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Potentiation of chloroquine by antiplasmodial fraction of *moringa oleifera* leaves in drug resistant *Plasmodium berghei* infection

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Abstract

Background: This study assessed changes in blood chemistry associated with co-administration of chloroquine with antiplasmodial fraction of crude methanol extract of *Moringa oleifera* leaves.

Methods: Partial phytochemical profiling of antiplasmodial extract was done using standard methods. Using Rane's test, 60 male albino mice were randomized into 10 groups of six mice each. Nine groups were inoculated with quinine resistant strains of *Plasmodium berghei* and treated with tween 80,10mg/kg chloroquine (CQ), 10mg/kg CQ co-administered with graded concentrations of fractions of *Moringa oleifera* leaves, and standard artemisinin combination therapy (ACT) drug, Artemether lumefantrin at a dose of 1.14/6.85mg/kg, while the tenth group which was uninfected was treated with tween 80. Aside from percentage parasitaemia suppression, changes in liver and kidney function parameters and lipid profile were determined from blood collected by ocular puncture using standard methods.

Result: The Flavonoid rich antiplasmodial fraction co-administration with 10mg/kg CQ significantly ($p < 0.05$) dose and time dependently suppressed parasitaemia in the treated groups with total parasite clearance observed in the ACT and CQ-fraction treated groups by day 9. The chloroquine- fraction treatment decreased hepatic function enzyme activities significantly ($p < 0.05$), but not mean plasma bilirubin ($p > 0.05$). The changes in mean plasma urea, uric acid, triacylglycerols, cholesterol and lipoproteins concentrations were not significant ($p > 0.05$) when compared to the ACT treated controls.

Conclusion: The antiplasmodial fraction of the leaf extract of *Moringa oleifera* enhanced the antiplasmodial activity of CQ in the rodent malaria pathogenesis

Keywords: Potentiate; *Moringa oleifera;* ACT; Chloroquine; blood chemistry.

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Background

Malaria has been adjudged to be by far the world's most important tropical disease second only to HIV/AIDS in sub-Saharan Africa [1]. It is responsible for more than 200 million clinical cases and is the cause of death of millions especially women and children under the age of five [1]. It is caused by parasites belonging to the genus plasmodium of which *P. falciparum, P. vivax, P. malariae, P. ovale,* and recently *P. knowlesi* are known to infect humans [2]. Malaria disease is transmitted by the bite of a female anopheles mosquito [3].

The current gold standard treatment is the fixed dose artemisinin combination therapy (ACT) which is made up of chemical derivatives of the natural product artemisinin obtained from the Chinese plant *Artemisia annua*, and a longer acting partner (an arylaminoalcohol or 4-amino quinoline), which ultimately traces its ancestry back to the natural product quinine [4]. Decline in the efficacy of most antimalarials as a result of development of resistance to antimalaria drugs which targets the blood stage of infection of the parasite has become widespread. Most antimalaria drugs have lost their efficacy to the prevalence of drug resistance to the drugs that used to be effective in clearing parasitaemia and even the ACTs have began to lose their efficacy to drug and cross resistance [5]. The management of malaria involving the use of artemisinin derivatives with other antimalarial agents is currently having problems with implementation in developing countries due to affordability and accessibility constraints [6]. The rising cost of prescription drugs in the maintenance of personal health has led to increased interest in the use of medicinal plants as a re-emerging health aid leading to re-awakened interest in the exploration of medicinal plants for the management of malaria [7, 8, 9]. *Moringa oleifera* (Family: *Moringaceae*) is a tree cultivated for different purposes such as medicine, vegetable, spice, for cooking and cosmetic oil among other things. Previous studies have indicated effective antiplasmodial activity and low level toxicity of *Moringa oleifera* leaves extracts in *in vitro* and *invivo* studies [10,11]. Potentiation of the antimalarial activity of conventional antimalaria drugs by antiplasmodial plant extracts with a view to overcome drug resistance in malaria infection has aroused scientific interest [7, 12, 13]. As usual, such assessment requires non human models at this stage. Since we were more particular about drug resistant specie this was done using quinine resistant parasite *Plasmodium berghei* due majorly to the unavailability of the ANKA chloroquine resistant strain. We assessed the herb drug interactions and changes in blood chemistry associated with co-administration of chloroquine and antiplasmodial extract of *Moringa oleifera* leaves in rodent model in this study.

