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SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS IN *PLASMODIUM BERGHEI* INFECTED MICE TREATED WITH ETHANOLIC AND AQUEOUS LEAF EXTRACT OF *PHYLLANTHUS AMARUS*

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ABSTRACT
Phyllanthus amarus is a medicinal plant used to treat several disease conditions including malarial infection. The antilipidemic effects of ethanolic and aqueous leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels in *Plasmodium berghei* infected mice is investigated. Forty two (42) adult male Swiss albino mice weighing 20-30g were divided into six (6) groups (n= 7). Group 1: Control (uninfected and untreated), Group 2: Negative control (infected with *P. berghei* and untreated), Group 3: *P. berghei* infected and treated with chloroquine (25mg/kg), Group 4-6: *P. berghei* infected and treated respectively with 150, 300 and 450mg/kg of ethanolic and aqueous leaf extract of *Phyllanthus amarus*. The animals were treated for seven days and sacrificed after an overnight fast on the 8th day by cervical dislocation. Blood samples were obtained by cardiac puncture, centrifuged to obtain serum for biochemical analysis. Data were statistically analyzed using analysis of variance (ANOVA) with significant level at P<0.05. Result shows that *P. berghei* malaria infection significantly (*P*<0.05) increased cholesterol (233.33±12.55mg/dl), but reduced (*P*<0.05) triglyceride levels (59.00±4.16mg/dl) when compared with control values (CHOL:145.67±7.88mg/dl; TAG:162.33±6.96mg/dl). Results indicate that malarial infection causes liver dysfunction. However treatment with *P. amarus* leaf extract and chloroquine restores the serum lipids toward normal control values as seen in this study.

KEYWORDS: *Phyllanthus amarus*; *Plasmodium berghei*; Cholesterol; Triglyceride.

INTRODUCTION
Malaria is a parasitic disease transmitted by the bites of the female Anopheles mosquitoes infected with *Plasmodium* species, four of which infect humans: *Plasmodium falciparum* (the most deadly), *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*.[1] *Plasmodioum knowles*, is a zoonotic and new malaria parasite transmitted from human to human via mosquito without an intermediate host.[2] Malaria is widespread in tropical and subtropical regions of the world and pandemic in Sub-Saharan Africa. In Nigeria, malarial infection is mostly caused by *Plasmodium falciparum* [75%] followed by *Plasmodium vivax* [20%][2] with about 60 million of the total population experiencing malaria attack at least twice a year. The exoerythrocytic stage infects the liver while the erythrocytic stage infects the blood and causes recognizable symptoms associated with the malaria infection. Due to the fact that the liver is a major organ in lipid metabolism, biochemical alterations especially glucose and lipids accompanying the infection have been reported.[3] Today, a variety of anti-malaria medications are available to combat malaria. Traditionally, medicinal plants are therapeutic resources used by residence of the African continent for health care, serving as the raw material for drugs,[4] and providing an alternative strategy in the research for new drugs. There is a rich abundance of plants repute in traditional medicine to possess antimalarial properties. Of such plant is *Phyllanthus amarus*, a plant which belongs to the family *Euphorbiaceae* (the spurge family), with about eight hundred (800) species found in tropical and subtropical countries of the world including Nigeria.[5] Numerous phytochemicals such as alkaloids, flavonoids, tannins, Saponins, anthroquinone and glycosides have been identified in the leaf extract of *Phyllanthus amarus*[6] which may have been purported to carry out the pharmacological activity of the plant. The present study evaluates the effect of ethanolic and aqueous leaf extracts of *Phyllanthus amarus* on serum cholesterol and triglyceride levels in *Plasmodium berghei* infected experimental mice.

MATERIALS AND METHODS

Experimental Animals

The mice used in this study were adult male Swiss albino BALB/c mice weighing between 20-30g. The animals were bred and procured from the Laboratory Animal Centre in the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The mice were housed in plastic cages under standard laboratory conditions and fed with standard mouse feed *ad libitum* with clean tap water. The animals were handled in compliance with the guidelines approved by the institution's Ethics Committee.

Source of *Phyllanthus amarus*

Fresh *Phyllanthus amarus* plant was obtained in Abraka, Ethiope East Local Government Area of Delta State, Nigeria and authenticated in the Forest Research Institute of Nigeria, Ibadan with Herbarium number FHI 109728.

