HSI Health Sciences Investigations Journal

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ISSN Online 2704-4890 ISSN Print 2720-7609

Online first publication

Original Research Article

HSI Journal (2024) Volume 5 (Issue 1): 635-644. https://doi.org/10.46829/hsijournal.2024.6.5.1. 635-644

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A combination of unsweetened natural cocoa powder and artemether/lumefantrine: A strategy to improve malaria treatment outcomes

Philip DEBRAH [1], Prince A AKOTUAH [2], Grace L ALLOTEY-BABINGTON [1], Seth K AMPONSAH [3], Mansa FREDUA-AGYEMAN [1], Joseph A SARKODIE [4], Emelia O BEKOE [4], Joshua BOATENG [6], Samuel ADJEI [5], Alexander K NYARKO [2], Benoit B N'GUESSAN [2], Isaac J ASIEDU-GYEKYE [2*]

[1]Department of Pharmaceutics and Microbiology, University of Ghana School of Pharmacy, University of Ghana, Legon, Ghana; [2]Department of Pharmacology and Toxicology, University of Ghana School of Pharmacy, University of Ghana, Legon, Ghana; [3]Department of Medical Pharmacology, University of Ghana Medical School, College of Health Science, University of Ghana, Korle-Bu, Accra, Ghana; [4]Department of Pharmacognosy and Herbal Medicine, University of Ghana School of Pharmacy, University of Ghana, Legon, Ghana; [5]Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research, College of Health Science, University of Ghana, Legon, Ghana; [6] School of Science, University of Greenwich, Medway, United Kingdom

Received February 2024; Revised March 2024; Accepted April 2024

Abstract

Background: Reports suggest that unsweetened natural cocoa powder (UNCP) has antiplasmodial activity and contains enough fat content to enhance the absorption of artemether/lumefantrine (A/L).

Objective: This study assessed the pharmacokinetic and pharmacodynamic properties of UNCP co-administered with A/L

Methods: Male Sprague-Dawley (SD) rats were infected with A/L-sensitive *Plasmodium berghei*. Rane's curative model was used to assess the effect of the excipient UNCP (300 - 1500 mg/kg) formulated with A/L on parasite clearance. Additionally, healthy non-malarious male SD rats were co-administered orally with the fixed doses of A/L (recommended therapeutic dose of 2 mg/kg artemether and 12 mg/kg lumefantrine) with varying doses of UNCP (300, 600, 900, 1200 and 1500 mg/kg), to assess the effect of UNCP on the disposition of A/L. The number of mice in each group that were given each dose was five (n = 5). Plasma lumefantrine concentration was assayed using HPLC/UV-Vis.

Results: Co-administration of UNCP (1200 and 1500 mg/kg) with A/L caused a significant difference in parasite clearance compared to conventional A/L (Coartem®-only) or UNCP alone. Pharmacokinetic analysis showed that the peak serum concentration (Cmax) of lumefantrine for the A/L+UNCP (1200 mg/kg and 1500 mg/kg) was higher than the Coartem®-only group. Additionally, the area under the lumefantrine concentration-time curve (AUC0→24) post-drug administration was higher for the A/L+UNCP (1200 mg/kg and 1500 mg/kg) groups compared to the commercially obtained conventional A/L Coartem®-only group.

Conclusion: UNCP, co-administered with A/L, increased the in vivo antiplasmodial activity of A/L enhanced lumefantrine disposition (peak concentration and total drug exposure) in rats. Thus, it can be exploited as an excipient in the formulation of A/L for the management of uncomplicated malaria in humans.

Keywords: Artemether/lumefantrine, pharmacokinetics, cocoa powder, malaria, *plasmodium berghei*

Cite the publication as Debrah P, Akotuah PA, Allotey-Babington GL, Amponsah SK et al. (2024) A combination of unsweetened natural cocoa powder and artemether/lumefantrine: A strategy to improve malaria treatment outcomes. HSI Journal 5 (1):635-644. https://doi.org/10.46829/hsijournal.2024.6.5.1.635-644

INTRODUCTION

The strategy of using pharmaceutical excipients that synergise the action of active pharmaceutical agents is recommended [1]. Some of these excipients exhibit the activity of the active ingredient, but the effects produced by their use alone are suboptimal. However, when used in combination with active pharmaceutical agents, they enhance the activity of the latter. In situations where enhanced activity is not desired, reduced quantities of the active ingredient can be used in combination with these

* *Corresponding author*
Email: ijasiedu-gyekye@ug.edu.gh

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excipients. Reducing the amounts of active ingredients leads to a reduction in the adverse (undesirable) effects caused by these active agents. Malaria still remains a major cause of morbidity and mortality worldwide [2,3]. The use of monotherapy for the management of infectious diseases has resulted in resistance to the agents used [3]. Thus, combination therapy has been adopted as a strategy to prevent multidrug resistance. The standard approach in the management of uncomplicated malaria is the use of Artemisinin-based Combination Therapy (ACT) [3,4].

