, be affordable.² As part of the effort at discovering new antimalarial agents, we evaluated on Methyl Jasmonate, a plant hormone for its therapeutic effect. Methyl jasmonate has been known since 1962 as a fragrant component in the essential oil from flowers of jasmine, *Jasminium grandiflorum*.³ The result of an *in vitro* study demonstrated the antiparasitic potential of Jasmonates using two major human blood parasites, *Plasmodium falciparum* and *Schistosoma mansoni*.⁴ Other studies also showed that Methyl Jasmonate (MJ) exhibited anticancer activity *in vitro* and *in vivo*.⁵⁻⁷

In this study, we evaluated possible *in vivo* antimalarial activity of MJ and its effect on lipid profiles in mice infected with *Plasmodium berghei*.

Materials

Chemicals

Methyl Jasmonate (MJ) was obtained from SERVA

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(Herdelberg). It was dissolved in 95% ethanol. Chloroquine and artheether were obtained from Laborate Pharmaceutical (India) and IPCA (India) respectively. Triglyceride and cholesterol kits were obtained from Vital Diagnostic Spb Ltd, Russia, HDL-cholesterol kit was obtained from Randox Laboratories Ltd United Kingdom while alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), and alkaline phosphatase (ALP) kits were obtained from Vital Diagnostic Spb Ltd, Russia. All other reagents were of analytical grade and purest quality available.

Method

Animals and parasite

Seventy BALB/c mice were obtained from the animal house of the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were housed in group of seven mice per cage, fed with standard mouse cubes (Ladokun feeds, Nigeria, Ltd) and supplied with clean drinking water *ad libitum*. *P. berghei* NK 65 (chloroquine sensitive) used in this study was a donation to the laboratory of one of us (AOG) by Malaria Research and Reference Reagent Resource centre (MR4) USA. The parasites were maintained in animals by serial blood passages in mice.

Study of the course of infection and antimalarial activity

The course of infection following intraperitoneal inoculation of mice was studied in each experimental mouse that received 1×10^7 parasitized red blood cells in 0.2ml inoculums. Thin films were made from the tail vein of infected mice, fixed with methanol and stained with 10% Giemsa stain using standard procedure. Parasitemia was monitored daily and screened under X 1000 magnification using a light microscope. Treatment commenced when parasitemia was established (about 10%). *In vivo* antimalarial activity against *P. berghei* infection in mice was monitored according to Rane's test as described by Elufioye *et al.*⁸ The Rane's test relies on the ability of standard inoculum of *P. berghei* to kill the recipient mouse within 12 days of inoculation. Extension of survival beyond 12 days is regarded as activity.

Study design

Mice weighing between 18 and 30g were distributed into 10 groups of seven animals each. Four groups of animals were uninfected; one group received the vehicle only (0.3ml ethanol) and served as normal uninfected

control group, while the three other uninfected groups received 10, 25 and 50 mg/kg body weight MJ respectively once daily for four consecutive days. Six groups were infected with *Plasmodium berghei* (NK 65 strain); one group was not treated while the remaining five groups were treated with 3 mg/kg arteether (AE), 10 mg/kg chloroquine (CQ), and MJ 10, 25 and 50 mg/kg body weight respectively. The groups that received AE and CQ were administered standard doses for three consecutive days while those groups that received MJ were treated for four consecutive days after infection was established. All administration was by oral gavage. Thin films were made from the blood collected from the tail of infected mice for the first 7 days to determine the percentage parasitemia. Films were made weekly for 28 days. The mice were sacrificed 24 hours after the administration of the last treatment (D4). All the procedures and the protocol conformed to the guidelines of the National Institute of Health (NIH) publication 85-23, 1985) for laboratory animal care and use. Two milliliter of blood was collected into plain bottles, centrifuged for 10 minutes at 3,000g using bench centrifuge and serum were collected to determine ALT, AST, and ALP activities, total cholesterol, triglyceride and high density lipoprotein- cholesterol (HDL-Cholesterol) levels. Liver, kidney and brain tissues were collected into test tubes containing 10% formalin stored at 4°C for histopathological examination.

