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Original Research Article

Efficacy of artemether-lumefantrine on malaria parasite and its effect on some lipid profile in mice infected with *Plasmodium berghei*

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Artemisinin Combination Therapy (ACT) was introduced by WHO to prevent drug resistance by the malaria parasite, unfortunately ACT treatment failures have been reported in some malaria endemic areas, hence this work studied the antimalarial activity of artemether-lumefantrine, and its effects on some lipid profile in serum and different organs of mice infected with *Plasmodium berghei.* Thirty five albino male mice were divided into seven groups with five mice in each group. Six groups were infected with *Plasmodium berghei* NK 65 intraperitoneally, the seventh group was not infected (normal control). Five of the infected groups were treated with 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg and 2.5 mg/kg artemether-lumefantrine per body weight, while the sixth group was not treated (negative control). The treatment was observed daily for four consecutive days through oral intubation. The animals were sacrificed on the 5th day from the commencement of the treatment and the whole blood was collected into EDTA bottle, while the organs were collected into plain bottle and later homogenized in ice cold normal saline. The parasitaemia was monitored for 4 days of the treatment. The effect of treatment on lipid profile was also determined in serum, liver, kidney and heart. The result showed that there was significant increase (P<0.05) in the parasitaemia counts in the negative control when compared with the groups treated with artemether-lumefantrine. The parasite clearance rate was highest in the group treated with 2.5 mg/kg (87%) and least in the group treated with 1.0 mg/kg (36%). The triglyceride level was significantly higher (P<0.05) in the negative control than in the group treated with 1.5 mg/kg, 2.0 mg/kg and 2.5 mg/kg, while HDL and Cholesterol levels were significantly reduced in the negative control than in the group treated with 1.5 mg/kg, 2.0 mg/kg and 2.5 mg/kg. This study concluded that the efficacy of artemether-lumenfatrine was dose related and has effect on lipid profile.

Key words: Artemether-lumefantrine, malaria parasite, combination therapy, lipid profile.

INTRODUCTION

Malaria is a mosquito borne infectious diseases of humans caused by eukaryotic protists of genus *Plasmodium*. The term "malaria" originates from medieval Italian (WHO, 2014). It is transmitted into human by the bite of infected female anopheles mosquito and it is endemic in tropical and sub-tropical regions including parts of America, Asia and Africa (WHO, 2016). Malaria is essentially a disease of the tropics and subtropics particularly the sub-Saharan

African region but it can also be found in some people in temperate areas due to migration from the tropics. Malaria represents a medical emergence because it may quickly progress to complication and death without prompt and appropriate treatment. Severe disease is caused by *Plasmodium falciparum* while *Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae* are generally responsible for a milder disease that is rarely fatal (Sutherland et al., 2010). Hence, *Plasmodium falciparum* remains a major public health problem in the developing world (WHO, 2016). In 2010, about 3.3 billion people were exposed to malaria and the highest risk was for people living in the Sub-Saharan African where approximately 81% of cases and 91% of death occurred mostly among children under five years of age and pregnant women (WHO, 2013; Akanbi et al., 2012). In 2013, Malaria infection was drastically reduced with estimation of 198 million clinical cases and 584,000 deaths (WHO, 2014). Malaria infection has the ability to activate the immune system which causes the release of reactive oxygen species (ROS) with the potency of inducing oxidative damage and cell destruction (Akanbi et al., 2009; Aja et al., 2015).

Several methods have been adopted for the control and treatment of malaria infection. Recently, WHO has introduced Artemisinin-based combination therapy (ACT) in treating uncomplicated malaria due to its efficacy against malaria parasite and quinine for management of acute or complicated malaria (WHO, 2008). The recent resistance of malaria parasites, especially disease caused by *P. falciparum* to the several synthetic drugs including monotherapy artemisinin derivative of antimalarial drugs in malaria endemic regions has necessitated the call for combination therapy of artemisinin derivatives with any other proved antimalarial drugs (Mishra et al., 2009; WHO, 2015). Artemisinin Combination Therapy was introduced by WHO to prevent drug resistance by the malaria parasite against the artemisinin and its derivatives and their drugs partners, but unfortunately ACT treatment failures have been reported in some places (WHO, 2015).

Though 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, it becomes important 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 locality and this should be based on the efficacy of the medicines against malaria parasites in every country, and there is a serious need for continuous global monitoring and reporting of drugs efficacy and parasite resistance.