In Ghana, the three commonly used ACTs for uncomplicated malaria include artemether/lumefantrine (A/L), artesunate/amodiaquine (A/A) and dihydroartemisinin/piperaquine (D/P), which are administered based on patient's body weight [2]. A/L is one of the first-line treatments for uncomplicated malaria in many jurisdictions. However, poor gastrointestinal (GI) absorption occasionally presents a major treatment challenge. This is because lumefantrine is highly dependent on dietary fat for GI absorption [5,6]. Studies have shown two-fold and sixteen-fold increases in systemic bioavailability of artemether and lumefantrine, respectively, when administered with fatty meals [7]. In order for patients to reap the full benefit of the use of A/L for the treatment of uncomplicated malaria, pharmacists usually advise patients to take the medication after a fatty meal. Appetite loss and vomiting are symptoms usually associated with malaria. Patients are unable to eat during this period; thus, poor gastrointestinal absorption is expected. It therefore comes as no surprise that drug resistance to A/L has been reported in some jurisdictions [3].

Unsweetened natural cocoa powder (UNCP) is a popular West African nutraceutical obtained from the beans of Theobroma cacao (cocoa). It is commonly consumed as a beverage in many African countries. UNCP possesses *in vitro* anti-plasmodial activity [8]. It has been reported to attenuate malaria-related liver damage in mice [9] and also mitigate high-dose A/L-induced hepatotoxicities, cardiotoxicities and nephrotoxicities in guinea pigs [10,11]. The chemical composition of Theobroma cacao has been widely studied and well documented to contain flavonoids and other phytochemicals [10]. There is also clear evidence of non-genotoxic and non-organ toxicity with the use of Theobroma cacao [12,13]. Although UNCP is prepared after the removal of the cocoa butter from powdered cocoa beans, there are indications to suggest that UNCP may contain enough fat to aid the absorption of some drugs when co-administered [14,15]. Co-administration of UNCP with A/L is envisaged to enhance the GI absorption of A/L. Additionally, a synergistic antiplasmodial effect is envisaged with the co-administration since UNCP is reported to possess antiplasmodial activity *in vitro* [8]. This study aimed to use an integrated approach to enhance the antiplasmodial effect of A/L by enhancing its absorption through co-administration with UNCP. Thus, plasma concentrations of lumefantrine after co-administration with UNCP and parasitemia clearance were evaluated in *Plasmodium berghei*-infected murine models.

MATERIALS AND METHODS

Materials

The test organism, *Plasmodium berghei* (NK65) malaria parasites, was obtained from the Immunology Department of the Noguchi Memorial Institute for Medical Research (NMIMR). UNCP was procured from Hords Company Ltd, Accra-Ghana (batch number 3010019SEP), under the brand name Brown Gold. The Lumefantrine reference standard was procured from Sigma Aldrich, USA, and the lumefantrine powder from IPCA Laboratories Limited, India. Ketamine injectable was obtained from the anaesthetic clinic of the Korle-Bu Teaching Hospital. Coartem was acquired from Korle Bu Hospital 24-Hour Pharmacy.

Animal Care and Housing

Eight-week-old male Sprague-Dawley (SD) rats with weights between 200 g and 250 g were used for this study. The rats were obtained from the Department of Animal Experimentation of the Center for Plant Medicine Research, Mampong, Ghana. The animals were kept in clean stainless-steel cages at the Animal House of the Department of Medical Microbiology, Korle-Bu, University of Ghana. The cages were kept in enclosed, well-ventilated rooms with proper lighting and under hygienic conditions. Each rat occupied a minimum space of 5.86 cubic meters (61 cm x 31 cm x 31 cm) with soft wood shavings as bedding for comfort. The animals were fed with a normal pellet diet (AGRIMAT, Kumasi), given water ad libitum, and maintained under standard laboratory conditions (temperature 25 ± 1°C, relative humidity 60 - 70%, and 12-hour light-dark cycle). All feeding and water troughs were regularly cleaned to prevent contamination. The animals were made to acclimatise for seven days under the aforementioned conditions before experimentation.

The study protocol was reviewed and approved by the Protocol Review Committee (PRC), College of Health Sciences, University of Ghana – Korle-Bu, Ghana (number: CHS-Et/M5-5.3/20190-2020). Clearance for animal experimentation was also obtained from the University of Ghana's Institutional Animal Care and Use Committee (UG-IACUC) (Number: 2016-02-2Q). All animal experiments were conducted in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [16] and the National Institute of Health guide for the care and use of laboratory animals [17] [18,19].