Methods

Collection and treatment of plant material

The plant material for this study (fresh leaves of *M. oleifera*) was collected randomly from trees from Afikpo community between February and May 2014 and authenticated by the plant taxonomist at Bioresources Diversity and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. It was shade dried for three weeks, ground to powder and stored in air tight plastic container until used.

Parasites

Quinine resistant *P. berghei* Q (N1923) strain obtained from the MR4/American type culture collection (ATCC), Manassas, USA maintained by serial passage in mice was used for the study. Preparation of inoculum was from donor mouse with *P. berghei* parasitaemia established by microscopic examination of thin blood film under oil immersion at x100 magnification and measured as percentage erythrocytes [13]. Each mouse was infected with a standard inoculum of approximately 10^7 parasitized erythrocytes suspension in normal saline (0.2 mL) from donor mouse.

Animals

In bred male Swiss albino mice weighing between 20.5 g and 29.5 g (mean 25.0 g) obtained from the animal house of the department of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were kept in well ventilated rodent cubicles under 12 hours light/ dark cycle and fed with mice mash (Top feeds, Nigeria) and water *ad libitum*. The caring and experimental uses of the mice were according to the guidelines of National Institute of Health guidelines for care of laboratory animals.

Drugs

The monotherapeutic drug administered for the control and treatment groups were chloroquine (200 mg) (CLARION, Nigeria) and the ACT drug administered was Lokmal® a brand of artemether lumefantrin (20/120 mg) (EMZOR, Nigeria). These were purchased from registered pharmacy in Afikpo Ebonyi state, Nigeria and were dissolved in 7% ethanolic tween 80 [14] to obtain fixed dose of 10 mg/kg and 1.14/6.85 mg/kg respectively used in the assay. The contents of the dosage forms were however not verified before use.

Extraction and column fractionation of plant materials [15, 16]

One hundred and fifty grams of dried ground sample of *M. oleifera* leaves were macerated with two litres analytical grade methanol for 72 hours with occasional stirring using a magnetic stirrer. The extract was then filtered with Whatman No 1 filter paper and concentrated by gentle warming in a water bath at 40°C. The concentrated crude extract was fractionated by means of column chromatography using silica gel (60–120 mesh) and eluted with chloroform at first then subsequently with chloroform containing increasing concentrations of methanol (80:20, 60:40,40:60 and 20:80). Fractions were monitored with thin layer chromatography at the end of which seven fractions were obtained. The fractions 2, 5 and 7 observed to show significant chemo suppression in the activity guided study reported elsewhere [16] were combined into a single fraction used for the study.

Quantitative phytochemical analysis of fraction

The bioactive fraction were quantitatively analyzed for the presence of alkaloids, tannins, flavonoids, terpenoids, saponins and glycosides the methods described by Sofowora [17], Trease and Evans [18] and Harborne [19].

Antimalaria assay of the bioactive fraction

The seven-day curative method (Rane's test) according to Iwalokun [13] was used with modifications. The animals were divided into 10 Groups of 6 mice each. Each mouse was intraperitoneally infected with 1.0 x 10^7 parasitized erythrocytes in

0.2 mL physiological saline suspension. Treatments commenced on day 3 (4th day after inoculation) and continued to day 7 via oral route using a cannula. Blood films prepared from tail of the infected animals were fixed in methanol, stained with Giemsa and examined microscopically under oil immersion (X100) on day 3 (just before treatment commenced), day 6 and 9 post inoculation. Density of the parasite and percentage reduction in parasitaemia with respect to standard drugs (control) was assessed on group by group basis.

Experimental design

Sixty (60) Swiss male albino mice were randomized into 10 Groups of six mice each.