Biochemical assays

In vitro quantitative determination of ALT, AST and ALP activities, total cholesterol and triglyceride levels in the serum were determined using Vital Diagnostics Spb Ltd kits while RANDOX Laboratories Ltd kit was used to determine high density lipoprotein- cholesterol (HDL-Cholesterol) level. The activities of ALT and AST were determined using Kinetic UV method (IFCC modification). The method is based on change in spectral absorption of NADH when converted to NAD. ALP activity was determined using End point method which is based on the formation of p-nitrophenol after increase in spectral absorption. The p-nitrophenol formed was proportional to the alkaline phosphate activity in the sample. Cholesterol level was determined by hydrolyses of cholesterol esters to free cholesterol by cholesterol esterase and resulted to red quinoneimine production. Triglyceride level was assayed by the chromagen production which is directly proportional to concentration of triglyceride. Supernatant fraction of cholesterol was used to determine HDL-cholesterol

using enzymatic method. LDL-cholesterol was determined using Friedewald's formula.⁹

Statistical analysis

The results were expressed as mean \pm standard deviation (SD) of seven mice per group. Data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS version (15.0) statistical software. Level of significant differences was set at $p < 0.05$.

Results

A progressive increase in percentage parasitemia was observed in infected untreated mice with maximum of

177% increase by day 3 (post treatment) which led to the mice death. AE and CQ gave maximum response in parasitemia reduction on day 3 (post treatment) by 90% and 91.9% respectively while parasitemia reduction on day 3 (post treatment) in 50 mg MJ treated group was 45.4%. The maximum percentage reduction in parasitemia was obtained on day 10 (post treatment) with 87.2%, 78.4% and 68.3% in 50, 25 and 10 mg/kg MJ treated groups respectively. Mean survival time for infected untreated group was significantly reduced due to the *P. berghei* infection, while a doubling effect was observed for AE, CQ and MJ treated groups when compared with infected untreated group (Table 1), (Fig 1).

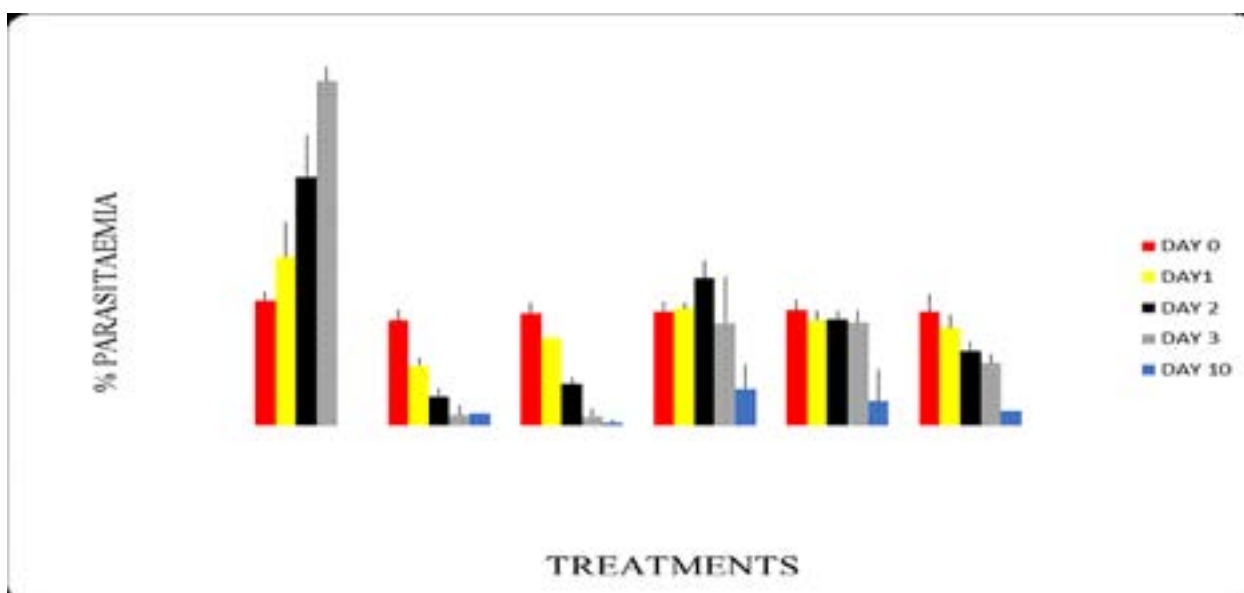
Table 1: Mean survival time of infected treated and infected untreated animals

Treatments Groups	Days
Infected untreated (IU)	9.3 \pm 4.9
P + AE	27.2 \pm 2.3
P + CQ	31.5 \pm 2.5
P+ MJ (10mg)	21.6 \pm 3.4
P+ MJ (25mg)	22.3 \pm 3.1
P+ MJ (50mg)	22.6 \pm 2.9

Values are reported as mean (n=7)

IU = Infected-untreated animals, P + CQ = *P.berghei* Infected animals – treated with 10mg/kg chloroquine, P + AE = *P.berghei* infected animals-treated with 3 mg/kg arteether, P + MJ (10mg) = *P.berghei* infected animals-treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P.berghei* infected animals- treated with 25mg/kg methi jasmonate, P + MJ (50mg) = *P.berghei* infected animals-treated with 50mg/kg methyl jasmonate.