Preparation of various formulations of A/L with UNCP

The medications used for the study were administered by oral gavage. The medications being investigated were formulated as pharmaceutical suspensions. This is because the various components, artemether, lumefantrine, and UNCP, are not readily soluble in distilled water. The only excipient used was UNCP. This was to ascertain that any

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effect observed would be due solely to the UNCP. In order to administer the same volume of drug to each animal, six different formulations were prepared. The first formulation (A/L plus 300 mg/kg UNCP) was prepared by mixing by geometric dilution in a mortar and pestle the powdered ingredients (1.725 g of UNCP, 11.5 mg of artemether and 69 mg of lumefantrine).

A primary suspension was prepared using part of the vehicle (distilled water), which was further diluted with the remaining distilled water to obtain 50 mL suspension. Thus, each 2 mL dose administered contained 69 mg of UNCP, 0.46 mg artemether and 2.76 mg of lumefantrine [Ratio of artemether to lumefantrine in the marketed product for the treatment of malaria is 1:6, and the recommended therapeutic dose is 2 mg/kg artemether and 12 mg/kg lumefantrine]. Like the method just described, five other formulations were prepared. Since the goal was to determine the effect of UNCP on A/L, the amounts of artemether and lumefantrine were the same as in the previous formulation, but the amount of UNCP was varied. The amounts of UNCP in formulations 2, 3, 4, and 5 were 138 mg, 207 mg, 276 mg, and 345 mg, respectively. These were for animals in groups administered UNCP concentrations of 600, 900, 1200 and 1500 mg/kg respectively [10,11]. Thus, each animal received 2 mL of medication, but they contained the same amount of A/L but different amounts of UNCP.

Culture of *P. Berghei* and infection of SD rats with the parasite

The parasites, *Plasmodium berghei*, were cultured by initial inoculation of donor rats (non-experimental rats) with 0.2 mL of 107 *P. berghei* parasitised red blood cells (RBCs) per µL via the intraperitoneal route. A series of passages were performed until the infection was fully established. Parasite monitoring was performed by obtaining blood from the tail vein of the infected SD rats and placing the blood on a clean glass slide (All Pro Processed Microscope Slide, Cat # 7105, Surgifriend Medicals, Middlesex, England). This was taken through routine thick and thin film preparations for examination under a light microscope (Leica, Galen III, Cat# 317505; Leica Microsystems, Wetzlar, Germany) with immersion oil and ×100 objective lens [9]. After the target parasite density was achieved, the donor rats were anaesthetised with ketamine (5 mg/kg), blood was then drawn from donor animals into heparinised tubes and immediately diluted using normal saline (0.9% NaCl) to obtain a standard inoculum, which was used to infect the test animals. To infect test animals, a volume of 0.2 mL of blood equivalent to 107 *P. berghei* parasitised-infected erythrocytes was introduced into all the experimental animals via intraperitoneal injection. Parasite density was then monitored for three days, where the target parasite range (29% - 32%) was obtained before the administration of test samples commenced [20,21]. These values served as baseline parasitemia for each group prior to treatment. The thick film helped in the determination of parasite density, which was calculated according to the formula recommended by the WHO manual [22]. The number of parasites relative to the number of leucocytes is calculated and expressed as 'parasites per microlitre of blood' from the mathematical formula as follows [22]:

$$\text{Parasite Density}/\,\mu\text{L of blood} = \frac{\text{Number of parasites}}{\text{200 white blood cells}} \times 800$$

The thin film was employed in calculating the percentage parasitemia as follows [22]:

$$\text{Percentage Parasitemia} = \frac{\text{Number of parasitised RBCs}}{\text{Total number of RBCs}} \times 100$$

Treatment of infected rats with various formulations

Prior to the administration of various treatments, it was ensured that all the SD rats in the various groups were infected with *P. berghei* (NK65) [23,24]. Rats were randomly divided into 12 groups (n = 5). Groups one to five received a combination of A/L and UNCP, groups six to ten received various concentrations of UNCP alone, and groups eleven and twelve were controls. Groups 1-5 were administered A/L (2/12 mg/kg) (at the recommended 1:6 artemether to lumefantrine ratios) in combination with different concentrations of UNCP. Group 1 received A/L (2/12) with 300 mg/kg of UNCP, Group 2 received the same concentration of A/L with 600 mg/kg of UNCP, Group 3 received A/L with 900 mg/kg of UNCP, Group 4, A/L with 1200 mg/kg of UNCP and finally, Group 5 received A/L with 1500 mg/kg of UNCP. Groups 6-10 were given varying doses of only UNCP: 300, 600, 900, 1200 and 1500 mg/kg respectively. Rats in Group 11 served as negative control and thus were administered (distilled water), whilst those in Group 12, the positive control, were administered coartem®- (2/12 mg/kg) (the innovator A/L brand). Treatments were administered per 2 mL oral gavage in fasted animals twice daily for three consecutive days. The animals that received 300 mg only, 600 mg only and vehicle only (negative control) were euthanised after the 24-hour samples were taken because they were in distress. After the third day, blood samples were drawn from the tail veins of rats at predetermined times (0,8,24,36,48 and 60 hours) to assess parasite clearance, which was done via microscopic examination. A two-hour gap of no feeding of the rats was maintained pre- and post-sample administration to ensure that the presence of food did not affect the absorption of various treatments. Additionally, rats were followed up to estimate survival times post-treatment. Animals were observed in compliance with all governing regulations involving humane care and the use of animals in research. Distressed rats were euthanised by placing them in a carbon dioxide (CO_2) chamber [25,26]. Signs of distress included, but were not limited to, decreased mobility, scruffy coat, and hunched posture.

