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ANTIMALARIAL ACTIVITY OF CAFFEINE, ORALLY ADMINISTERED WITH A LIPID-BASED FORMULATION IN A MURINE MODEL

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ABSTRACT

Despite Caffeine being known essentially for its psychoactive properties, an attempt was made in this study to investigate its potential antimalarial activity considering that it's an alkaloid and that the malaria parasite is a purine auxotroph. In the baseline experiment, caffeine was administered at three doses (5, 10 and 20 mg/kg) once daily in suppressive and curative models. The observed chemosuppression activity was comparable to that of chloroquine (CQ) in both experiments. In the baseline suppressive experiment, a chemosuppression of 70.39% for CQ (at 10 mg/kg) was observed, while caffeine had 78.90%, 80.73% and 81.95% at the respective doses. However, the survival index estimated based on the rate of survival of the experimental animals for 28 days post infection, was very low (33%, 29% and 43% respectively, and the same trend was observed in the baseline curative experiment). This initial result showed that caffeine had potential as an antimalarial agent relative to the standard drug, chloroquine, and the lipid-based formulation must have played a role in ensuring that the caffeine had enhanced bioavailability. A follow-up experiment was conducted in which the caffeine was administered twice daily (at 20 mg/kg) in suppressive and curative experiments. The observed chemosuppression in the suppressive test (with twice daily administration), showed that caffeine at 20 mg/kg had similar antiplasmodial activity with chloroquine (10 mg/kg). Both had chemosuppression of 53.6% and 54.36%, respectively and a survival index of 100% was recorded for both compounds. The curative experiment that followed (also with twice daily administration) further showed that caffeine compares favourably with chloroquine. Caffeine exhibited 45.92%, 72.00%, 69.87% chemosuppression as compared to chloroquine with 20.97%, 65.64%, 60.95% for 3, 5 and 7 days of treatment respectively. Caffeine's survival index was very high and much better than what was observed in the once daily administration experiment. A survival index of 93% was observed in the twice daily administration curative experiment against the 53% survival index observed in the once daily curative experiment. It is assumed that apart from the fact that the lipid-based oral delivery system ensured that the caffeine was effectively absorbed, bypassing liver first-pass, the twice daily administration also helped to sustain large concentrations of the caffeine in the blood to offset the rapid clearance that caffeine is known for.

Keywords: chemosuppression, curative, antimalaria, caffeine, purine auxotroph, bioavailability.

INTRODUCTION

Purines are the most naturally occurring nitrogen-containing heterocyclic compounds [Rosemeyer, 2004]. Purine is made up of two rings which are pyrimidine and imidazole fused together. They are found in high concentration in meat but generally low in plant-based diets [Chiu, 2020]. The naturally occurring purines include nucleobases adenine and guanine, xanthine, theobromine, caffeine, uric acid and isoguanine (Figure 1). Caffeine (1,3,7-trimethylxanthine alkaloid), is a bitter, white crystalline purine that is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Caffeine is a naturally occurring CNS stimulant [Evans, 2023] and the most used psychoactive drug in the world [Nehlig, 1999]. Caffeine has been shown to possess antifungal [Sugiyama *et al*., 2016; Kobetičová *et al*., 2020], antimicrobial [Al-Janabi, 2011], muscle relaxant, diuretics, analgesics, powerful antioxidant effect [Paşaoğlu *et al*., 2011; Carelli-Alinovi *et al*., 2016] and inhibition of monoamine oxidase, MAO [Vlok *et al*., 2006; van den Berg *et al*., 2007; Pretorius *et al*., 2008; Mostert *et al*., 2012]. Methylxanthines, including caffeine were also studied (*in-silico*) as a group by Rolta *et al*. (2022) investigating their potential to Inhibit SARS-CoV-

2 [Rolta *et al*., 2022]. There is no record of caffeine being investigated as a potential antimalarial agent except for a study by Akinyinka *et al*., 2000, that studied the effect of acute falciparum malaria on the disposition of caffeine [Akinyinka *et al.,* 2020], an investigation of how caffeine is metabolized by malaria patients (human subjects) without describing any possible antimalarial activity. This study therefore reports for the first time, the potential for caffeine to serve as an antimalarial agent using a murine model.