Figure 1 Effect of treatment on percentage parasitaemia



IU= infected - untreated animals, P + CQ = *P.berghei* infected animals – treated with 10mg/kg chloroquine, P + AE = *P.berghei* infected animals – treated with 3mg/kg arteether, P + MJ (10mg) = *P.berghei* infected animals – treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P.berghei* infected animals – treated with 25mg/kg methyl jasmonate, P + MJ (50mg) = *P.berghei* infected animals – treated with 50mg/kg methyl jasmonate. Note that all animals in the infected untreated had died by day 10.

As expected a decrease in packed cell volume (PCV) was observed in infected untreated mice as percentage parasitemia increased. The PCV of groups treated with

AE and CQ were not reduced markedly when compared with infected untreated and MJ treated groups. (Table 2)

Table 2: Effect of MJ, AE, and CQ treatments on packed cell volume (PCV) in *P. berghei* infected and uninfected mice

Days	Infected untreated (IU)	P+AE	P+CQ	P+10mg MJ	P+25mg MJ	P+50mg MJ	control	10mg MJ	25mg MJ	50mg MJ
0	50±3.6	46.6±3.3	42.8±5.6	49±4.2	47±10.5	49.8±3.3	51.4±3.1	47±2.7	49.6±0.9	48±3.1
7	41.5±2.5	40.6±3.7	37.8±5.6	40.2±3.3	44.6±6.9	46.2±4.7	51.4±3.1	48.6±1.9	49.2±1.3	48±3.1
8	33.6±3.6*	42.2±2.1	38.2±4.7	37±2.2	41.4±5.3	40.6±9	50.6±1.3	48.2±2.5	50±0.00	48.2±3.3
9	29.6±5.2*	43.8±3.6	41.1±3.2	33.4±2	35.6±4.7	38.2±7.3	50.6±1.3	48.2±2.5	49±2.2	47.4±4
10	22.8±5.9*	47.4±4	43.3±4.6	28.4±3*	32.8±6.4	35.4±8.3	50.6±1.3	47.8±3.0	49.4±2.5	47.6±4

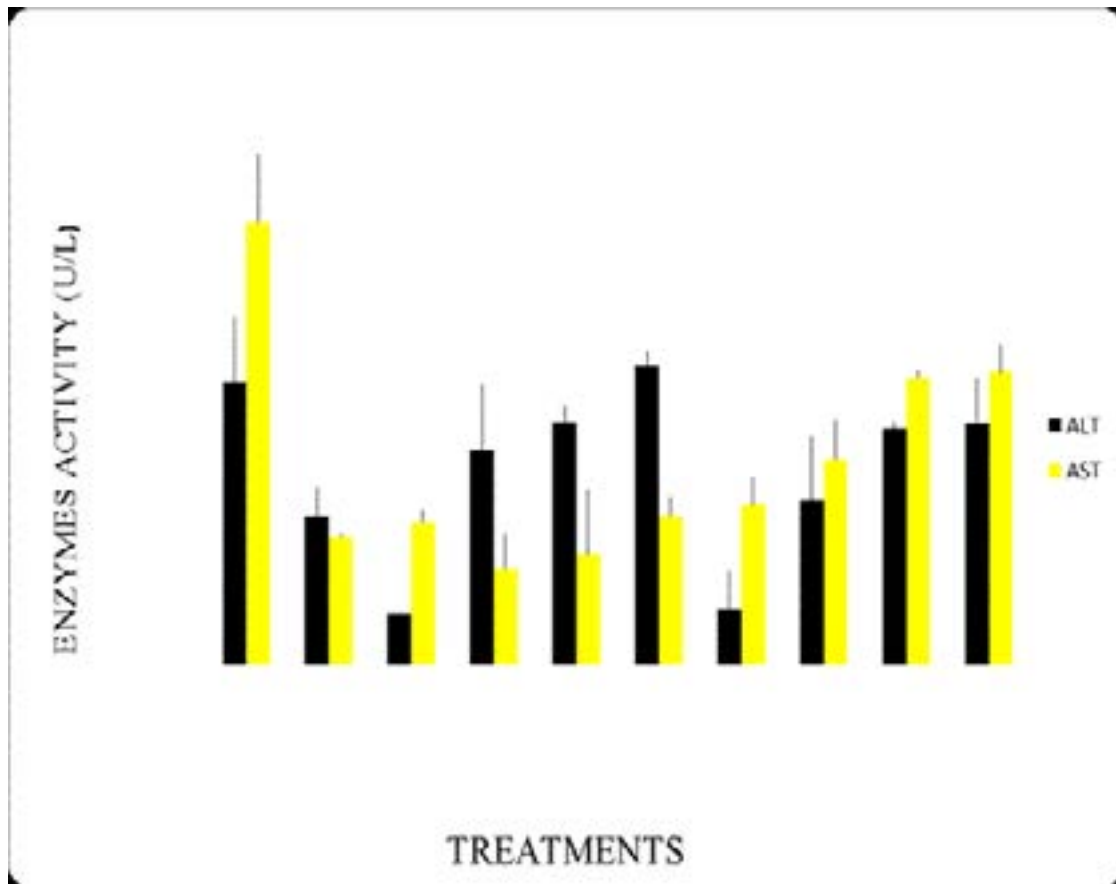
Values are reported as mean± S.D. (n=7) * = Significant when compared with base line PCV on day 0