Pharmacokinetic estimation of UNCP and A/L formulations

This aspect of the study was performed in healthy non-malaria-infected animals. Thirty-five SD rats were randomly put into seven groups (n = 5). Groups 1-5 were

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administered A/L (at the recommended therapeutic doses of 2 mg/kg artemether and 12 mg/kg lumefantrine) formulated with varying concentrations of UNCP: 300, 600, 900, 1200 and 1500 mg/kg, respectively, via the oral route as done previously [10,11]. Rats in Group 6 were given coartem®-(2 mg/kg artemether and 12 mg/kg lumefantrine, the innovator A/L brand). Mice in each group received a single dose of the treatment assigned, after which blood samples were collected. The first blood sample was drawn 30 minutes after the last dose. This was done by taking 500 µL whole blood from the tail vein of the rats at times of 0.5,1, 2,4,8,12, and 24 hours into heparinised tubes. Samples were kept on ice packs immediately and centrifuged at 2,500 rpm for 15 minutes within one hour after collection. Plasma was collected into cryotubes, labelled, and stored at -20ºC for subsequent high-performance liquid chromatography (HPLC) analyses.

The peak plasma drug concentration (Cmax) and the time to achieve this peak (Tmax) of lumefantrine were extrapolated from concentration-time curves. The elimination rate constant (Ke) was determined by linear regression analysis of the terminal-linear part of the log plasma concentration-time curves. The elimination half-life (t1/2e) was calculated from 0.693K-1. The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal rule. Pharmacokinetic analysis was conducted using GraphPad Prism version 7.0 for Windows, GraphPad Software, www.graphpad.com.

Plasma lumefantrine concentrations determined by high-performance liquid chromatography

The stored plasma samples were thawed at room temperature for about 1-2 hours. Samples were vortexed in seconds. Acetonitrile, 1.5 mL, was added to precipitate plasma proteins, vortexed for 1 minute, and then centrifuged for 5 minutes at 10,000 × g [34]. The supernatant was transferred into clean tubes and evaporated to dryness at 37°C under a steady stream of nitrogen gas. The residue was reconstituted with 500 µL acetonitrile, filtered using a 0.4 µm syringe filter and 20 µL injected into the HPLC column. HPLC analysis was performed on an LC-20AT (Shimadzu, Japan) liquid chromatograph equipped with an SPDM-20A UV detector (Shimadzu, Japan).

Separation was performed on a YMC-Pack Pro C18, 5 µm, 12 nm, length 150 mm and internal diameter 3.0 mm. The column temperature was set at 30 ºC, and the flow rate was 1.0 ml/minute. The detection conditions were as follows: UV set at 380 nm. A UV-visible spectrophotometer model-1700 (Shimadzu, Japan) was used to determine UV spectra. Analysis was carried out for lumefantrine alone due to the fact that artemether absorption is not known to be a challenge. The chromatographic analysis was carried out using the International Pharmacopoeia (IP) method [27,28]. A similar experiment using the same equipment has been performed in the same laboratory previously [29].

Liver function test

After the last time point, blood was also collected for liver function test (LFT). Whole blood (1 mL) was collected in clot activator gel tubes and allowed about 10 minutes to clot. This was followed by centrifugation at 1000 g for 10 minutes to obtain serum. Biochemical analysis was performed using an autoanalyser (URIT-8021AVet, China). Specific biochemical markers such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and albumin were determined.

Statistical Analyses

Results were reported as a Means. Error bars are indicative of the standard deviation of uncertainty. Statistical analysis was performed using one-way (ANOVA) followed by Bonferroni's multiple comparison test. Analysis was done using GraphPad Prism version 8.0.2 for Windows, GraphPad Software, and www.praphpad.com. Statistical significance was set at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

RESULTS

Mean parasitemia levels after treatment with UNCP only

There was an initial increase in parasitemia 24 hours after UNCP administration across all the UNCP-only groups shown in Figure 1A. The animals that received 300 mg only, 600 mg only and vehicle only (negative control) were euthanised after the 24-hour samples were taken because they were in distress. There was a difference in parasitemia for the higher doses of the UNCP (1200 mg/kg and 1500 mg/kg) compared to the vehicle group at 24 hours (Figure 1B)

Mean parasitemia levels after treatment with UNCP and A/L

Varying UNCP doses, co-administered with a fixed dose of A/L, appeared to decrease parasitemia with increasing UNCP. Total parasitemia clearance was obtained for almost all the groups in 60 hours (Figure 2A).