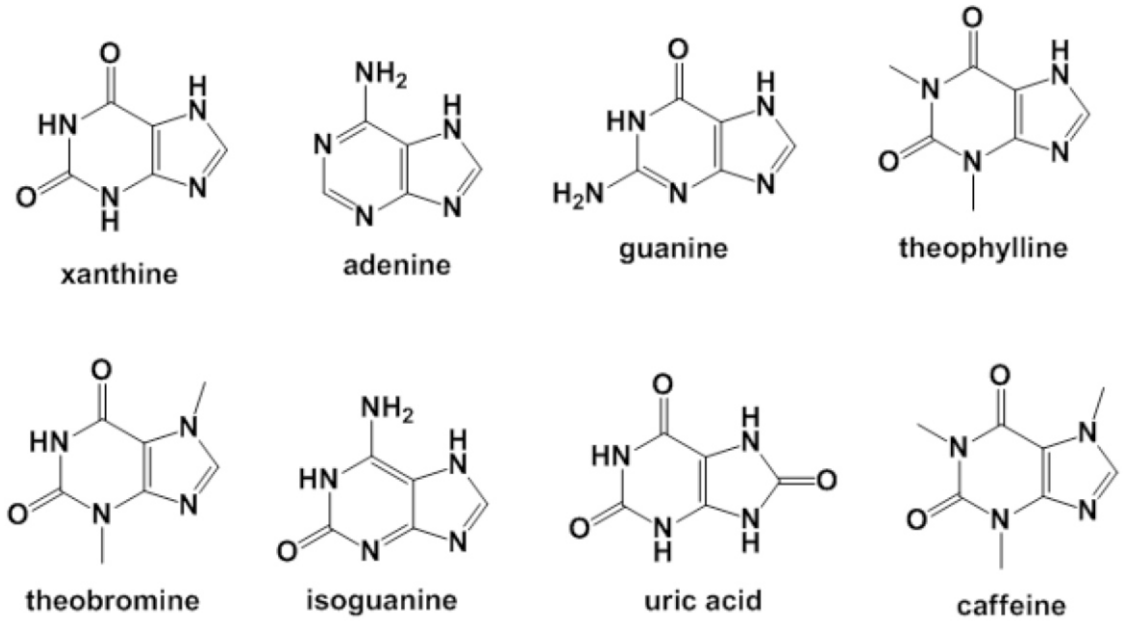

Figure 1: Common purines found in nature

Many purine derivatives have been reported in literature to possess promising antimalarial activity [Kicska *et al*., 2002; Tyler *et al*., 2007]. The malaria parasite is incapable of synthesizing purines de no vo [de Koning *et al*., 2005; Hyde, 2007] as the purine metabolic pathway of the parasite relies on salvaged nucleosides and nucleobases imported from the bloodstream of the host and transported through the erythrocyte equilibrative nucleoside transporters (ENT), thus it is the parasite's ENT that is incapable of de no vo purine synthesis [de Koning *et al*., 2005]. Hypoxanthine is the main source of purine in the parasite, which is converted upon uptake into inosine-5'-monophosphate (IMP), a precursor of all purine nucleotides. Hypoxanthine is the key precursor of other purines in *Plasmodium* metabolism and is commonly used as a nutritional supplement in malarial culture media. A key source of hypoxanthine in-vivo is from the erythrocyte purine pool where ATP is in dynamic metabolic exchange with hypoxanthine via ADP, AMP, IMP, inosine, and adenosine [Hyde, 2007]. In human erythrocytes, adenosine is efficiently salvaged by adenosine kinase (hAK). This allows human erythrocytes to utilize serum adenosine to maintain erythrocyte ATP levels. In *P. falciparum*, adenosine is salvaged by conversion to hypoxanthine using adenosine deaminase (PfADA) and purine nucleoside phosphorylase (PfPNP). Hypoxanthine is then converted to IMP by hypoxanthine-guanine-xanthine phosphoribosyltransferase (PfHGXPRT). PfADA, PfPNP, and PfHGXPRT are highly expressed proteins in the parasite [Reyes *et al*., 1982; Shi *et al*., 2004]. No AK gene has been found in the *P. falciparum* genome [Gardner *et al*., 2002]. As a result, the parasite cannot directly convert adenosine to AMP. *P. falciparum* cell growth and division demands robust purine salvage, in particular adenosine, because the parasite contains the most (A + T)-rich genome sequenced to date (~ 80%), Weber *et al*., 1987. Immucillin-H (ImmH) and DADMe-ImmG are purine-based compounds that are potent inhibitors of malaria parasite in-vitro – in the same vein some purine analogs (Coformycin, 2'-deoxycoformycin (pentostatin) and 5'-methylthiocoformycin) have also been found to possess potent antimalaria activity in-vitro as well [Kicska *et al*., 2002; Shi *et al*., 2004; Cassera *et al*., 2011; Evans *et al*., 2018;] Figure 2.