Asterisk indicate, significant difference from infected untreated($p < 0.05$); IU= *P. berghei* infected - untreated animals, P + CQ = *P. berghei* infected animals – treated with 10mg/kg chloroquine, P + AE = *P. berghei* infected animals – treated with 3mg/kg arteether, P + MJ (10mg) = *P. berghei* infected animals – treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P. berghei* infected animals – treated with 25mg/kg methyl jasmonate, P + MJ (50mg) = *P. berghei* infected animals – treated with 50mg/kg methyl jasmonate, control – administered with ethanol, M J(10mg), MJ (25mg) and MJ (50mg/kg) = uninfected animals administered with 10mg,25mg and 50mg/kg methyl jasmonate, day 7,8,9 and 10=post infection

ALT and AST activity in infected untreated group increased relative to normal control (Fig 2). CQ and AE

inhibited the increase caused by *P. berghei* infection while MJ on the other hand inhibited the increase in AST levels but had no effect on ALT (Fig 2).

Figure 2 Effect of treatment on ALT and AST activity among various groups

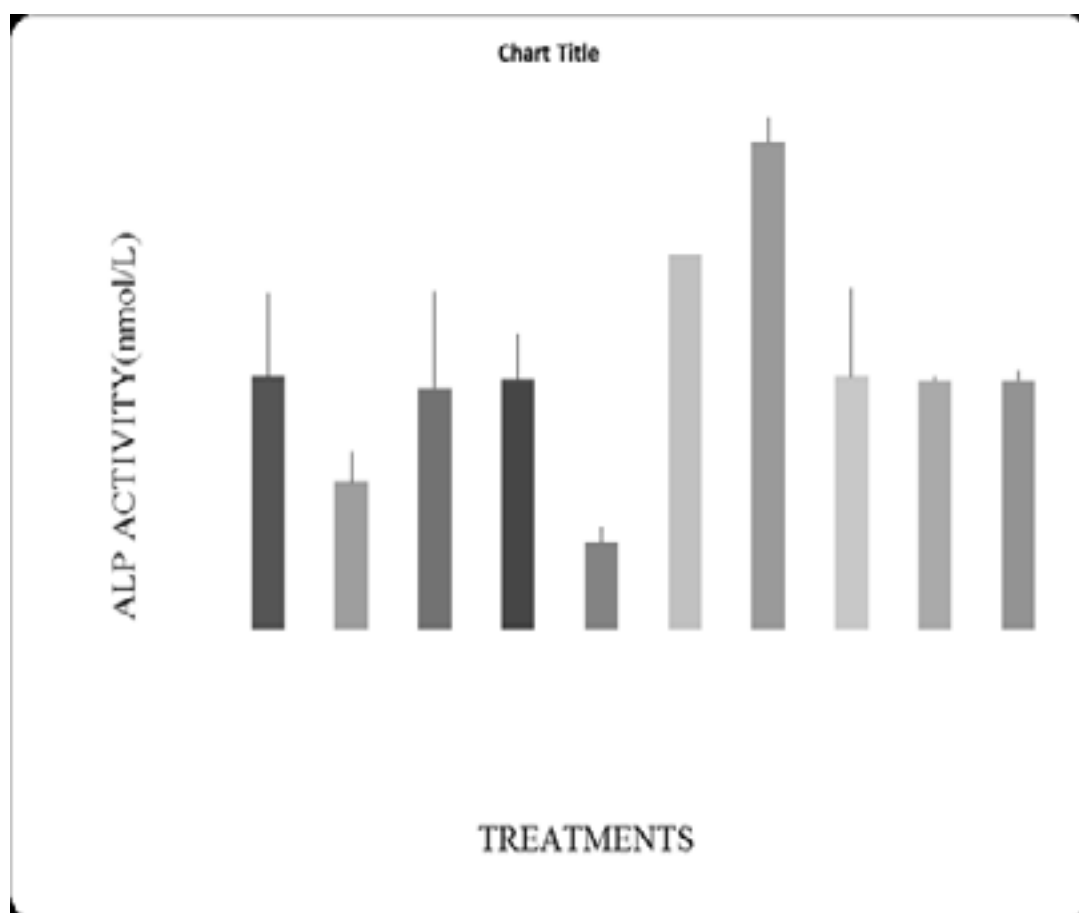


IU= *P. berghei* infected - untreated animals, P + CQ = *P. berghei* infected animals – treated with 10mg/kg chloroquine, P + AE = *P. berghei* infected animals – treated with 3mg/kg arteether, P + MJ (10mg) = *P. berghei* infected animals – treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P. berghei* infected animals – treated with 25mg/kg methyl jasmonate, P + MJ (50mg) = *P. berghei* infected animals – treated with 50mg/kg methyl jasmonate , control – administered with ethanol, M J(10mg), MJ (25mg) and MJ (50mg/kg) = uninfected animals administered with 10mg,25mg and 50mg/kg methyl jasmonate

It was observed that there was increase in ALT and AST activity in uninfected group administered with MJ relative to control(fig 2). Although there was elevation in ALT and AST in uninfected animals administered with MJ but the elevation was not significant ($p > 0.05$)

when compared with infected untreated animals relative to control (Fig 2). ALP in infected untreated group increased markedly compared with infected treated and control groups (Fig 3).