For all the groups, there was a significant difference between the baseline parasitemia levels and the treated groups at predetermined time points. Twenty-four hours after treatment commenced, there was no observable difference between the treatment groups (Figure 2A). The drop in parasite levels of the positive control was comparable to that of the group treated with 900 mg UNCP + A/L. Groups that received 1200 mg and 1500 mg had much lower parasite levels than the positive control group (Figures 2B and 2C).

Pharmacokinetic estimation of UNCP and A/L formulations

A plasma concentration-time plot for lumefantrine in the A/L+UNCP groups showed a general increase in plasma occurring within 4 - 8 hours, followed by a general decrease within 24 hours post-dose administration (Figure 3).

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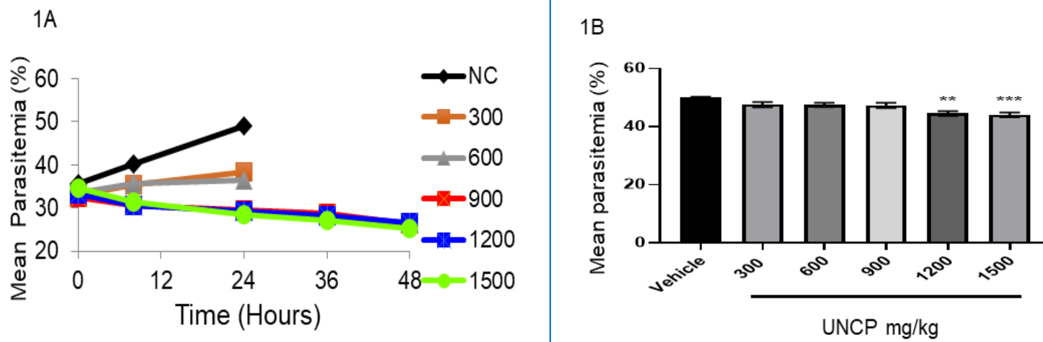

Figure 1. In vivo antiplasmodial activity of UNCP-only (300, 600, 900, 1200 and1500 mg/kg) on *Plasmodium berghei* (NK65) parasitemia; (A) Parasitemia trends from baseline till death of animals for UNCP-only formulations. (B) In vivo effect of higher doses of UNCP (1200 mg/kg and 1500 mg/kg) on parasitemia compared to the negative control group (no treatment) at 24 hours post exposure. (n = 5) and *p < 0.05, **p < 0.01 and ***p < 0.001 using two-way ANOVA followed by Bonferroni's multiple comparison post hoc analysis

