Comparative Clinical Pathology
https://doi.org/10.1007/s00580-018-2780-8

ORIGINAL ARTICLE

Evaluation of antimalarial and biochemical profiles of Abaleria® in *Plasmodium berghei*-infected mice

Abiodun Humphrey Adebayo[1] · Omolara Faith Yakubu[1] · Jacob O. Popoola[2] · Lawrence Chibuike David[1] · Gloria Okenze[1] · Amarachi Grace Agbafor[3] · Olajuwon Okubena[4]

Received: 6 February 2018 / Accepted: 19 June 2018

Abstract

The rate of increasing resistance to most antimalarial drugs suggests a need for better alternatives. Hence, the present study evaluates the in vivo antimalarial and biochemical profiles of a locally formulated herbal antimalarial therapy, Abaleria® on mice infected with *Plasmodium berghei*. Eight groups of five mice each were used. The control groups include uninfected, infected with 1.0×10^7 *P. berghei* parasites but not treated, infected, and treated 3 days after inoculation with 25 mg kg^{-1} chloroquine diphosphate (CDP). Other groups were infected and treated with 50, 100, 200, 300, and 500 mg kg^{-1}/day of Abaleria® for 4 days. On the 5th day, blood smears were prepared and evaluated for parasitemia microscopically, and animals were thereafter sacrificed; serum obtained from blood samples collected through cardiac puncture was used for biochemical assays. There was a significant ($p < 0.05$) reduction in parasitemia at the highest dose of the drug which compared favorably with CDP. Infection led to elevated liver function indices while treatment with Abaleria® normalized these parameters; a dose dependent increase in HDL-cholesterol was detected in the groups treated with Abaleria® and CDP. The study shows that Abaleria® displayed a dose-dependent in vivo antiplasmodial and biochemical properties as well as improvement of lipid profiles of mice infected with *P. berghei.*

Keywords Abaleria® · Malaria · In vivo antimalarial studies · *Plasmodium berghei* · Biochemical profiles

Introduction

Malaria disease is among the most fearful that is killing people around the globe (Abdulelah et al. 2011; Ojurongbe et al. 2015; Somsak et al. 2016a). In sub-Sahara Africa, it remains a major public health problem affecting over 90% of the populace (WHO 2012; Somsak et al. 2015). Recent estimates indicate new instances of malaria infection around the world ranging from 149 to 303 million cases with 88% of such cases in Africa, 10% in South-East Asia, and 2% in East Mediterranean region (WHO 2014). Also, over 400,000 deaths caused by malaria were reported in 2015, and 90% of these deaths occurred in Africa where malaria is endemic (White et al. 2014; Somsak et al. 2016b). Malaria is regularly found in over 100 developing countries accounting for about 45 million disability-adjusted life years (DALYs) (Zofou et al. 2014). The disease originates from the *Plasmodium* species and transmitted to the victims by a bite of female Anopheles mosquito (Somsak et al. 2016a).

Malaria control over the years has hinged on two main approaches including effectiveness in case management and controlling vector mosquito (Nicholas 2004; Zofou et al. 2014). However, the strategies are not without limitation. For instance, the case management is limited in the provision of drugs due to side effects, resistance, or inadequate compliance (Zofou et al. 2014; Somsak et al. 2015). Several reports and surveys indicated one of the highest impediments in fighting malaria is its resistance to drugs (Barik 2015; Moghadamtousi et al. 2015). In addition, the challenges encountered in producing effective

✉ Abiodun Humphrey Adebayo
abiodun.adebayo@covenantuniversity.edu.ng

[1] Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria

[2] Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria

[3] Department of Biochemistry, College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