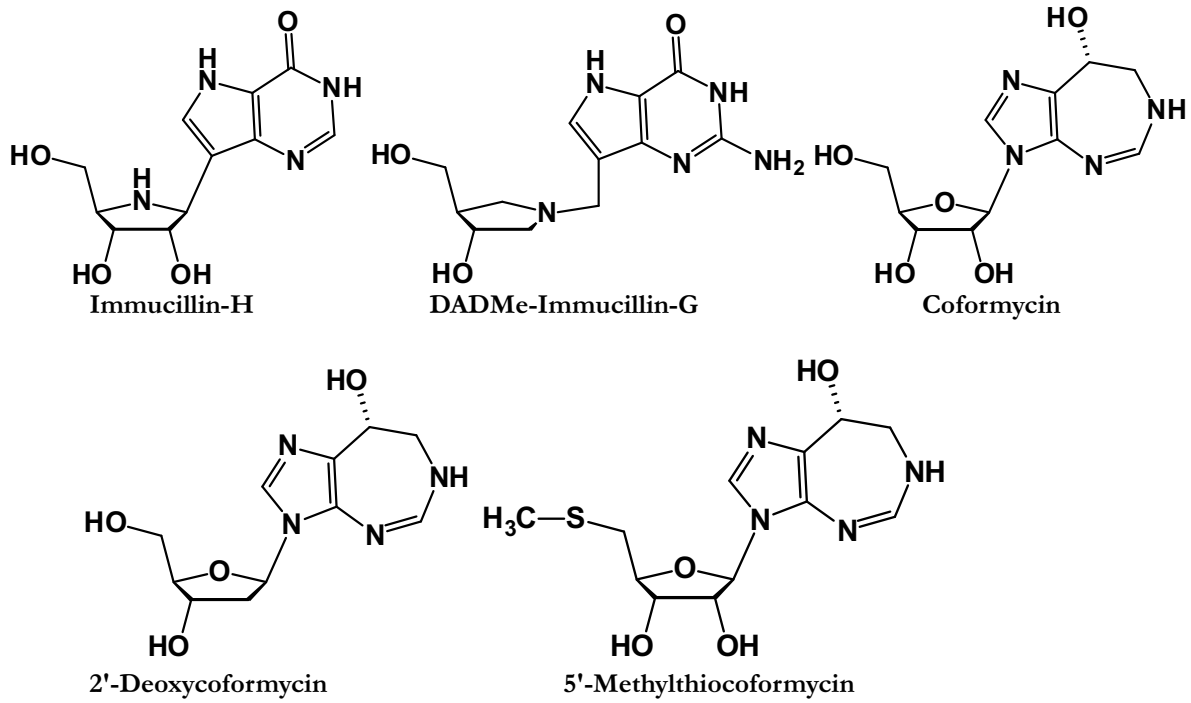

Figure 2: Purine and Purine analogs that have been found to be potent antimalarials in-vitro.