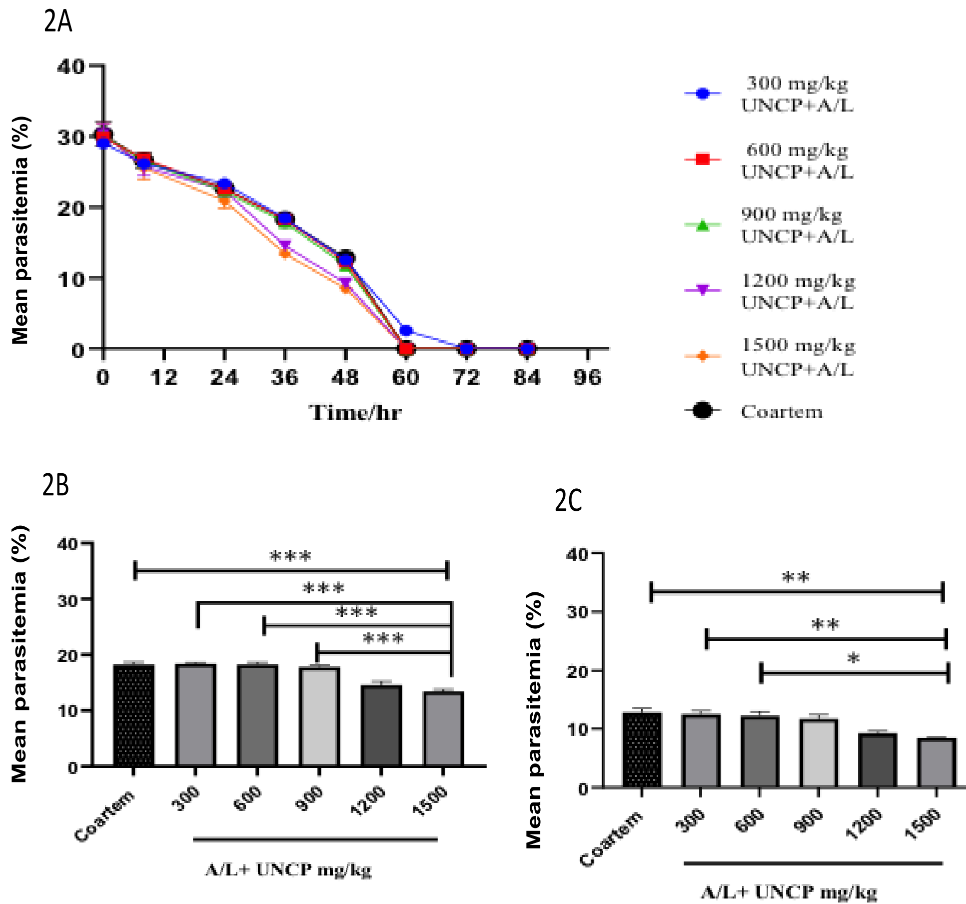

Figure 2A. *In vivo* antiplasmodial activity of A/L+UNCP (300, 600, 900, 1200 and 1500 mg/kg) in rats infected with *Plasmodium berghei* (NK65).

*The overall antimalarial trend over a 96-hour time course. Comparison of parasitemia clearance of A/L + UNCP (varying amounts) at time points 36 hours (Figure 2B) and 48 hours (Figure 2C) respectively. (n = 5). *P < 0.05, **P < 0.01 and ***P < 0.001 using Two-way ANOVA followed by Bonferroni's multiple comparison post hoc analysis).*

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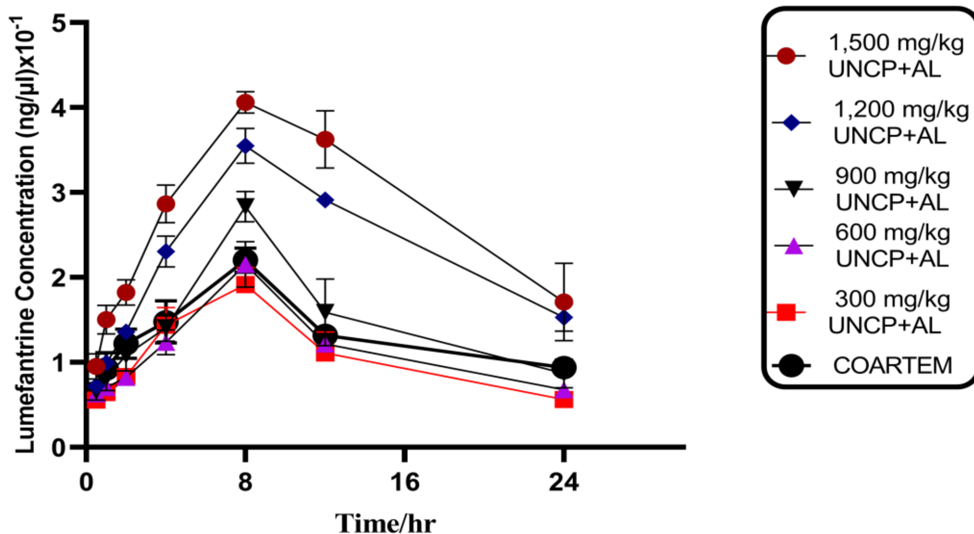

Figure 3. A 24-hour plasma concentration-time curve of lumefantrine in various treatment groups

Table 1. Pharmacokinetic Parameters of lumefantrine in the various treatment groups

PK Parameter	Treatment Group [Mean ± SEM]						Overall *p*-value
	A/L+300 mg/kg UNCP	A/L+600 mg/kg UNCP	A/L+900 mg/kg UNCP	A/L+1200 mg/kg UNCP	A/L+1500 mg/kg UNCP	COARTEM	
T_{max} (hr)	8.17±0.04^B	8.10±0.03^B	8.31±0.13^B	8.10±0.04^B	8.28±0.04^B	8.19±0.05	0.1667
C_{max} (ng/µl*10^{-1})	1.92±0.20^B	2.15±0.27^B	2.83±0.18^B	3.55±0.21^A	4.06±0.13^A	2.20±0.14	<0.000
$AUC_{0\rightarrow 24}$ (ng/µl*10^{-1}*hr)	26.11±3.01^A	28.04±1.59^A	35.89±4.79^A	56.57±2.13^A	68.21±6.11^A	32.12±1.85	<0.0001
K_e (hr^{-1})	0.05±0.02^B	0.05±0.00^B	0.05±0.01^B	0.06±0.01^B	0.07±0.02^B	0.03±0.01	0.3879
$t_{1/2}$ (hr)	17.75±0.20^B	14.27±0.27^B	14.65±0.18^B	13.26±0.21^B	11.86±0.13^B	26.23±0.14	0.2002