- **NCTRL:** infected control group treated with 0.2 mL of 7% methanolic Tween 80 solution.
- **CQCTRL:** positive control group consisting of *Plasmodium* passaged mice treated with chloroquine at a dose of 10mg/kg body weight/24 hr.
- **CQFL:** *Plasmodium* passaged mice treated with CQ (10 mg/kg body weight) combined with 100 mg/kg bw/24hr of bioactive fraction of *M. oleifera* leaves.
- **CQFM:** *Plasmodium* passaged mice treated with CQ (10 mg/kg body weight) combined with (150 mg/kg bw /24hr of bioactive fraction of *M. oleifera* leaves.
- **CQFH:** *Plasmodium* passaged mice treated with CQ (10mg/kg body weight) combined with 200 mg/kg bw/24hr of bioactive fraction of *M. oleifera* leaves.
- **FL:** *Plasmodium* passaged mice treated with 100mg/kg bw/24 hr of bioactive fraction of *M. oleifera* leaves**.**
- **FM:** *Plasmodium* passaged mice treated with 150mg/kg bw/24 hr of bioactive fraction of *M. oleifera* leaves.
- **FH***: Plasmodium* passaged mice treated with 200mg/kg bw/24 hr of bioactive fraction of *M. oleifera* leaves.
- **ACTCTRL**: *Plasmodium* passaged mice treated with 1.14/6.85 mg/kg bw/24 hr of artemether lumefantrine
- **UIFCTRL:** Uninfected control group treated with 0.2 mL 7% ethanolic Tween 80 solution.

Biochemical assays

Blood was collected on day 10 post inoculation by ocular puncture and the following biochemical parameters were assessed in serum and plasma samples: Liver function (aspartate aminotransferase (AST), alanine aminotransferase (ALT) by the method of [20], alkaline phosphatase (ALP) kidney function, plasma creatinine, urea [21] and uric acid by method described by Vijayalakshmi et al., [22]. Lipid profile (HDL, LDL, VLDL, triacylglycerols and total cholesterol were determined as described by Ayodele E and Oyedele [23].

Data analysis

Data from phytochemical analysis of antiplasmodial fraction of the crude extract of leaves were expressed as mean $\pm$ standard deviation of triplicate determinations while that from the antimalarial assays were expressed as mean $\pm$ standard error of mean and analyzed for significance of disparity using a One-way analysis of variance (ANOVA). $p < 0.05$ was regarded as significant.

Results

Table 1 shows the phytochemical composition of the antiplasmodial fraction of *M. oleifera* leaves. The fraction wasfound to be high in flavonoids and terpenoids with significant amount of tannins and alkaloids. There were trace amounts of glycosides and saponins in the antiplasmodial fraction of *Moringa oleifera* leaves

There were significant ($p < 0.05$) dose and time dependent increase in percentage chemosuppression in all the drug and extract treated groups (Table 2). Total parasite clearance was observed by day 9 in the ACT, CQ-extract and 200mg/kg extract treated groups while CQ treatment failed to clear parasitaemia by day 9.

The changes in serum activity of liver function enzymes (ALT, AST and ALP) revealed that the CQ-fraction treatment decreased significantly ($p < 0.05$) the activity of these enzymes when compared to untreated and ACT treated controls. Mean serum total and direct bilirubin concentrations were non-significantly decreased ($p > 0.05$) in the CQ- extract treated groups when compared with the ACT treated group but significantly decreased ($p < 0.05$) when compared to the infected group treated with Tween 80 (Table 3).

CQ–fraction co-administration decreased significantly ($p < 0.05$) mean plasma urea, uric acid and creatinine concentrations when compared to the infected group treated with Tween 80 but the changes were not significant ($p > 0.05$) when compared to the ACT treated control group (Table 4).

The CQ-fraction co-administration did not change the mean plasma triacylglycerols, total cholesterol, LDL and HDL-cholesterol concentrations significantly ($p > 0.05$) when compared with the infected group treated with Tween 80 and ACT treatment controls (Table 5).