[4] Health Forever Ltd, Ikeja, Lagos, Nigeria

Published online: 28 June 2018

vaccines along with the aftermath of the available antimalarial drugs emphasizes the demand for more acceptable and innovative antimalarial drugs for both its prevention and cure (Ramazani et al. 2010; Zofou et al. 2014; Ojurongbe et al. 2015). Globally, there are increased chloroquine resistance (Gonçalves et al. 2014) to which alternative new and potent antimalarial therapies are needed. Several reports have shown that three quarter of the whole world depend on alternative treatment such as herbal formulations as their principal source of treatment (Petros 2011; Maroyi 2013; Adebayo et al. 2017). In Africa, where malaria is mostly endemic, it has become a common practice to use herbs in treating the disease. Common antimalarial drugs such as quinine and artemisinin were either developed by using their chemical structures as templates or obtained from plants (Ajaiyeoba et al. 2006; Ojurongbe et al. 2015). Recent research has focused on plant secondary metabolites and/or plant derived products which are selected based on their ethnobotanical applications (Nawal et al. 2012; Somsak et al. 2016a). This study explores a locally formulated antimalarial herbal drug known as Abaleria® for the treatment of chronic and drug resistant malaria. Abaleria® essentially consists of extracts from *Caesapinia bonduc* and *Calotropis procera*. The pharmacological activities of the two plants including antimalarial, antiinflammation, antimicrobial, antiparasitic, antidiabetic, antihyperlipidemia, hepatoprotective, and anticancer activities have been reported at various times (Alencar et al. 2004; Kannur et al. 2012; Kanerkar et al. 2015). Extensive phytochemical evaluations of the species revealed the presence of polyphenols, alkaloids, flavonoids, essential oils, cyclopeptides, and kaempferol (Ogunlana et al. 2015). Several studies have shown that these phytochemicals possess antimalarial activities (Ovenden et al. 2011). Therefore, this study aims to revalidate the indigenous claims of the use of *Caesalpinia bonduc* and *Calotropis procera* plants as compounded in Abaleria® to treat malaria, and this is being investigated in *Plasmodium berghei*-infected mice.

Materials and method

Drug and reagents

Reagents used were purchased from Randox laboratories (Crumlin, UK) unless otherwise stated. Abaleria® herbal formulation was obtained in syrup form from a Pharmaceutical outlet at Ikeja, Lagos, Nigeria.

Experimental animals

Four-week-old Swiss albino male mice were procured from the National Institute for Medical Research (NIMR), Lagos, Nigeria and fed with standard rat feed (Graceland feeds) and water ad libitum. The animals were kept in aerated wooden cages in an environment with average temperature of 25 °C. All experimental animals were handled following standard protocols officially accepted by the Research ethics committee of the Biological Science Department of Covenant University, Ota, Ogun State, Nigeria.

Malaria parasites

The strain of *Plasmodium berghei* (NK-65) parasite was obtained from National Institute for Medical Research (NIMR), Lagos, Nigeria and sustained via constant intraperitoneal reinfestation of parasitized erythrocytes which were sourced from a donor-infected mouse by the tail via a heparinized syringe and made up to 20 mL with normal saline. The animals were inoculated with 0.2 mL of infected blood suspension having 1.0×10^7 parasitized erythrocytes intraperitoneally on day 0. All the infected mice with 5–7% parasitemia were further assigned into groups of five mice each and observed carefully for any changes in behavior and appearances.

Experimental design

Forty mice were used and assigned into eight groups of five mice each. Group 1 was not infected but administered normal saline (normal control), group 2 was infected with standard intraperitoneal inoculums of 1.0×10^7 *P. berghei* parasites via a 1-mL disposable syringe and administered 0.2 ml of normal saline (negative control), group 3 was infected and treated with chloroquine diphosphate (CDP) (positive control) (25 mg kg^{-1} bw/day) for four consecutive days while the remaining five groups were also infected and orally administered the different doses of Abaleria® (50, 100, 200, 300, and 500 mg kg^{-1} bw/day) for four consecutive days. In determining the parasitemia, blood smears were collected on the fifth day, their films fixed in methanol and stained with Giemsa and thereafter examined microscopically.

Biochemical assay

The animals were fasted over night after treating with the last dose and were sacrificed by cervical dislocation. Blood samples were obtained via cardiac puncture under mild anesthesia using diethyl ether and collected in labeled lithium heparin sample bottles. The liver was also excised and homogenized. Serum obtained from each blood sample collected from the mice were used to ascertain the activities of aspartate aminotransferase (AST) (Bergmeyer et al. 1986a), alkaline phosphatase (ALP) (Bergmeyer et al. 1986b), alanine aminotransferase (ALT)(Tietz et al. 1983), bilirubin (Doumas et al. 1971), and albumin (Doumas et al. 1973), while the liver homogenate was used to determine the level of triglycerides (Doumas et al. 1973), total cholesterol, HDL-cholesterol, LDL-cholesterol (Zoppi and Fellini 1976).