Preparation of plant extracts

Fresh leaves of *Phyllanthus amarus* were washed with tap water, air-dried and ground to powder. Extraction was done with a Soxhlet apparatus with ethanol. The extract obtained was evaporated to dryness using rotary evaporator (Buchi R-210) under decreased pressure. The dried extract obtained was dissolved in distilled water and the volume administered is given by the formula;[6]

V (ml) = D (g/kg) x P (kg)/C (g/ml)
D= Dose used (g/kg b.wt),
P= Body weight (kg),
C= Concentration of the extract (g/ml),
V= Volume of extract (ml) administered.

Preparation of drug

Five hundred milligram (500mg) Chloroquine phosphate was used. Each tablet (500mg) was dissolved in 100ml of distilled water. The mixture is centrifuged to obtain clear chloroquine solution (3mg/ml), which was then administered orally (25 mg/kg) for seven days.[7]

Inoculation with *Plasmodium berghei*

Five (5) *Plasmodium berghei* (NK 65 strain) infected mice (donor) were obtained from the Department of Parasitology, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. Three to four (3-4) drops of parasitized blood is obtained from the cut tail of an infected (donor) mouse. Thereafter, 0.1ml of the infected blood was diluted in 0.9ml of phosphate buffer, pH 7.2. Then the mice were inoculated intraperitoneally with 0.1ml of the parasitized blood containing about twelve thousand (12,000) parasites. Seventy two hours (3 days) later, parasitemia was assessed in all injected mice by making thin blood films from the infected mice, stained with Giemsa stain[8] and viewed under the light microscope at x100 magnification before commencing treatment with the extract and Chloroquin.

Animal Grouping and Administration of extract

Forty two (42) adult male mice obtained were divided into 6 groups (n=7).

Treatment with the ethanolic and aqueous *P. amarus* extract and Chloroquin was done once a day (only in the morning) using orogastric cannula for a period of seven (7) days with 150mg/kg, 300mg/kg and 450mg/kg for the extract and 25mg/kg for Chloroquin.

Group 1: Control
Group 2: Negative control (*P. berghei infected* and untreated)
Group 3: Chloroquine (25mg/kg)
Group 4: *P. berghei infected* + *Phyllanthus amarus* leaf extracts (150mg/kg)
Group 5: *P. berghei infected* + *Phyllanthus amarus* leaf extracts (300mg/kg)
Group 6: *P. berghei infected* + *Phyllanthus amarus* leaf extracts (450mg/kg)

Analysis of sample

Total cholesterol was determined according to the enzymatic method described by Allan et al.1979.[9] Triglyceride was determined according to the method of Fossati and Prencipe, 1982.[10]

Sacrificing of animals and Collection of sample

On the 7th day, the animals were fasted overnight and sacrificed by cervical dislocation on the 8th day. Laparotomy was carried out on each animal to access the internal organs. Blood was collected by cardiac puncture using 2ml syringes and centrifuged to obtain blood sample for serum cholesterol and triglyceride level.

Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis were performed by one way analysis of variance (ANOVA) using the SPSS software (Version 21). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Effect of ethanolic leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels in *Plasmodium berghei* infected mice

The effects of ethanolic leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels is represented in the Fig. 1. From the Fig. 1 below, *Plasmodium berghei* infected mice shows significantly increased **(**$P<0.05^{a}$) serum cholesterol and a significantly decreased **(**$P<0.05^{c}$) triglyceride levels when compared with control. However, treatment with 150, 300 and 450mg/kg of ethanolic leaf extract of *P.amarus* restored the cholesterol and triglyceride levels in *Plasmodium berghei* infected mice toward values obtained in the control and chloroquine treatment group.

Effect of aqueous leaf extract of *Phyllanthus amarus* on serum triglyceride levels in *Plasmodium berghei* infected mice

The effects of aqueous leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels is represented in the Fig. 2. In the Fig. 2 below, *Plasmodium berghei* infected mice shows significantly

increased (P<0.05[a]) serum cholesterol and a significantly decreased (P<0.05[c]) triglyceride levels when compared with control. Also, administration of 150, 300 and 450mg/kg of aqueous leaf extract of *P.amarus* restored the cholesterol and triglyceride levels in *Plasmodium berghei* infected mice toward normal.