Discussion

This study determined the changes in blood chemistry associated with potentiation of chloroquine by antiplasmodial extract fraction of *Moringa oleifera* leaves. Changes in parasitaemia and percentage chemosuppression were observed to be dose and time dependent in all the treated groups. The parasitaemia increased significantly ($p < 0.05$) in the infected group treated with the 7% ethanolic tween 80 vehicle while in the CQ control groups, the treatment with a fixed dose of the CQ (10mg/kg) failed to achieve total parasite clearance by day 9. This may not be unconnected with the drug resistant nature of the parasite used. In related antiplasmodial activity study of drug-extract combination using drug sensitive strains of *P. berghei,* Adegbolagun et al. [7] reported that artesunate and artesunate co-administered with extract of *Telfairia occidentalis* leaves at 100mg/kg cleared parasitaemia by day 7 while the crude extract alone cleared parasitamia by day10. Ukwe et al. [12] reported total parasite clearance in animal groups treated with 5mg/kg of chloroquine combined with 100mg/kg of extract of *Ageratum conyzoides* leaves by day 14 while 2mg/kg of artesunate co-administered with 100mg/kg of *A. conyzoides extract* achieved total parasite clearance by day 7. The differences in the observations in this study may be due in part to the higher dose and multi-drug resistant nature of the parasite used in this study. Additive interactions between the components of the fraction and CQ may be directly or remotely responsible for the enhanced antiplasmodial activity observed in this study. The modulation or inhibition of the drug transporter by components of the CQ-fraction

combination and the ACT, artemether lumefantrin may be responsible for the total parasite clearance observed in those groups. Parasitaemia clearance by day 9 of the fraction at 200mg/kg suggests the presence of synergy of more than one active principle in the fraction. The findings of this study corroborate other reports of herb-drug interactions that showed potentiating effect of herbs on the antimalarial action of some standard drugs [7,12,13].

The potential of drug-extract interaction of CQ and antiplasmodial extract to clear parasitaemia in drug resistant infection was evaluated in this study. The higher antiplasmodial activity of the extract-drug combination may be due to the additive interactions of the drug and the partially characterized components of the fraction. Flavonoids, a major constituent of the fraction are believed to exert antiplasmodial activity by inhibiting the fatty acid biosynthesis (FASII) of the parasite [23].

The result of the ALT, AST and ALP activities suggests an amelioration of hepatic damage due to the degeneration and necrosis of parasitized hepatocytes. Increased activities of the transaminases have been associated with untreated malaria infection in studies involving human subjects and in rodent models [24]. Dose related decreased in the activities of these enzymes suggest that the ameliorative effect on liver function is attributable to the additive interaction of the bioactive components and CQ-fraction. The reason for the higher activities of these enzymes in the ACT treated group may not be unconnected with the free radical mediated mechanism of action of the artemisinins.

The increase in the total and direct bilirubin concentrations in the untreated control group when compared to the treated groups indicating ameliorative impact of the various treatments on plasmodium induced dimunition of hepatobilliary function of the liver. The CQ-extract treatment was found to be associated with increased bilirubin concentration relative to CQ treatment suggestive of a negative interaction of the CQ and the extract. The dose related mean plasma bilirubin concentration observed in the CQ-extract treatment was observed not to be significantly different from the values obtained for the ACT treatment indicating that the ACT had no therapeutic advantage over the CQ-extract treatment in this regard. Rapid haemolysis or red blood cells break down has been suggested to lead to increased plasma bilirubin levels in addition to the failure of the hepatic cells to excrete the bilirubin as rapidly as it is formed [25,26]. The mechanism by which the drug-extract interactions decreased the bilirubin levels is unclear but may not be unconnected with the plasmocidal activity of the treatment which reduced the parasite presence and parasite induced hyperbilirunaemia.

Dose dependent decreases in plasma urea and uric acid concentration in the group treated with CQ fraction combination observed in this study suggests that the treatments were associated with improved excretory function of the kidney. Impairment of renal function during malaria infection has been reported and it is an important life-threatening complication of the disease [27]. Changes in mean plasma concentration of creatinine, a relatively diet independent indicator of renal function suggests that the CQ–fraction treatment ameliorated impairment of kidney function occasioned by malaria parasite infection.