Among the combination therapy drugs adopted by WHO is Artemisinin-lumefantrine and it is one of the artemisinin combination therapy well adopted in Nigeria. Artemether-lumefantrine is a combination therapy containing 20 mg artemether/120 mg lumefantrine per tablet, used for treating uncomplicated malaria in patients weighing ≥5 kg (Mutabingwa and Adam, 2013). It is unfortunate that due to limited availability and affordability of orthodox medicine in many tropical countries, the majority of the populations depend on traditional medical remedies (Bankole et al., 2015).

Relationship of serum cholesterol levels and malaria parasites has drawn the attention of various workers. Since it has been shown in in-vitro studies that parasites like *Giardia* and *Entamoeba* can grow in lipid rich media in the absence of serum (Bansal et al., 2005), it would be interesting to determine the mechanism of lipid/cholesterol utilization in malaria infected person. Recent studies have shown elevated levels of lipoproteins like high density lipoproteins (HDL), low density lipoproteins (LDL) and total cholesterol in patients suffering from parasitic infection (Faucher et al., 2005). Though it has been confirmed that patients with malaria often exhibit laboratory abnormalities in lipid profile due to an acute phase response, but little is known about serum lipid profile changes in malaria. The report of transient lipid profile changes in six returning travelers with malaria caused by *Plasmodium vivax* has suggested for the first time that changes in high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) in human serum are related to the lipid metabolism of the parasite (Bansal et al., 2005). It was hypothesized that the malaria parasite uses cholesterol and phospholipids from its host, resulting in a decrease of serum HDL (Bansal et al., 2005).

MATERIALS AND METHODS

Parasites

The parasites used (*Plasmodium berghei* NK 65) was donated by Professsor Ademowo O.G from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, Univerity of Ibadan, Ibadan, Nigeria. The parasites were maintained in animals by serial passage of blood collected from a patent donor mouse to a naïve recipient.

Experimental animals

The experimental animals used for this study (adult Swiss male albino mice) weighing between 18-22 g were obtained from the Animal unit of Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, Univerity of Ibadan, Ibadan, Nigeria. The animals were kept in well aerated wire cages, fed with standard mouse feed bought from Ladokun feed and flour mill, Mokola, Ibadan, Nigeria. The animals were allowed to drink water freely and were kept for two weeks to acclimatize to the new environment before they were infected with malaria parasites (*Plasmodium berghei*).

Acquisition and preparation of drug

The drug (artemether-lumefantrine) was manufactured by

Bliss gvither™ tablets pharmaceutical limited and was obtained from Therapy pharmaceutical strore in Akungba-Akoko Ondo-State, Nigeria. Artemether-lumefantrine was ground into powder and 0.01g of powdered form was weighed and then dissolved in 2ml distilled water to make a treatment solution.

Experimental design

A 4-day suppressive test against *Plasmodium berghei* infection in mice was carried out. Thirty- five mice were distributed into seven groups and each group comprised of five animals. The first group was not infected with *Plasmodium berghei* (normal control). The second group was infected with 0.2ml of the parasite and was not treated (negative control). The third, fourth, fifth, sixth and seventh groups (treatment groups) were infected with 0.2 ml of *P.berghei* and treated with 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, and 2.5 mg/kg of artemether-lumefantrine respectively. All treatment was administered orally once daily by gavage using intubator for four consecutive days. Blood was taken from the tail vein of mice before treatment once a day during the 4 days of treatment for the assessment of daily parasitaemia count.

Blood and organ collection

On the 5th day of treatment mice was stunned using chloroform. Blood sample was collected by heart puncture into an EDTA and plain bottles. The liver, heart and kidney were removed and weighed, and the organs were later homogenized with 5ml of saline water, centrifuged at 5000 mph for 5 minutes. The supernatant was stored in the freezer till when needed. The blood in plain bottle was centrifuged and serum was collected for the determination of lipid profile.

Parasitaemia counts determination

Blood was collected from the tail vein of the infected mice on slide before the treatment for the four days of treatment and on the fifth day before the animals were sacrificed. Both thick and thin smears were prepared on a microscope slide and were labelled accordingly. The thin film was fixed with 30% methanol. Staining was done with Giemsa stain and the slides were viewed under the light microscope with X100 magnification and malaria parasitaemia counts were determined.