Statistical analysis

Data obtained were presented as mean ± standard error of mean (SEM) and analysis of variance (ANOVA) was used for group comparisons with the Turkey's post hoc test. Values with $p < 0.05$ were considered statistically significant.

Results

Estimation of percentage parasitemia

Percentage parasitemia decreased significantly ($p < 0.05$) in all treatment groups when compared with the parasitized untreated group (Table 1). The infected group treated with 300 mg kg^{-1} bw of Abaleria® showed a significant decrease in the percentage parasitemia when compared with both the parasitized untreated and parasitized and treated with CDP. Percentage parasitemia count after treatment showed that the treatment groups of 50, 300, and 500 mg kg^{-1} bw Abaleria® exhibited 21.95, 10.18, and 18.29% respectively whereas the drug control group (CDP) showed 25% parasitemia count after treatment (Table 1).

Biochemical studies

There were increases in the level of alanine aminotransferases in the groups treated with 100, 200, and 300 mg kg^{-1} bw of Abaleria® although not significant while no changes was observed for aspartate aminotransferases activity. Alkaline phosphatase activity increased significantly ($p < 0.05$) in the infected group treated with CDP, 50, 300, and 500 mg kg^{-1} bw of Abaleria®. Albumin decreased in the CDP-treated group when compared with the uninfected group while a significant increase ($p < 0.05$) was observed in the group administered 300 mg kg^{-1} of Abaleria® (Fig. 1). LDL cholesterol level decreased significantly ($p < 0.05$) in all groups when compared with the uninfected control group; similarly, a significant decrease was observed in the group administered with 200 mg kg^{-1} of Abaleria® when compared with untreated group. However, a significant increase in HDL-cholesterol was observed in the 500 mg kg^{-1} of Abaleria® as compared with the uninfected, infected untreated, and with the CDP-treated group (Fig. 2). A rise in triglyceride was observed in the negative control group (infected untreated); however, all treated groups (both with CDP and Abaleria®) showed significant ($p < 0.05$) reduction in triglycerides levels as compared with the normal control (Fig. 2).

Discussion

Malaria still remains an important health issue in most African countries with respect to the number of people affected, levels of morbidity, and mortality (WHO 2016). Exploring of new plant-derived antimalarial drugs has increased after the success of artemisinin. However, reported and documented cases of resistance to these drugs including artemisinin made the research and development of new antimalarial drugs inevitable (Wichmann et al. 2004). Increased use of herbal plants and formulations to treat malaria among other diseases has led to the need for scientific investigation and documentation of plants that possesses anti-plasmodia and/or antimalarial activities in a bid to validate the claims for their use in folklore (Adebayo and Krettli 2011). Plants are major sources of bioactive compounds with potential for developing new and original antimalarial drugs (Newman et al. 2003). Hence, this study focused on evaluating the antimalarial and biochemical profiles of Abaleria®, an herbal mixture used in the treatment of malaria, on mice infected with *Plasmodium berghei*. The treatment of the infected mice with CDP and Abaleria® significantly ($p < 0.05$) dropped the parasite count in the infected

Table 1 Effect of parasitemia counts following treatment with Abaleria®in mice infected with *P. berghei*

Parameters	Pre-treatment parasite count	Post-treatment parasite count	% Parasitemia count after treatment
Uninfected + no treatment	0.00 ± 0.00	0.00 ± 0.00	–
Infected + no treatment	6.60 ± 0.68	6.40 ± 0.51	96.97
Infected + CDP	7.20 ± 0.97	1.80 ± 0.86	25.00
Infected + 50 mg kg^{-1} bw Abaleria®	8.20 ± 1.02[a]	1.80 ± 1.36[b]	21.95
Infected + 100 mg kg^{-1} bw Abaleria®	7.00 ± 0.70[a]	2.60 ± 0.51[ab]	37.14
Infected + 200 mg kg^{-1} bw Abaleria®	8.80 ± 1.02[a]	2.40 ± 1.03[b]	27.27
Infected +300 mg kg^{-1} bw Abaleria®	7.40 ± 0.93[a]	0.80 ± 0.37[b,c]	10.18
Infected + 500 mg kg^{-1} bw Abaleria®	8.20 ± 1.39[a]	1.50 ± 1.39[ab]	18.29

Values are expressed as mean ± SEM of eight replicates. *CDP* chloroquine diphosphate