Changes in mean plasma TAG and total cholesterol suggest that possible synergistic interactions of the drug and fraction components elevated the parasite induced hypolipidaemia. Relationship between serum cholesterol levels in man/animals and parasitic infections has drawn the attention of several authors [28, 29].

Positive drug –extract interaction of the CQ-extract treatment was not observed to be associated with significant difference ($p>0.05$) in mean plasma HDL and LDL concentration when compared to the CQ and ACT treatments. The pathogenesis of malaria infection is characterized by oxidation of low density lipoprotein [29] which has been observed to promote endothelial expression of adhesion molecules in malaria infected patients and is a critical stage in the progression of the disease [30] The CQ, ACT and CQ-extract treatments in this study was not associated significant difference in these lipoproteins when compared to the infected untreated treated with Tween 80. Lipoproteins for example chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and free fatty acids (FFA) are major lipid macromolecules that originate from the liver [31] and thus depends on cellular integrity and functionality of the hepatocytes which in malaria pathogenesis had been compromised.

Table 1. Quantitative phytochemical composition of the fraction.

Phytochemicals	Composition(mg/100g)
Alkaloids	53.65 ± 0.002
Terpenoids	130.59 ± 0.010
Flavonoids	172.60 ± 0.004
Tannins	93.99 ± 1.341
Saponins	0.66 ± 0.020
Glycosides	1.53 ± 0.056

Values are mean ± standard deviation of triplicate determinations

Table 2. The mean percentage parasitaemia and chemosuppression in the different Groups.

GROUPS	DRUGS		% PARASITAEMIA			%CHEMOSUPPRESSION		
		mg/Kg bw	DAY 3	DAY 6	DAY 9	DAY 3	DAY 6	DAY 9
NCTRL	TWEEN 80	0.2 mL	16.67±2.10	25.33±1.66	32.00±2.24	-	-	-
CQTRL	CQ	10	16.00±1.78	8.20±1.03[a]	10.76±2.21[a]	-	48.33±6.22[a]	65.50±3.80
CQFL	CQ+FL	10+100	18.33±0.84[a]	10.33±1.22	0.00±0.00	-	44.50±11.81	100.00±0.00
CQFM	CQ+FL	10+150	16.67±2.17	8.00±1.36	0.00±0.00	-	50.50±2.22	100.00±0.00
CQFH	CQ+FL	10+200	20.00±1.46[abc]	9.33±0.66[a]	0.00±0.00	-	55.67±5.87	100.00±0.00
FL	FRACTION	100	20.66±1.90	11.66±0.66	3.67±1.67[ab]	-	44.17±5.29	82.5±4.33
FM	FRACTION	150	19.66±1.97	9.54±1.33	1.67±0.00[ab]	-	51.67±4.42	93.10±0.21
FH	FRACTION	200	19.33±1.22[ab]	8.66±0.86[a]	0.00±0.00	-	55.00±2.24[a]	100.00±0.00
ACTCTRL	ACT	1.14/6.85	16.66±2.40	10.00±1.36	0.00±0.00	-	39.67±3.97	100.00±0.00
UICTRL	TWEEN 80	0.2 mL	-	-	-	-	-	-

n=6. Mean values ± SEM. a=significant at $p < 0.05$ when compared to untreated control NCTRL, b=significant at $p < 0.05$ when compared to CQ treated control CQCTRL, c=significant at $p < 0.05$ when compared to ACT treated control ACTCTRL in the same column. CQFL, CQFM, CQFH are groups treated with 10mg/kg CQ combined with 100,150,200 mg/kg dose extract while FL, FM and FH Groups treated with 100, 150, 200mg/kg dose of fraction and UIFCTRL, uninfected control group treated with Tween 80.