Figure 3 Effect of treatment on ALP activity among various groups

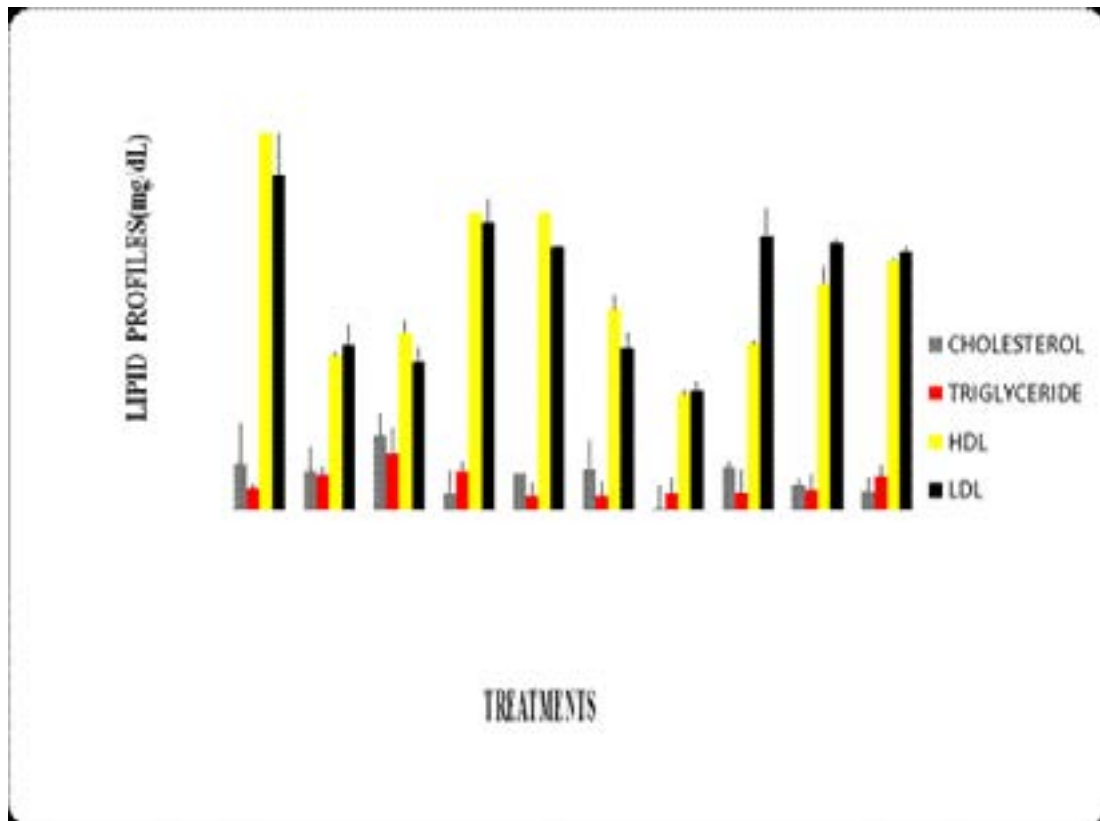


IU= *P. berghei* infected - untreated animals, P + CQ = *P. berghei* infected animals –treated with 10mg/kg chloroquine, P + AE = *P. berghei* infected animals – treated with 3mg/kg arteether, P + MJ (10mg) = *P. berghei* infected animals – treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P. berghei* infected animals – treated with 25mg/kg methyl jasmonate, P + MJ (50mg) = *P. berghei* infected animals – treated with 50mg/kg methyl jasmonate , control – administered with ethanol, M J(10mg), MJ (25mg) and MJ (50mg/kg) = uninfected animals administered with 10mg,25mg and 50mg/kg methyl jasmonate

Infected untreated group had the highest level of HDL and LDL compared with infected groups treated with either AE, CQ and MJ, uninfected and control group (Fig 4). An increase in HDL and LDL was observed in groups administered with MJ in both infected and uninfected animals relative to control (Fig 4). An in-

crease in cholesterol was observed in the groups treated with MJ compared with control but triglyceride levels were similar in MJ treated groups and control. Infected group treated with AE showed a significant increase ($p < 0.05$) in cholesterol and triglyceride in comparison to control (Fig 4).