$p < 0.05$, significantly different from the control groups [a] $p < 0.05$ as compared with uninfected + no treatment group, [b] $p < 0.05$ as compared with infected + no treatment group, [c] $p < 0.05$ as compared with infected + CDP group

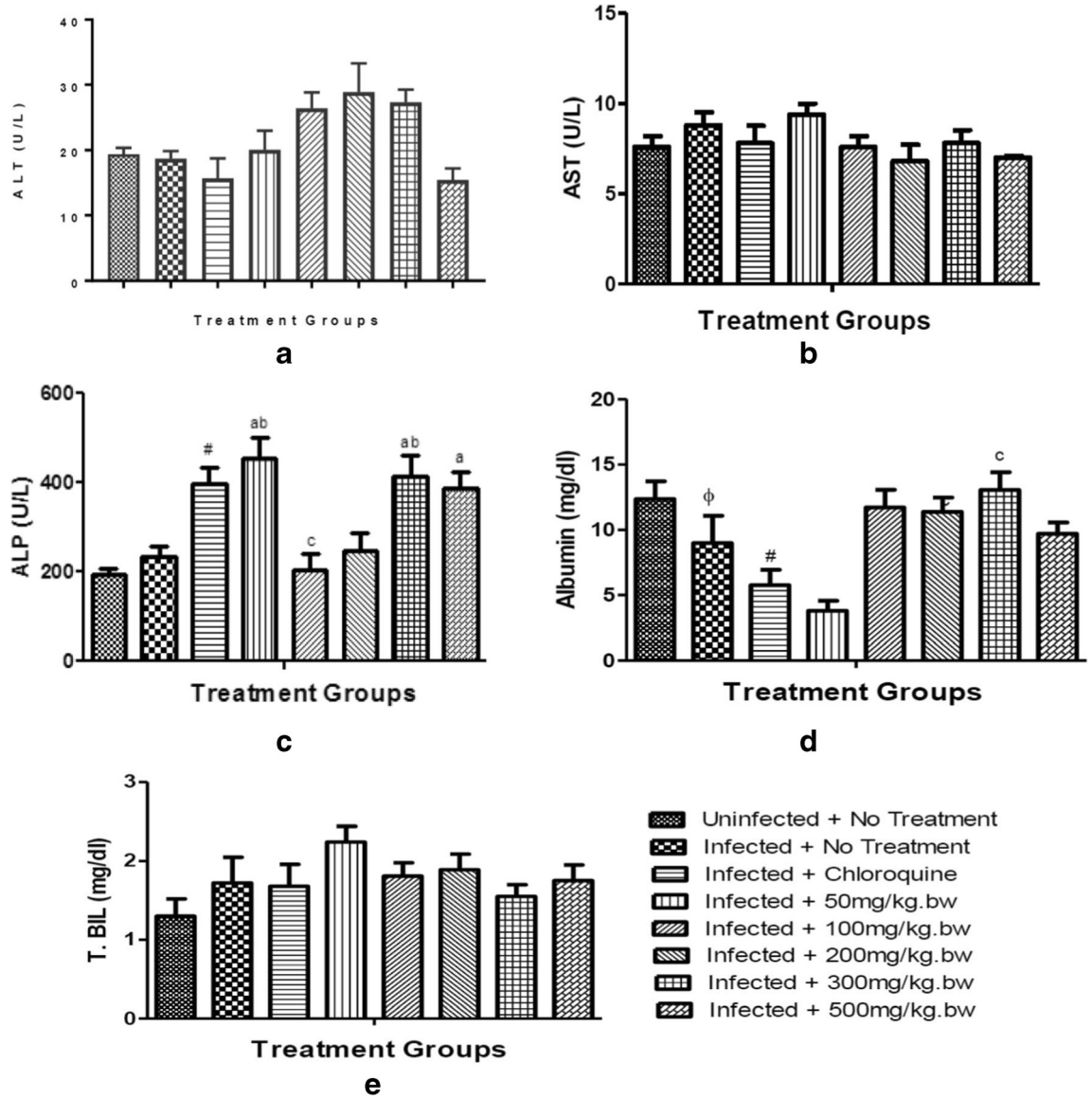

Fig. 1 The effect of Abaleria on liver functions parameters in mice. **a** ALT alanine aminotransferase. **b** AST aspartate aminotransferase. **c** ALP alkaline phosphatase. **d** Albumin. **e** Bilirubin. Values are presented as mean ± SEM of eight replicates. $p < 0.05$, significantly different from the control groups; $^{a}p < 0.05$ as compared with uninfected + no treatment group, $^{b}p < 0.05$ as compared with infected + no treatment group, $^{c}p < 0.05$ as compared with infected + CDP group. $^{\#}p < 0.05$, $^{\phi}p < 0.05$ as compared with uninfected + no treatment group; *CDP*, chloroquine diphosphate