Table 3. The mean serum liver function enzymes activity and bilirubin concentrations for the different groups

GROUPS	DRUGS		LIVER FUNTION PARAMETERS				
			ALT	AST	ALP	TBIL	DBIL
		mg/Kgbw	(U/L)	(U/L)	(U/L)	(mg/dl)	(mg/dl)
NCTRL	TWEEN 80	0.2mL	63.83±1.40	79.50±2.54	155.33±3.17	3.08±0.14	1.34±0.12
CQTRL	CQ	10	36.67±0.33[ac]	48.67±1.12[a]	113.83±3.67[a]	1.58±0.97[a]	1.12±0.89
CQFL	CQ+FL	10+100	39.00±1.45	54.33±0.56	125.50±2.68	2.42±0.02	1.17±0.10
CQFM	CQ+FL	10+150	38.83±2.61	51.50±2.29	121.50±5.27	2.27±0.15	1.03±0.10
CQFH	CQ+FL	10+200	45.83±1.62[a]	46.00±3.48[ab]	138.50±10.37[bc]	2.02±0.12[ac]	0.96±0.09[ab]
FL	FRACTION	100	42.33±2.46	41.33±2.20	114.33±11.13	2.19±0.12	1.09±0.14
FM	FRACTION	150	40.17±2.32	49.50±1.93	123.33±17.35	2.08±0.15	1.02±0.14
FH	FRACTION	200	46.67±2.30[ac]	56.33±1.48	102.17±1.14[abc]	1.85±0.18[ac]	1.18±0.18
ACTCTRL	ACT	1.14/6.85	52.83±3.10	54.00±4.73[ab]	147.17±12.02[b]	2.30±0.16[ab]	1.01±0.15[a]
UICTRL	TWEEN 80	0.2 ml	27.33±1.26	46.33±2.58	50.00±11.51	1.06±0.05	0.77±0.86

n=6. Mean values ± SEM . a=significant at $p < 0.05$ when compared to untreated control NCTRL, b=significant at $p < 0.05$ when compared to CQ treated control CQCTRL, c=significant at $p < 0.05$ when compared to ACT treated control ACTCTRL in the same column. CQFL, CQFM, CQFH are groups treated with 10mg/kg CQ combined with 100,150,200 mg/kg dose extract while FL, FM and FH groups treated with 100, 150, 200mg/kg dose of fraction and UIFCTRL, uninfected control group treated with Tween 80.

Table 4. The mean plasma concentrations kidney function parameters for the different Groups

GROUPS	DRUGS	DOSE	KIDNEY FUNCTION PARAMETERS		
			CREATININE	UREA	URIC ACID
		mg/Kgbw	(mg/dl)	(mg/dl)	(mg/dl)
NCTRL	TWEEN 80	0.2 mL	26.62±3.15	5.17±0.36	1.23±0.18
CQTRL	CQ	10	20.00±1.00[a]	5.58±0.22[c]	0.61±0.10[a]
CQFL	CQ+FL	10+100	16.07±1.52	5.88±0.26	0.58±0.06
CQFM	CQ+FL	10+150	15.88±1.08	5.82±0.42	0.84±0.04
CQFH	CQ+FL	10+200	15.58±0.41[abc]	5.57±0.53[a]	0.61±0.03[ac]
FL	FRACTION	100	17.15±2.75	5.56±0.35	0.85±0.02
FM	FRACTION	150	18.85±3.41	6.62±0.25	0.80±0.07
FH	FRACTION	200	19.98±2.13[a]	5.28±0.31[c]	0.55±0.03[ac]
ACTCTRL	ACT	1.14/6.85	19.55±0.25[a]	6.32±0.38[ab]	0.92±0.02[a]
UICTRL	TWEEN 80	0.2 ml	19.14±0.57	4.58±0.23	0.70±0.06

n=6. Mean values ± SEM . a=significant at $p < 0.05$ when compared to untreated control NCTRL, b=significant at $p < 0.05$ when compared to CQ treated control CQCTRL, c=significant at $p < 0.05$ when compared to ACT treated control ACTCTRL in the same column. CQFL, CQFM, CQFH are groups treated with 10mg/kg CQ combined with 100,150,200 mg/kg dose extract while FL, FM and FH Groups treated with 100, 150, 200mg/kg dose of fraction and UIFCTRL, uninfected control group treated with Tween 80.