Figure 4 Effect of treatment on lipid profiles in various groups



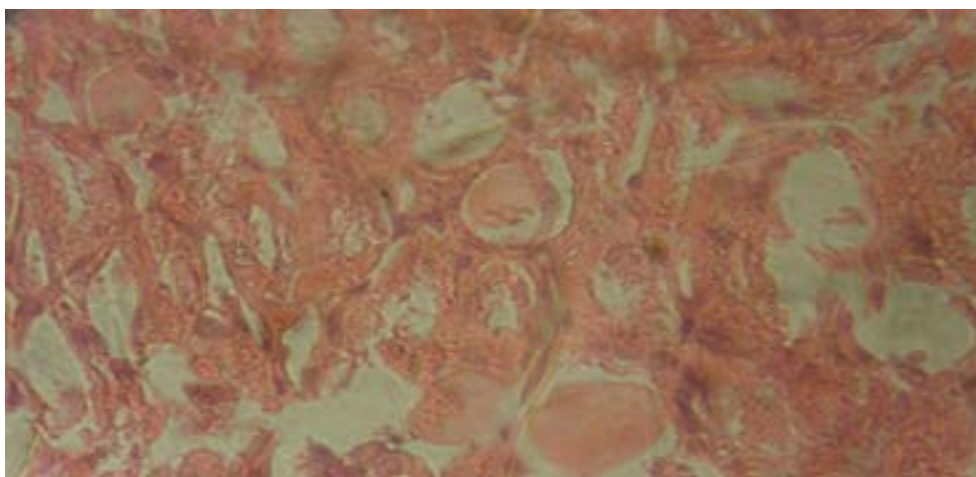
IU= *P. berghei* infected - untreated animals, P + CQ = *P. berghei* infected animals – treated with 10mg/kg chloroquine, P + AE = *P. berghei* infected animals – treated with 3mg/kg arteether, P + MJ (10mg) = *P. berghei* infected animals – treated with 10mg/kg methyl jasmonate, P + MJ (25mg) = *P. berghei* infected animals – treated with 25mg/kg methyl jasmonate, P + MJ (50mg) = *P. berghei* infected animals – treated with 50mg/kg methyl jasmonate, control – administered with ethanol, M J(10mg), MJ (25mg) and MJ (50mg/kg) = uninfected animals administered with 10mg,25mg and 50mg/kg methyl jasmonate

No significant changes in histology of the brain was observed in all the groups relative to control. The kidney of treated and untreated mice were normal except for *P. berghei* infected mice treated with CQ that showed presence of protein cast (Fig 5{a}).

Perivascular mononuclear cell infiltration occurred in the liver of all *P. berghei* infected mice treated and untreated (Fig 5{b}), while necrosis was observed in the liver of infected untreated mice (Fig 5{c}).