Table 2. Biochemical analysis of blood collected from rats in various groups

Liver function test	A/L +300 mg/kg UNCP	A/L +600 mg/kg UNCP	A/L +900 mg/kg UNCP	A/L +1,200 mg/kg UNCP	A/L +1,500 mg/kg UNCP	Coartem Only	Vehicle Only	p-value
AST U/L	478.20 ± 47.02	497.60 ± 39.90	624.32 ± 187.35	483.90 ± 33.38	564.52 ±194.41	774.16 ±198.61	863.01 ±117.6	0.3790
ALT U/L	99.02± 23.62	104.82± 20.41	204.42± 43.76	187.88 ±25.07	263.04± 92.58	278.48 ±44.51	204.90 ±23.78	0.1962
ALP U/L	119.14 ±32.22	131.04 ±35.33	188.72 ±25.46	118.04 ±24.07	204 ±49.94	305.80 ±13.47	225.34 ±15.20	0.0043
ALBUMIN g/L	67.58 ±20.34	70.12 ±23.29	159.40 ±53.88	56 ±18.65	200.48 ±110.74	200.48 ±46.25	60.8± 15.81	0.3495

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Liver function tests

Relative to the vehicle-only group (no treatment), ALT and AST levels did not appear elevated. Hence, it may be difficult to suggest liver damage after treatment.

DISCUSSION

In vitro, antiplasmodial activity of UNCP has been previously reported in mice [8]. However, *in vitro*, pharmacological activity does not always translate into in vivo activity due to pharmacokinetic factors such as drug absorption. This study sought to investigate the in vivo antiplasmodial activity of UNCP co-administered with A/L in SD rats. *Plasmodium berghei*-infected rodent models usually typify *P. falciparum* malaria in man and are commonly used for such studies. After establishing a baseline parasitemia of 29-32%, UNCP-only formulations (different concentrations) were administered to the animals. Twenty-four hours after treatment was initiated, there was no observed effect. Instead, the percentage of parasites in the blood continued to rise (Figure 1A). The groups that received lower doses (300, 600 mg/kg) were euthanised after the 24-hour time point. Even though UNCP showed parasite suppression activity at higher doses, it was observed that all the animals showed signs of distress between 24 and 48 hours.

The animals in the groups that received 900, 1200 and 1500 mg/kg of UNCP remained alive till the 48-hour time point and were also euthanised. The difference in survival time between these animals was not significant. The *in vitro* antiplasmodial property of UNCP has been attributed to the presence of flavonoids [9, 13,46] and other phytochemicals like alkaloids and tannins [8,15,27,30]. These same phytochemicals may be responsible for the observed in vivo activity. This can be postulated to mean that even though UNCP possesses some antiplasmodial activity, it is not efficient in clearing the parasites from the blood of the infected rats. Thus, UNCP alone may not be curative in cases of malaria. Trace minerals such as manganese, zinc, and copper found in UNCP have been reported to possess immunomodulatory properties [31,32] that may inhibit malaria parasite growth. This effect may have augmented the activity of the phytochemical constituents in UNCP in inhibiting parasite growth [15,33,34].

When higher doses of UNCP were co-administered with a fixed dose of A/L, a general dose-dependent decrease in parasitemia at various time points was observed (Figures 2B and 2C). This was, however, not coherent with the trend of activities of AST and ALT in Figure 2A. This observation may be due to the possible synergistic antiplasmodial activity of UNCP and A/L or an enhancement of the absorption of A/L by UNCP, which invariably would increase plasma concentration (bioavailability) of A/L. Other studies have shown the varying effects of AL in murine models [35,36,37]. Total parasite clearance was observed for almost all the groups within 60 hours (Figure 2A). From the results in Figures 2B and 2C, parasitemia clearance for A/L with UNCP 900 mg/kg and positive control (coartem®) were comparable. The conventional A/L (coartem®) used as positive control has been formulated with excipients to yield adequate absorption and bioavailability. However, the new formulations used contained just the active ingredients A/L and UNCP. This was done to ensure that any effect observed on the A/L was solely due to the UNCP. Combinations of UNCP (1200 and 1500 mg/kg) and A/L showed better parasite clearance than the positive control (Figures 2B and 2C). The findings of this research suggest that formulating A/L with 900 mg/kg of UNCP will produce results similar to the innovator brand (coartem®). Although the higher doses of UNCP (1200 and 1500 mg/kg) with A/L produced better results, the authors are not recommending its use in the formulation of a unit dosage form. This is due to the fact that the most convenient adult dosage forms are tablets or capsules.

From the formulation standpoint, tablets or capsules will not be appropriate in this instance as their sizes (due to the quantities of UNCP to be used) will pose swallowing challenges to patients. Authors are therefore proposing the formulation of individually divided granules (powders) that will contain A/L plus 900 mg of UNCP packaged in sachets to be reconstituted just before administration. Further studies need to be done on doses of UNCP between 600 mg/kg and 900 mg/kg in order to optimise the appropriate dose. Even though there was an observable difference in parasite clearance, this was only significant after 36 hours (Day 2), which corroborates the assertion of stimulation of an immune response by the UNCP [15], a process that requires time. Cocoa has been established to be rich in antioxidants and has immune modulatory properties [31,32]. Its immunomodulation has been linked with the presence of flavonoids with regulatory effects on acquired immune response in both *in vivo* and *in vitro* studies [38-40].