mice when compared with the infected untreated group (Table 1). The significant ($p < 0.05$) reduction in parasite count by Abaleria® was found to be dose-dependent and also in conjunction with previous study that proved the dose-dependent effect of some antimalarial herbs (Ogbonna et al. 2008) and consistent with its folklore use as a herbal treatment for malaria in West, East Southern Nigeria (Adebayo and Krettli 2011). The herbs activity could be accrued to the phyto-constituents contained in them as flavonoids, alkaloids, and anthraquinones which have been reported to display antiplasmodia activity (Philipson and Wright 1990).

Serum levels of AST and ALT normally gives a clue to the functional status of the liver which is the primary organ affected when malaria parasites are transmitted into a host system; they infect the red blood cells as they incubate in the liver which leads to alterations in the flow of blood through the organ as parasitized red blood cells cling to endothelial cells thus obstructing the intra-hepatic blood flow. There will also be impaired bilirubin transport arising from hepatocyte necrosis. All these will affect the liver's function indices as well as enzyme activities (Viriyavejakul et al. 2014). Some of the above changes were observed in this present study as infection led to elevated AST and ALT activities in the serum although not significant when compared to the normal control (Fig. 1); such increases could be as a result of damaged or altered liver membranes. This is in conjunction with the

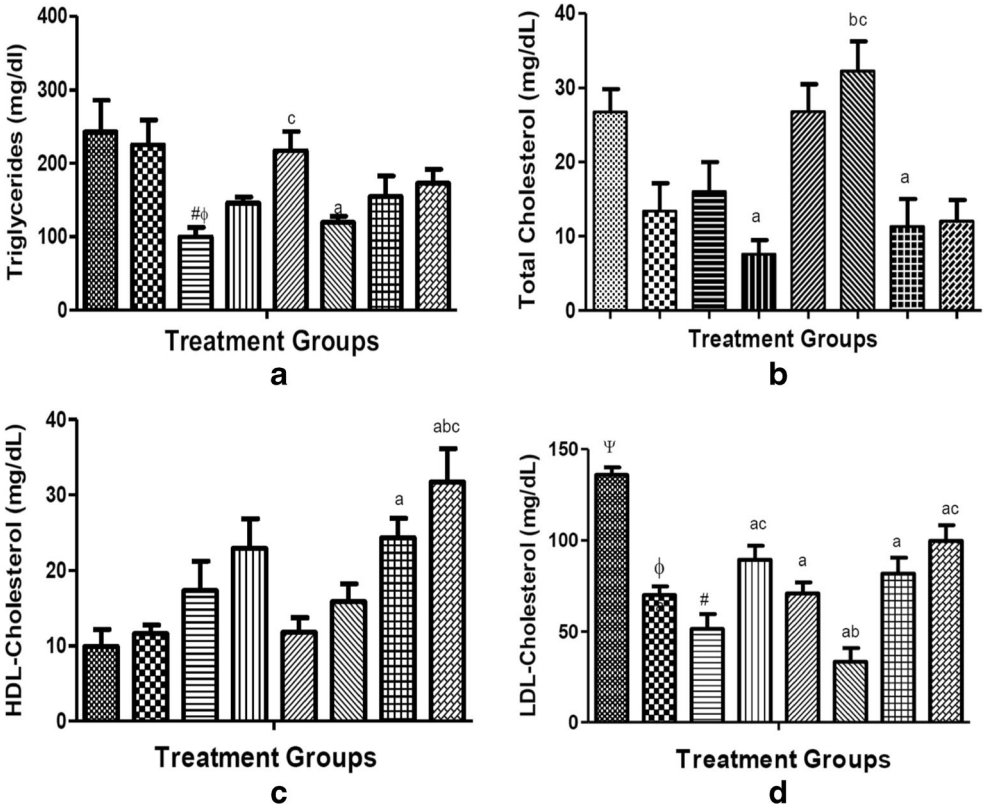

Fig. 2 The effects of Abaleria on lipid profile parameters in liver homogenate. Values are presented as mean ± SEM of eight replicates. $p < 0.05$, significantly different from the control groups; $^{\Psi}p < 0.05$, $^{\#}p < 0.05$, $^{a}p < 0.05$ as compared with uninfected + no treatment group, $^{\phi}p < 0.05$, $^{b}p <$ 0.05as compared with infected + no treatment group, $^{c}p < 0.05$ as compared with infected + CDP group; *CDP*, chloroquine diphosphate