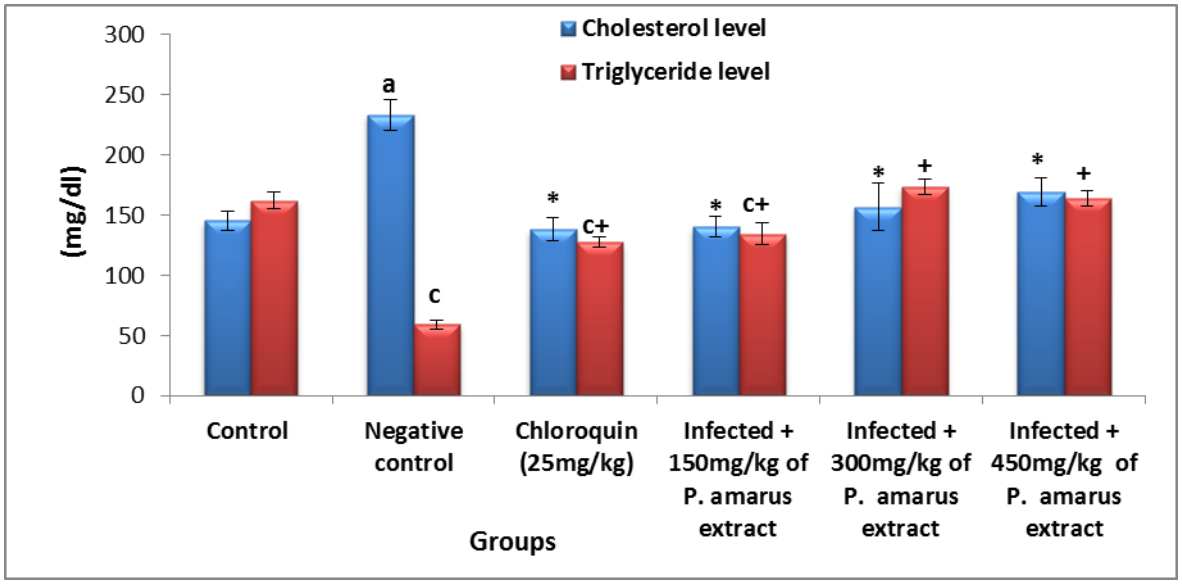

Fig. 1. Showing the effects of ethanolic leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels in *Plasmodium berghei* infected mice.

Values are expressed as Mean ± Standard Error of the Mean (SEM), n=5.

(a) *Significant increase when compared with Control.*

(c) *Significant decrease when compared with Control.*

(*) *Significant decrease when compared with Negative control.*

(+) *Significant increase when compared with Negative control.*

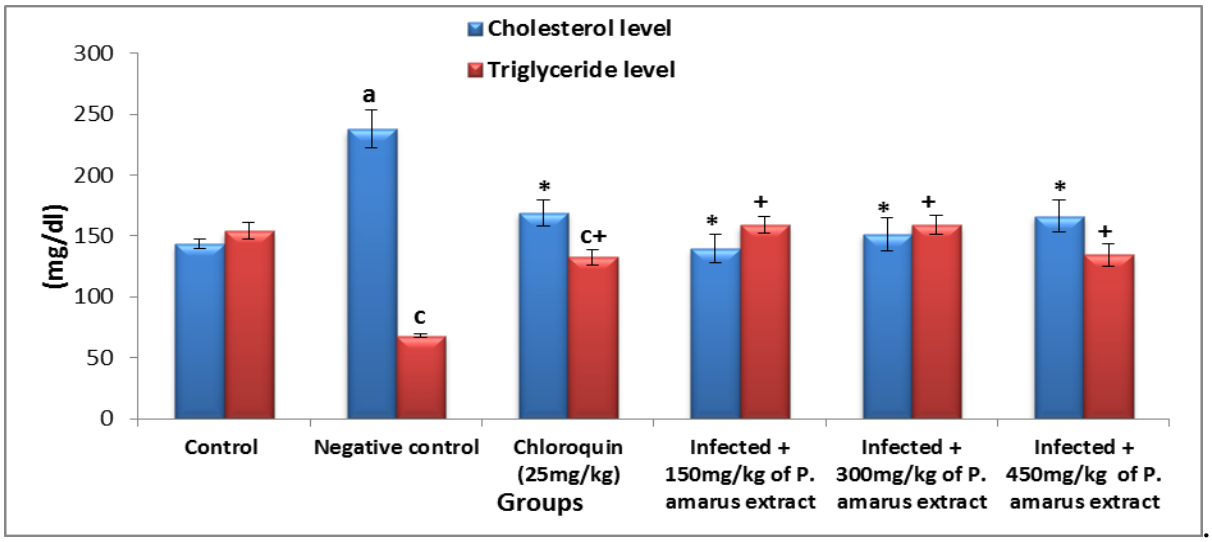

Fig. 2. Showing the effects of aqueous leaf extract of *Phyllanthus amarus* on serum cholesterol and triglyceride levels in *Plasmodium berghei* infected mice.