Table 5. Changes in lipid profile for the different groups

GROUPS	DRUGS	DOSE	LIPID ROFILE				
			TAG	T.CHOL	HDL	LDL	VLDL
		mg/Kgbw	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)
NCTRL	TWEEN 80	0.2mL	1.10±0.98	2.95±0.17	1.56±0.11	1.46±0.16	0.97±0.17
CQTRL	CQ	10	1.08±0.01^{c}	5.00±0.01^{a}	1.35±0.19	1.21±0.20	0.67±0.08^{a}
CQFL	CQ+FL	10+100	1.16±0.89	4.64±0.12	1.02±0.12^{a}	1.60±0.10	0.68±0.44
CQFM	CQ+FL	10+150	1.22±0.62	4.16±0.37	1.23±0.07	1.72±0.13^{abc}	0.61±0.03
CQFH	CQ+FL	10+200	1.15±0.39	2.92±0.10^{bc}	1.52±0.12	1.45±0.05	0.57±0.02^{ab}
FL	FRACTION	100	1.24±0.12	3.20±0.24	1.88±0.19	1.57±0.19^{c}	0.61±0.06
FM	FRACTION	150	1.40±0.08	3.24±0.18	2.00±0.15	1.21±0.15	0.69±0.04
FH	FRACTION	200	1.54±0.14^{b}	4.05±0.36^{ab}	1.85±0.18	1.53±0.86	0.77±0.07^{a}
ACTCTRL	ACT	1.14/6.85	1.48±0.08^{b}	4.42±0.30	1.65±0.28	1.23±0.09^{a}	0.74±0.04^{a}
UICTRL	TWEEN 80	0.2 mL	0.97±0.07	5.14±0.41	1.22±0.13	1.06±043	0.47±0.07

n=6. Mean values ± SEM. a=significant at $p < 0.05$ when compared to untreated control NCTRL, b=significant at $p < 0.05$ when compared to CQ treated control CQCTRL, c=significant at $p < 0.05$ when compared to ACT treated control ACTCTRL in the same column. CQFL, CQFM, CQFH aregroups treated with 10mg/kg CQ combined with 100,150,200 mg/kg dose extract while FL, FM and FH groups treated with 100, 150, 200mg/kg dose of fraction and UIFCTRL, uninfected control group treated with Tween 80.

Conclusions

This study demonstrated that extract-drug interactions of antiplasmodial fraction of *Moringa oleifera* leaves with CQ enhanced the antiplasmodial activity of the monotherapy resulting in total parasite clearance comparable to the ACT treatment. Changes in blood chemistry during the course of the co-administration indicated a herb-drug interaction that apart from clearing parasiteamia by day 9 also ameliorated the *Plasmodium* induced liver, kidney damage and dislipidaermia in a manner not significantly different from the standard CQ and ACT treatments.

Declaration

Supporting data relating to this publication is available in the PhD thesis 'Reversal of chloroquine and artesunate drug resistance by methanol leaf extract of *Moringa oleifera* lam. (March, 2016) University of Nigeria Nsukka. It can also be assessed on request from the corresponding author.

Abbreviations

ACT: artemisinin combination therapy; WHO WMR :World health organization world malaria report ; CQ : chloroquine; NCTRL and CQCTRL are infected groups treated with vehicle tween 80 and 10mg/kg CQ respectively while CQFL, CQFM, CQFH are groups treated with 10mg/kg CQ combined with 100,150,200 mg/kg dose extract fraction; FL, FM and FH are groups treated with 100, 150, 200mg/kg dose of fraction ; UIFCTRL, uninfected control group treated with vehicle Tween 80.

Authors' Contribution

OSS conceived the concept and designed the study, OSS and SOT participated in data gathering. OSS wrote the first draft, did data analysis and wrote the discussion and conception of the paper under the supervision of LUSE and PNU who also read through the manuscript. All authors read and approved the final article.

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Conflict of interest

The authors declare that they have no competing interests.

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