results of Fabbri et al. (2013) where he gave an account that a lot of malaria patients show elevated levels of serum activities of AST, ALT, and ALP which indicates liver damage. ALP, a biomarker of obstructive jaundice and liver disease, was found to be significantly increased ($p < 0.05$) (Fig. 1) in the groups treated with CDP, 50, and 300 mg kg^{-1}; this suggests that the observed damaged or altered liver membrane might be linked to biliary obstruction of the liver. An increase in serum ALP may be as a result of enhanced synthesis of the enzyme to increase biliary pressure (Iweala and Odidoa 2009). The highest dose of the drug (500 mg/kg) reduced the activities of ALP to the level of the standard antimalarial drug, CDP. Concentration of serum bilirubin is a common feature linked to liver damage and hemolysis of liver cells (Beckwith et al. 1975). In this study, there was an increase in the concentration of total bilirubin in the infected control group although not significant when compared to the control. The total bilirubin levels were also observed to increase in all treated groups suggesting symptoms of jaundice.

It has been reported that lipids play keypart in the pathological changes associated with disease conditions (Adekunle et al. 2007). This study also reveals the impact of Abaleria® on lipid profile in mice infected with *P. berghei*. A rise in triglyceride has been associated as a risk factor for artherosclerosis; this was observed in the negative control group (Fig. 2) (Akanbi 2013). However, all treated groups (both with CDP and Abaleria®) showed significant ($p < 0.05$) reduction in triglycerides levels as compared with the normal control; this implies that Abaleria® can help improve the lipid profile of malaria patients as an increase in triglycerides could indicate impaired metabolism of chylomicrons (Krishna et al. 2009). Akanbi (2013) reported that hyperlipidemia is one of the signs of malaria infection and could diminish natural antioxidants in the body. Malaria parasite has been reported to cause elevated levels of triglyceride and reduction in HDL-cholesterol concentration in man (Mohanty et al. 1992; Akanbi et al. 2012) and that HDL-cholesterol could be ascertained by the parasite load in an organism (Akanbi et al. 2012).A dose-dependent increase in the HDL-cholesterol was detected in the groups treated with Abaleria®, and also an increase was observed in the positive control treated with CDP as compared with the negative control (Fig. 2); these suggests that Abaleria® has the potential to boost HDL-cholesterol level in vivo. An increase in HDL-cholesterol in all treated groups could be due to the decrease in parasite loads in these groups. This is in conjunction with the work as reported by Akanbi (2013) where he also observed an increase in HDL-cholesterol in the infected mice treated with a standard antimalaria drug. The significant increase in HDL-cholesterol in the groups administered 300 and 500 mg kg^{-1} of Abaleria® as compared with the negative control indicates that Abaleria® has the ability to boost *good cholesterol* thus capable of preventing an infected person from artherosclerosis. The level of LDL-cholesterol was significantly ($p < 0.05$) reduced in the negative control as compared with the normal control (Fig. 2).

This reduction could be as a result of the high parasite load in the negative control (Table 1). This is in line with the previous study where it was reported that increase in malaria parasite can reduce LDL-cholesterol level in man (Mohanty et al. 1992). In addition, malaria infection has been linked to reduced serum levels of both LDL and HDL cholesterol (Krishna et al. 2009). This study has thus provided scientific credibility to the ethnomedicinal use of Abaleria® as an antimalarial therapy and may therefore serve as potential sources of safe, affordable, and effective drug formulation as well as agents of improving lipid profiles.

Acknowledgements The authors appreciate the support of the technical staff of the Department of Biochemistry, Covenant University.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research was approved by the Ethics Committee of the Department of Biological Sciences Covenant University, Nigeria. All animals were also treated in line with the National institute of Health (NIH) guidelines for the use and care for animals in the laboratory (NIH 2011).