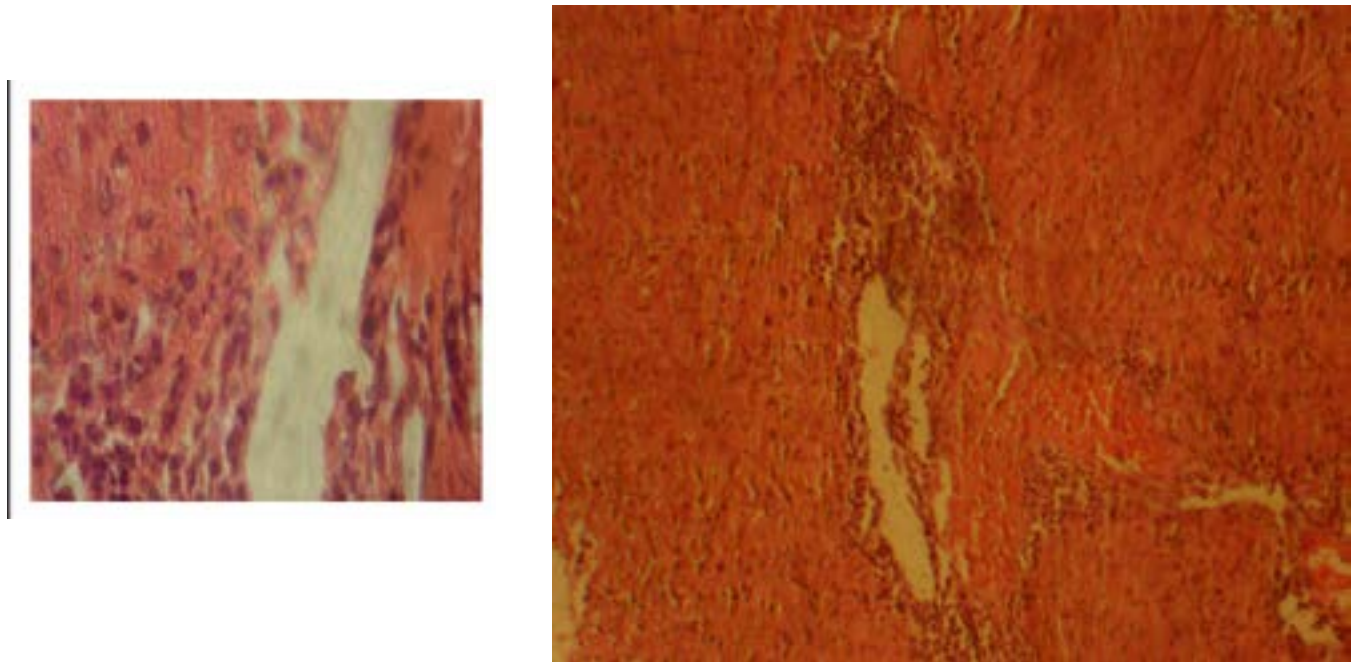
Discussion

Figure 5 (a) Effect of chloroquine treatment in kidney



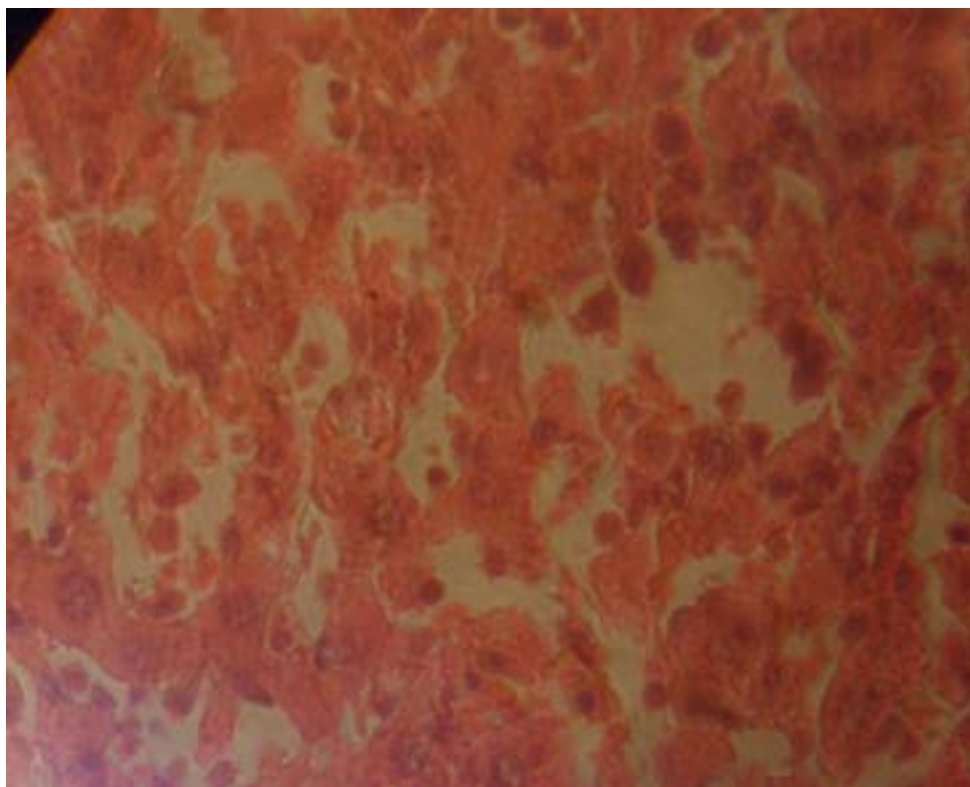
The photomicrograph of the kidney of mice passaged with *P. berghei* and treated with chloroquine 10mg/kg body weight showing protein casts (Arrow), (H&E Stain)