Biochemical Determination

Determination of Lipid Profile

Total triglycerides assay

Serum total triglycerides concentration was measured by the Tietze (1990) method, as described in the manual of the Randox Total triglycerides kit (Randox Laboratories Limited, United Kingdom).

Total cholesterol assay

Serum total cholesterol level was measured by the Trinder (1969) method, as described in the manual of the Randox Total cholesterol kit (Randox Laboratories Limited, United Kingdom).

HDL-cholesterol assay

Serum HDL-cholesterol concentration was measured by the NIHCDCS (1992) method, as described in the manual of the Randox HDL-cholesterol kit (Randox Laboratories Limited, United Kingdom).

Statistical analysis

The difference among the groups were analyzed by the one way analysis of variance test and the significant test was done using Microsoft excel 2007 and SPSS 17.0, software (SPSS Inc., Chicago,IL,USA) and descriptive and inferential statistics for this analysis. The result were expressed as Mean± Standard Error (SE), where the ANOVA level of significance was considered as $P<0.05$.

RESULTS

The result showed an increase and decrease in parasite density in the negative control and treatment groups as compared to the initial parasites at day 1. The parasitemia level on day 1 was used as base line and this was compared with the parasitemia level in subsequent days, i.e. from day 2 to day 5 in order to know the level of increase or suppression in parasitemia level. In the negative control the parasite density increased from 706.80±72.93 in day 1 to 1126.00±60.74 at the end of the treatment (Table 1). The parasitaemia count in the group treated with 0.5 mg/kg body weight of artemether-lumefantrine was slightly increased from 321.80±122.89 in day one to 349.60±145.01 in day 4. The results showed that there was reduction in the parasitaemia count in the groups treated with 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, and 2.5mg/kg from day 2 to day 5 when compared with day 1 of the treatment (Table 1). The increase and parasite clearance rate is shown in Table 2. The percentage increase in parasitemia counts in the negative control was 13%, 33% and 57% in day 2, day 3 and day 4 respectively, when compared with day 1. Among the treated groups, the reduction in parasitaemia counts started from the group treated with 1.5 mg/kg to the group treated with 2.5 mg/kg when compared the paarasitaemia counts in the last day of the treatment with the first day (Table 2).

The serum triglyceride level was slightly higher in the normal control (1.37±0.55) than in all the treated groups. The serum HDL and total Cholesterol levels were also slightly higher in the normal control than in all the treated

Table 1. Effect of artemether-lumefantrine on the parasitaemia counts

Concentation	day 1	Day2	Day3	Day4
Negative control	706.80±132.93^{a}	811.80±83.53^{ab}	951.80±106.63^{c}	1126.00±90.74^{d}
0.5mg/kg	321.80±122.89^{a}	334.80±128.17^{a}	352.60±135.28^{a}	349.60±145.01^{a}
1.0mg/kg	3634.00±911.97^{a}	3310.20±849.67^{a}	3166.40±830.23^{a}	2734.60±798.43^{a}
1.5mg/kg	992.20±256.86^{a}	930.60±232.95^{a}	799.00±209.31^{a}	649.80±220.36^{a}
2.0mg/kg	2234.80±352.95^{d}	1763.20±256.14^{c}	1125.40±166.87^{b}	709.80±85.32^{a}
2.5mg/kg	938.60±209.06^{d}	616.00±261.61^{c}	343.80±128.80^{b}	125.80±84.98^{a}

- Means with the same superscripts are not significantly different.
- The means are compared along the column

Table 2. Effect of artemether lumefantrine on the parasite clearance rate

Days	Day 1	Day2	Day3	Day4
Negative control	100	113	133	157
0.5 mg/kg	100	103	109	108
1.0 mg/kg	100	93	79	64
1.5 mg/kg	100	66	63	54
2.0 mg/kg	100	70	45	28
2.5 mg/kg	100	67	37	13

Table 3. Effect of different dosage of artemether-lumefantrine and malaria parasite on some serum lipid profile

Treatments	Triglycerides	Hdl-cholesterol	Cholesterol
Negative control	1.49±0.13^{c}	0.27±0.11^{a}	0.53±0.49^{a}
Normal control	1.37±0.15^{bc}	0.42±0.22^{ab}	0.70±0.53^{a}
0.5 mg/kg	1.16±0.21^{bc}	0.42±0.09^{bc}	0.56±0.52^{a}
1.0 mg/kg	1.31±0.39^{ab}	0.40±0.12^{bc}	0.39±0.48^{a}
1.5 mg/kg	0.94±0.48^{ab}	0.46±0.22^{ab}	0.61±0.69^{a}
2.0 mg/kg	0.93±0.65^{ab}	0.42±0.18^{ab}	0.60±0.55^{a}
2.5 mg/kg	0.93±0.25^{ab}	0.39±0.19^{ab}	0.64±0.49^{a}