References

Abdulelah HA, Hesham MA, Adel AA, Rohela M (2011) Antimalarial activity of methanolic leaf extract of *Piperbetle* leaves. Molecules 16:107–118

Adebayo JO, Krettli AU (2011) Potential anti-malarials from Nigerian plants: a review. J Ethnopharmacol 133:289–302

Adebayo AH, Yakubu OF, Adegbite OS, Okubena O (2017) Haematopoietic induction and hepatic protective roles of Hepacare® in CCl_4 induced hepatic damaged rats. Comp Clin Pathol 26:679–688

Adekunle AS, Adekunle OC, Egbewale BE (2007) Serum status of selected biochemical parameters in malaria: an animal model. Biol Res 18:109–113

Ajaiyeoba E, Falade M, Ogbole O, Okpako L, Akinboye D (2006) In vivo antimalarial activity and cytotoxic properties of *Annona senegalensis* extract. Afr J Tradit Complement Altern Med 3:137–141

Akanbi OM (2013) *In vivo* study of anti-plasmodia activity of *Terminalia avicennioides* and its effect on Lipid profile and oxidative stress in mice infected with *Plasmodium berghei*. Br Microbiol Res J 3(4): 501–512

Akanbi OM, Omonkhua AA, Cyril-Olutayo CA, Fasimoye RY (2012) The anti-plasmodial activity of *Anogeissus leiocarpus* and its effect on oxidative stress and lipid profile in mice infected with *Plasmodium berghei*. Parasitol Res 110:219–226

Alencar NM, Figueiredo IS, Vale MR, Bitencurt FS, Oliveira JS, Ribeiro RA, Ramos MV (2004) Anti-inflammatory effect of the latex from *Calotropis procera* in three different experimental models: peritonitis, paw edema and hemorrhagic cystitis. Planta Med 70:1144–1149

Barik T (2015) Antimalarial drug: from its development to deface. Curr Drug Discov Technol 12(4):225–228

Beckwith R, Schenkel RH, Silverman PH (1975) Qualitative analysis of phospholipids isolated from nonviable Plasmodium antigen. Exp Parasitol 37(2):164–172

Bergmeyer HU, Herder M, Rej R (1986a) International Federation of Clinical Chemistry (IFCC) scientific committee, analytical section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6 1:1). J Clin Chem Clin Biochem 24(7):497–510

Bergmeyer HU, Herder M, Rej R (1986b) International Federation of Clinical Chemistry (IFCC) scientific committee, analytical section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6 1:2). J Clin Chem Clin Biochem 24(7):481–495

Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31(1):87–96

Doumas BT, Perry BW, Sasse EA, Straumfjord JV Jr (1973) Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clin Chem 19(9):984–993

Fabbri C, de Cassia Mascarenhas-Netto R, Lalwani P, Melo GC, Magalhaes BML et al (2013) Lipid peroxidation and antioxidant enzymes activity in *Plasmodium vivax* malaria patients evolving with cholestatic jaundice. Malar J 12:315. https://doi.org/10.1186/1475-2875-12-315

Gonçalves LA, Cravo P, Ferreira MU (2014) Emerging *Plasmodium vivax* resistance to chloroquine in South America: an overview. Mem Inst Oswaldo Cruz 109(5):534–539

Iweala EEJ, Odidoa O (2009) Effect of a long term consumption of a diet supplemented with leaves of *Gongronema latifolium* (Benth.) on some biochemical and histological parameters in male albino rats. J Biol Sci 9:859–865

Kanerkar UR, Bhogaonkar PY, Indurwade NH (2015) Antispermatogenic effect of *Caesalpinia bonduc* (L.) Roxb. Seeds. Int Res J Sci Eng 3(4):173–178

Kannur DM, Paranjpe MP, Sonavane LV, Dongree PP, Khandelwal KR (2012) Evaluation of *Caesalpinia bonduc* seed coat extract for anti-inflammatory and analgesic activity. J Adv Pharm Technol Res 3(3): 171–175

Krishna AP, Chandrika SA, Manasa A, Shrikant LP (2009) Variation in common lipid parameters in malaria infected patients. Ind J Pharmacol 53:271–274

Maroyi A (2013) Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. J Ethnobiol Ethnomed 9:31–49

Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA (2015) *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. Int J Mol Sci 16(7):15625–15658

Mohanty S, Mishra SK, Das BS, Satpathy SK, Mohanty D, Patnaik JK, Bose TK (1992) Altered plasma lipid pattern in falciparum malaria. Ann Trop Med Parasitol 86:601–606