Figure 5 (b) Effect of methyl jasmonate (MJ) 25 mg/kg/body weight in liver



The photomicrograph of the liver of mice passaged with *Plasmodium berghei* ,and treated with 25mg/kg body weight MJ,showing perivascular mononuclear cell infiltration(arrow), (H&E Stain)

Figure 5 (c) Effect of *plasmodium berghei* in the liver of untreated mice



The photomicrograph of the liver of mice passaged with *P.berghei* ,without treatment, showing mononuclear cell infiltration and necrosis (arrow), (H&E Stain)

Antimalarial drug resistance is a major public health problem, which hinders the control of malaria. There is an urgent need to increase the armamentarium against malaria. As part of this effort, we investigated the antimalarial activity of Methyl Jasmonate in *P. berghei* infected mice. Results of our studies demonstrated that MJ exhibited a dose-dependent significant reduction in percentage parasitemia from day 3 (post treatment). MJ at a dosage of 50 mg/kg reduced parasitemia better than 10 and 25 mg/kg doses. However MJ was less effective when compared to CQ and AE for which considerable reduction in parasitemia was observed by day 2 (post treatment). There was however resurgence in parasitemia in AE treated mice on day 10 (post treatment), which is in agreement with the recrudescence that occurs to artemisinin monotherapy.¹⁰

As the percentage parasitemia increased in infected untreated mice, the PCV as expected decreased markedly until the mice died. This is consistent with previous studies.¹¹ A decrease in PCV was observed in infected mice treated with MJ relative to base line PCV before infection while the PCV obtained in uninfected mice administered with MJ were not affected. *P. berghei* caused significant increase in cholesterol and triglyceride in infected untreated mice relative to control. Similarly a significant increase in HDL-C and LDL-C was observed in infected untreated mice in comparison to infected treated and uninfected groups. This finding is in concordance with report of Sherman *et al.*¹² that rats infected with *P. berghei* had elevated lipid profiles.

A significant increase in HDL-C and LDL-C occurred in infected and uninfected mice administered with MJ relative to AE and CQ treated and control groups this may be related to the lipid nature of MJ.¹³ Liver enzymes (ALT, AST and ALP) are used as markers of liver function and can be used for drug safety evaluation.^{14,15} They are involved in intermediary metabolism and present in high concentration in the liver and are released rapidly into the plasma in cases of acute destruction of tissue as in myocardial infarction or hepatocellular necrosis.¹⁵ This study demonstrated significantly high activity of ALT, AST and ALP in infected untreated group relative to control which is an indication of hepatocellular function impairment.¹⁵ MJ inhibited the increase in AST and ALP level but had no effect on ALT in infected mice. The increase in ALT is the most sensitive marker for liver cell damage while AST and ALP elevation may be caused by other disease conditions apart from hepa-

tocellular damage.¹⁶ MJ treatment of uninfected mice however caused elevation in ALT, AST and ALP compared with control.

The changes observed in ALT, AST and ALP levels among the uninfected mice might be due to the vehicle used for Methyl Jasmonate (10% ethanol). Protein cast was observed in the kidney of infected mice treated with CQ. Perivascular mononuclear cell infiltration was observed in the livers of infected treated mice with CQ, MJ 25 and 50 mg/kg, while necrosis was observed in the livers of untreated group. However, livers of infected mice treated with AE were normal, which supports the report of Edington¹⁷. Gilles *et al.*¹⁸ reported that changes in histopathology occur if *P. berghei* penetrate the cerebrum.

Conclusion

It is concluded that MJ has appreciable antimalarial activity in *P. berghei* infected mice although reduced relative to AE and CQ. Administration of MJ in *P. berghei* infected and uninfected mice showed no significant toxicity in the brain, liver and kidney. MJ will be a good candidate for consideration as a new antimalarial agent. Further in vitro and in vivo evaluation and possible structural modification and drug combination will be required.

Acknowledgement

We acknowledge Prof. E.A. Bababunmi of ENHICA International for introducing and donating Methyl Jasmonate for this work.

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