- Means with the same superscripts are not significantly different.
- The means are compared along the column

groups (Table 3). There was significant increase (P<0.05) in the serum triglyceride level in the negative control when compared with groups treated with 1.5, 2.0 and 2.5 mg/kg body weight, while serum HDL and cholesterol levels were reduced in the negative control when compared with the treated groups.

The mean heart triglyceride level was significantly higher (P<0.05) in the normal control and negative control (0.81±0.29 and 0.73±0.56), respectively than in all the groups treated with artemether-lumefantrine. While the liver triglyceride level was not significantly higher in the normal control (1.63±0.11) as compared with treated groups, the liver triglyceride level was significantly lower (P<0.05) in the negative control (0.62±0.37) than in all the treatment groups. The kidney triglyceride level was significantly higher in the normal control (1.65±0.13) as compared with the groups treated with 1.5, 2.0 and 2.5 mg/kg (0.98±0.24, 1.30±0.28 and 1.37±0.11), respectively (Table 4).

The heart HDL level was significantly higher in the normal control than in all the treated groups. Among the treated groups, the HDL was significantly lower in the group treated with 1.0 mg/kg than other treated groups. The heart HDL level was significantly lower (P<0.05) in the negative control than in the groups treated with 1.5, 2.0 and 2.5 mg/kg. In the liver, the HDL was significantly lower in the group treated with 2.0 mg/kg than in all other treated groups. The HDL cholesterol was significantly higher in normal control (0.33±0.07) when compared with group treated with 1.5 and 2.5 mg/kg (0.02±0.01 and 0.20±0.13), respectively (Table 5).

The cholesterol level in the heart was significantly higher in the normal control than in all other groups. Among the treated groups, it was higher in the group treated with 2.5 mg/kg than in all other groups. The level of heart cholesterol was lowest in the negative control than in the other groups. In the liver, the cholesterol level was significantly higher in the normal control (0.81±0.03) as

Table 4. Effect of different dosages of artemether lumefantrine on the triglyceride level in the organs of mice infected with *Plasmodium berghei*

Treatments	Hearts	Liver	Kidney
Negative control	0.73±0.26^{bc}	0.62±0.37^{a}	1.57±0.06^{c}
Normal control	0.81±0.19^{ab}	1.63±0.11^{c}	1.65±0.13^{cd}
0.5mg/kg/bw	0.28±0.14^{a}	1.59±0.10^{c}	1.44±0.34^{bc}
1.0mg/kg/bw	1.01±0.19^{b}	1.37±0.47^{bc}	1.53±0.07^{c}
1.5mg/kg/bw	0.44±0.20^{a}	1.33±0.44^{bc}	0.98±0.24^{a}
2.0g/kg/bw	0.32±0.18^{c}	1.66±0.08^{c}	1.30±0.28^{bc}
2.5mg/kg/bw	0.27±0.09^{a}	1.60±0.11^{c}	1.37±0.11^{b}

- Means with the same superscripts are not significantly different.
- The means are compared along the column

Table 5. Effect of different dosages of artemether lumefantrine on the Hdl level in the organs of mice infected with *Plasmodium berghei*

Treatments	Hearts	Liver	Kidney
Negative control	0.24±0.29^{a}	0.34±0.06^{b}	0.21±0.09^{b}
Normal control	0.67±0.12^{bc}	0.27±0.09^{b}	0.33±0.07^{c}
0.5 mg/kg/bw	0.34±0.11^{a}	0.51±0.03^{c}	0.41±0.03^{d}
1.0 mg/kg/bw	0.42±0.21^{ab}	0.34±0.02^{b}	0.44±0.05^{d}
1.5 mg/kg/bw	0.51±0.03^{b}	0.26±0.11^{b}	0.02±0.01^{a}
2.0 mg/kg/bw	0.45±0.16^{b}	0.14±0.06^{a}	0.38±0.14^{cd}
2.5 mg/kg/bw	0.51±0.03^{b}	0.24±0.19^{ab}	0.20±0.13^{b}

- Means with the same superscripts are not significantly different.
- The means are compared along the column