Nawal MA, Ramzi AM, Matheeussen A, Paul PC (2012) *In vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsula region. BMC Complement Altern Med 12:49

Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period of 1981-2002. J Nat Prod 66:1022–1037

Nicholas JW (2004) Antimalarial drug resistance. J Clin Invest 113: 1084–1092

Ogbonna DN, Sokari TG, Agomuoh AA (2008) Antimalarial activities of some selected traditional herbs from South Eastern Nigeria against *Plasmodium species*. Res J Parasitol 3:25–31

Ogunlana OO, Kim H, Wataya Y, Olagunju JO, Akindahunsi AA, Tan NH (2015) Antiplasmodial flavonoid from young twigs and leaves of *Caesalpinia bonduc* (Linn) Roxb. J Chem Pharm Res 7(1):931–937

Ojurongbe O, Ojo JA, Adefokun DI, Abiodun OO, Odewale G, Awe EO (2015) In vivo antimalarial activities of *Russelia equisetiformis* in *Plasmodium berghei* infected mice. Ind J Pharm Sci 77(4):504–510

Ovenden JR, Morgan JAT, Street R, Tobin A, Simpfendorfer C, Macbeth W, Welch D (2011) Negligible evidence for regional genetic population structure for two shark species *Rhizoprionodon acutus* and *Sphyrna lewini* with contrasting biology. Mar Biol 158:1497–1509

Petros Z (2011) The need of standardized herbal remedies as alternate sources of antimalarial products in Ethiopia - updated review. Pharmacology 3:1440–1447

Philipson JD, Wright CW (1990) Anti-protozoal compound from plant source. Planta Med 57:553–559

Ramazani A, Zakeri S, Sardari S, Astaran KN, Djadidt N (2010) *In vitro* and *in vivo* anti-malarial activity of *Boerhavia elegans* and *Solanum surattense*. Malar J 9:124

Somsak V, Noilod J, Chachiyo S, Kraithep S (2015) Antimalarial activity of ethanolic leaf extract of *Bauhinia strychnifolia* in mice infected with *Plasmodium berghei*. Malar Cont Elimination 4:131. https://doi.org/10.4172/2470-6965.1000131

Somsak V, Borkaew P, Klubsri C, Dondee K, Bootprom P, Saiphet B (2016a) Antimalarial properties of aqueous crude extracts of *Gynostemma pentaphyllum* and *Moringa oleifera* leaves in combination with artesunate in *Plasmodium berghei* infected mice. J Trop Med 2016:1–6. https://doi.org/10.1155/2016/8031392

Somsak V, Polwiang N, Chachiyo S (2016b) *In vivo* antimalarial activity of *Annona muricata* leaf extract in mice infected with *Plasmodium berghei*. Journal of Pathogens 2016:1–5. https://doi.org/10.1155/2016/3264070

Tietz NW, Rinker AD, Shaw LM (1983) International Federation of Clinical Chemistry. IFCC methods for the measurement of catalytic concentration of enzymes, Part 5. IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1). J Clin Chem Clin Biochem 21(11):731–748

Viriyavejakul P, Vasant K, Chuchard P (2014) Liver changes in severe Plasmodium malaria: histopathology, apopotosis, and nuclear factor kappa B expression. Malar J 13:106. https://doi.org/10.1186/1475-2875-13-106

White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM (2014) Malaria. Lancet 383(9918):723–735

Wichmann O, Muhlen M, Grub H, Mockenhaupt FP, Suttorp N, Jelinek T (2004) Malarone treatment failure not associated with previously described mutations in the cytochrome-b gene. Malar J 3:14

World Health Organization (2012) World malaria report. Geneva, Switzerland

World Health Organization (2014) Malaria in Africa. Roll Back Malaria Infosheet

World Health Organization (2016) Monitoring health for sustainable development goals. World Health Statistics, France, Europe

Zofou D, Nyasa RB, Nsagha DS, Ntie-Kang F, Meriki HD, Assob JCN, Kuete V (2014) Control of malaria and other vector borne protozoan diseases in the tropics: enduring challenges despite considerable progress and achievements. Infect Dis Poverty 3(1):1

Zoppi F, Fellini D (1976) Enzymatic colorimetric cholesterol determination. Clin Chem 22:690–691