A/L, as an antimalarial agent used in the treatment of uncomplicated *falciparum* malaria, is fraught with the problem of poor GI absorption and this challenge is known to improve when the drug is administered with fatty food [5,41,42]. The observed increase in parasitemia clearance when A/L was co-administered with UNCP could be due to the fat constituent (28%) in UNCP (Figure 2). As absorption increases, there is a corresponding increase in systemic drug concentration [5,41]. The study focused on lumefantrine absorption and not artemether against the background of the aforementioned advantage that a fatty meal has on A/L absorption and/or bioavailability pertaining to lumefantrine and not artemether. It is worth mentioning that patients could benefit from the energy offered by the other components of UNCP as well.

The use of UNCP as an additive or excipient in the formulation of A/L powders may be an option worth employing to improve the therapeutic outcomes of A/L. West Africa produces about 70% of the world's cocoa, with

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Ghana and the Ivory Coast being the leading producers. Africa is one of the most malaria-endemic regions despite its rich medicinal plant sources [43,44]. Thus, employing UNCP as an excipient in the pharmaceutical formulation of antimalarial drugs may improve malaria treatment outcomes. From the observations made in this study, it is evident that administering A/L with UNCP translates to better systemic peak lumefantrine concentration, total lumefantrine exposure and parasite clearance. The strategy of incorporating UNCP into A/L malaria management in Africa is likely to be more cost-effective. The use of these nutraceuticals may be beneficial in malaria prophylaxis [43, 45, 46]. This can be recommended in addition to malaria treatment, especially in children below five years, a vulnerable group to malaria [45,47,48]. Paediatric dosage forms of A/L are sometimes presented as divided powders (individually packed) for reconstitution into suspensions before use, and UNCP could be used as an excipient. Data from the pharmacokinetic aspect of the study showed that AUC, a surrogate for total drug exposure, was found to be higher among rats administered UNCP with A/L. AUC can also be indicative of the extent of absorption of a drug or its bioavailability.

Overall, the amount of the A/L absorbed when co-administered with UNCP was greater than the conventional formulation in a fasted murine model. Additionally, the peak plasma concentrations (Cmax) of the AL+UNCP groups were higher than the coartem® group, which sometimes gives an indication of the overall absorption of the drug. Hence, we can postulate that absorption was better when UNCP was combined with A/L (Table 1). The LFT results showed a significant increase in ALP for only the AL+UNCP (300 mg/kg) group (Table 2). High serum ALP for this group could be from sources other than the liver, such as bone and kidney. Nevertheless, further investigations may be needed to explain this observation. This potential use of UNCP as an excipient in the formulation of A/L can be exploited in the management of uncomplicated malaria in humans in the form of nanoparticles. A limitation of this study might be the fact that liver enzymes were examined, but other toxicities (renal) were not assessed. Additionally, the animals that were given low-dose UNCP had to be euthanised between 24-48 hours. This posed a challenge in comparing the parasite levels with those of the other groups.

Conclusion

UNCP alone was not efficient in reducing parasitaemia in rats. A combination of UNCP with A/L was found to improve *P. berghei* parasite clearance in SD rats. A/L formulated with 900 mg/kg of UNCP produced results comparable with the innovator brand, Coartem. Co-formulation of A/L with UNCP, a nutraceutical, would ensure that adequate plasma concentrations of the antimalarial agent are achieved independent of the patient's ability to ingest a fatty meal.

DECLARATIONS

Ethical consideration

The study protocol was reviewed and approved by the Protocol Review Committee (PRC), College of Health Sciences, University of Ghana – Korle-Bu, Ghana (number: CHS-Et/M5-5.3/20190-2020). Clearance for animal experimentation was also obtained from the University of Ghana's Institutional Animal Care and Use Committee (UG-IACUC) (Number: 2016-02-2Q).

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

PD and IJAG conceived the study. PD, PA, SKA, IJAG and GLAB designed the methodology. PA performed the experiments under the supervision of PD, IJAG, SKA, JB and GLAB. PD, PA, SKA and AKN summarised the data. PD, PA, GLAB. BBN, PD, OAD, AKN, EOB, JES, SKA, MFA and IJAG were involved in the analysis of data and writing of the manuscript.

Acknowledgement

The authors wish to thank the Noguchi Memorial Institute for Medical Research, Food and Drugs Authority-Ghana and all laboratory technicians at the School of Pharmacy, University of Ghana, for their help and technical support. Additionally, the authors thank the UG-Carnegie "Building a New Generation of Academics in Africa" (BANGA-Africa) Project for their Write Shop support, which afforded authors the opportunity to complete the draft manuscript and receive critical reviewer inputs.

Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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