Table 6. Effect of different dosages of artemether lumefantrine on the cholesterol level in the organs of mice infected with *plasmodium berghei*

Treatments	Hearts	Liver	Kidney
Negative control	0.11±0.05^{a}	0.31±0.01^{b}	1.15±0.21^{ab}
Normal control	0.25±0.11^{b}	0.81±0.03^{d}	1.03±0.49^{bc}
0.5 mg/kg/bw	0.13±0.13a^{b}	0.35±0.22^{ab}	1.21±0.25^{b}
1.0 mg/kg/bw	0.13±0.07^{a}	0.38±0.42^{ab}	0.78±0.65^{a}
1.5 mg/kg/bw	0.14±0.06^{a}	0.63±0.13^{bc}	1.50±0.05^{c}
2.0 mg/kg/bw	0.15±0.26^{ab}	0.22±0.10^{ab}	1.13±0.52^{bc}
2.5 mg/kg/bw	0.18±0.02^{b}	0.49±0.22^{b}	1.24±0.07^{b}

- Means with the same superscripts are not significantly different.
- The means are compared along the column

compared with the group treated with 0.5 mg/kg (0.35±0.22), 1.0 mg/kg (0.38±0.42), 2.0 mg/kg (0.22±0.10) and 2.5 mg/kg (0.49±0.22) (Table 6).

DISCUSSION

The use of artemether-lumefantrine as combination therapy for the treatment of malaria infection has become necessary both to meet the challenges of malaria infection and to prevent the parasite resistance to drugs. Despite all efforts to reduce the episode of malaria infections, it still poses a great threat to those living in endemic areas. This may be due to the development of drug resistance to monotherapy and potent drugs by the parasite (Okeola et al., 2010). Combination therapy is believed to slow parasite developing resistance to drug (Bloland, 2001; WHO, 2010). The rationale for the drug combination (artemether lumefantrine) was to combine the benefit of the fast onset of action of artemether with the long duration of action and high cure rate of lumefantrine in a single oral formulation (Lefevre et al., 2001). The rate of parasitaemia suppression

by the artemether-lumefantrine in this study when compared with negative control confirmed that artemether-lumefantrine is highly potent in the elimination of malaria parasite as it has been confirmed by other studies (Chotivanich et al., 2012; Hamed and Grueninger, 2012), hence it may still be maintained as first line of treatment in malaria endemic regions as adopted by WHO (WHO, 2008). Our study also revealed that the efficacy of artemether-lumefantrine is dose related (Table 1and 2), therefore, it is necessary for the patients to adhere to the dosage as it has been advised by the manufacturer.

It has been reported that the level of certain lipoprotein may affect the survival of malaria parasite (Okeola et al., 2010). Increase in triglyceride level with decrease in HDL and cholesterol levels have been reported to support the growth of the malaria parasite (Chotivanich et al., 2012;Krishna et al., 2009 and Akanbi, 2013). The increase in triglyceride level in the negative control than in all the treated groups as discovered in this study may be responsible for the increase in the parasitaemia count in the negative control when compared with the groups treated with artemether-lumefantrine. This agrees with previous study that malaria parasite thrives well in the presence of high level of triglyceride (Chotivanich et al., 2012). The sharp reduction in parasitaemia count among the groups treated with 1.5, 2.0 and 2.5 mg/kg may be a function of triglyceride level in those groups (Table 1). The reduction in HDL and Cholesterol in the negative control as compared with the groups treated with 1.5, 2.0 and 2.5 mg/kg where the parasitaemia counts have been reduced drastically confirmed the previous report that malaria parasite cannot thrive at high concentration of HDL and Cholesterol in the body of organism (Faucher et al., 2005). The increase in the dosage treatment of artemether-lumefantrine from 1.5 mg/kg to 2.5 mg/kg in this study may be responsible for the decrease in triglyceride and increase in HDL and Cholesterol level in this study. Further study could be done to ascertain this. It was also revealed in our study that treatment with artemether-lumefantrine had effect on the organ lipid profile level. The level of heart, liver and kidney HDL, Cholesterol and triglyceride level were found to be higher in normal control than in the treated groups (Table 4, 5, and 6).

CONCLUSION

This study concluded that the effectiveness of treatment of malaria parasite with artemether-lumefantrine is dose related and it was also deduced that the administration of artemether-lumefantrine alters the serum and organs lipid profile.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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