Values are expressed as Mean ± Standard Error of the Mean (SEM), n=5.

(a) *Significant increase when compared with Control.*

(c) *Significant decrease when compared with Control.*

(*) *Significant decrease when compared with Negative control.*

(+) *Significant increase when compared with Negative control.*

DISCUSSION

Malaria is a debilitating infection in humans which has caused overwhelming morbidity and mortality rates especially in Africa where it is endemic. The *Plasmodium* parasite infects different tissues and organs of the body especially the liver (exoerythrocytic stage)

and erythrocytes (erythrocytic stage) thus causes recognizable symptoms associated with the infection. Lipids have been discovered to play significant roles in pathological changes seen in diseases.[11] However, serum lipids primarily bound to lipoproteins such as cholesterol can be raised by an increase in synthesis or by a decrease removal, which contributes to the hyperlipidemia observed by some pathological conditions. Increase in erythrocyte lipid component in malaria infection has not been correlated to the lipid content of the parasite.[12] However, some notable biochemical changes in lipids accompanying the malaria infection have been reported.[3]

Mice infected with *Plasmodium berghei* and treated with ethanolic and aqueous leaf extract of *Phyllanthus amarus* were studied to assess serum cholesterol and triglyceride levels. Elevated serum cholesterol is a risk factor for atherosclerosis[13] which underlies the development of coronary heart disease.[14-15]

Results show increased serum cholesterol level and a concomitant decrease in triglyceride level in parasitized untreated mice (Fig.1 and 2). These results suggest compromise in the normal functioning of the liver by the *Plasmodium berghei* malaria infection. This observation with experimental mice is consistent with earlier findings which showed increased serum cholesterol in malarial patients compared with apparently healthy subjects.[16-17] The increase in serum cholesterol level in malarial subjects is consistent with the degree of parasitemia.[18] Although, malaria disturbs liver function, there is no evidence of hepatic insufficiency. Hepatomegaly has been observed in malarial infection with congested parasitized red cells, swollen parenchymatous and Kupffer cells,[19] resulting in derangement of cholesterol synthesized by the liver. Increased lipolysis induced by a high threshold of parasitemia promotes synthesis of very low density lipoprotein (VLDL) and their metabolism into low density lipoprotein (LDL), which upon cholesterol incorporation causes the increased overall LDL-cholesterol.[20] It is also likely that the elevation in LDL-cholesterol may be due to decrease breakdown of LDL by the infected liver tissue and a decrease uptake by the infected erythrocytes which is caused by the increased levels of parasitemia.[20]

Results also showed lower triglyceride level in malaria infection experimental mice (Fig.2). This observation may be attributed to higher requirement of lipids by the *Plasmodium* parasite for growth,[21] a relationship observed between lipid synthesis and *Plasmodium* infection. The augmented utilization of the host nutrients by the *Plasmodium* parasite could also contribute to the observed decrease in triglyceride level in malaria infection.[3]

The present study showed that treatment of malaria infected mice with *Phyllanthus amarus* and chloroquine resulted in an increase serum triglyceride level. It is also observed that *Phyllanthus amarus* is a potent anti-malarial herbal medicine, whose anti-plasmodial activities have been reported in earlier studies.[6, 22] This observation is corroborated with previous studies where anti-malarial treatment resulted in an increase in serum triglyceride level.[23,24] Result of the present study showed that extract of *Phyllanthus amarus* has significantly serum cholesterol lowering effect on the increased level of total cholesterol caused by *Plasmodium berghei* infection. The observed low cholesterol lowering effect of *Phyllanthus amarus* may be attributed to the intra-luminal interactive effect of its phytochemicals, especially saponin. Saponins are known anti-nutritional factors which reduce the uptake of certain nutrients including glucose and lipids especially cholesterol across the gut luminal membrane through a physicochemical interaction. Hence Saponins have been reported to have hypocholesterolemic effect.[25] Presence of Saponins has been reported in aqueous extract of *Phyllanthus amarus* and this may explain the hypolipidemic effect observed in this study.[26, 27]

CONCLUSION

This study validates the fact that *Plasmodium berghei* malaria infection increases serum cholesterol level, but reduces serum triglyceride level. However, treatment of *Plasmodium berghei* malaria infection with *Phyllanthus amarus* ameliorated the malaria in a manner likened to chloroquine treatment and causes subsequent reduction in cholesterol and an increase in triglyceride level.

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