Lipophilic drugs will normally have enhanced bioavailability when administered orally with a lipid-based formulation. An alternative route for delivering drugs to the systemic circulation is the intestinal lymphatic pathway. Lipophilic drugs are known to be transported via the lymphatic system. The intestinal lymphatic pathway can bypass first-pass metabolism in the liver, thus increasing drug bioavailability (Figure 3). Furthermore, the co-administration of drugs with lipids can enhance their lymphatic transport [Ali Khan *et al.*, 2013]. In a postprandial state, lipid–drug conjugates and lipid-based nanoparticles have been widely studied for the delivery of lipophilic drugs via the lymphatic pathway. Co-administration with lipids can increase the level of drug transport through the intestinal lymphatic system and the administration of a single capsule of long-chain lipids can stimulate significant lymphatic transport of drugs. Exogenous lipids from various formulations enhance lymphatic transport by assembling into chylomicrons for endogenous lipid transport. Thus, drugs together with exogenous lipids can be delivered more efficiently to the intestinal lymphatic system through an increase in chylomicrons. Caliph *et al.*, (2000) showed lipid-based nanoparticles with long-chain lipids increased the oral bioavailability of lipophilic drugs [Caliph *et al.*, 2000]. Other studies reported that docetaxel nanocapsules consisting of long-chain triglycerides were transported in the form of lipoproteinated nanocapsules [Attili-Qadri *et al.*, 2013; Fang *et al.*, 2015]. These nanocapsules were recognized as triglyceride-rich particles in enterocytes, which resulted in them being transported through the intestinal lymphatic pathway. Chylomicron-mimicking carriers have also been developed to enhance the intestinal lymphatic pathway [Paliwal *et al.*, 2009]. Carriers made of Compritol 888 ATO and soybean PC were recognized as chylomicrons in enterocytes. Methotrexate loaded in the carriers was delivered efficiently to the systemic circulation via the lymphatic route.

When drugs are orally administered, they can bypass first-pass metabolism through the lymphatic pathway. During intestinal lymphatic drug transport, long-chain and unsaturated lipids are assembled into chylomicrons in enterocytes. These chylomicrons are then exocytosed from the cell and enter the lymphatic route. If lipophilic drugs are co-administered with these lipids, they are prone to incorporation into chylomicrons and can be delivered to the lymphatic system in the form of chylomicron–drug complexes. Thus, co-administration with lipids can enhance the lymphatic transport of lipophilic drugs. There are

several methods to enhance such lymphatic drug transport: administration during a postprandial state and the use of lipidic prodrugs and lipid-based nanoparticles.

Malaria, caused by Protozoan Parasites, remains a global health problem that is responsible for the high morbidity and economic loss in the world today [Andrade *et al.*, 2022]. The increasing resistance of the parasites to existing drugs especially the human genus *P. falciparum* and *P. vivax* creates a surging demand for the development of new antimalarial agents that not only act on new targets but have distinct modes of action.

Caffeine being a purine that is abundant in nature and consumed regularly as a beverage is being proposed as a potential antimalarial agent because the malaria parasite is a purine auxotroph and it is already established that some purine based compounds are potent inhibitors of the parasite not exactly druglike. And the lipophilic nature of the caffeine (though considered as amphiphatic in many cases) makes it a prime candidate for oral administration using a lipid formulation. Lipid-based drug delivery systems (LBDDS) which include emulsions, vesicular system and lipid particulate system [Brigger *et al.*, 2002], have attracted a lot of attention by formulation scientists as the poorly water-soluble drugs poses great challenges such as solubility, in the process of formulation and eventually, bioavailability. The LBDDS provide a safe, site specific, time-controlled delivery of drugs with different molecular weight [Brigger *et al.*, 2002; Panyam and Labhasetwar, 2003], as well as vaccines, nutraceuticals and diagnostics [Müller *et al.*, 2002]. The LBDDS also have the advantage of low cost, ease of preparation, high bioavailability and facilitates improved dissolution within the gastrointestinal tract (GIT) by imitating the pharmaceutical food effect [Kim *et al.*, 2013; Ahn and Park, 2016]. This study is therefore premised on the notion that if the Iwanaga *et al.*, 1997caffeine is delivered orally with a lipid based formulation, potent antimalarial effects might be observed.

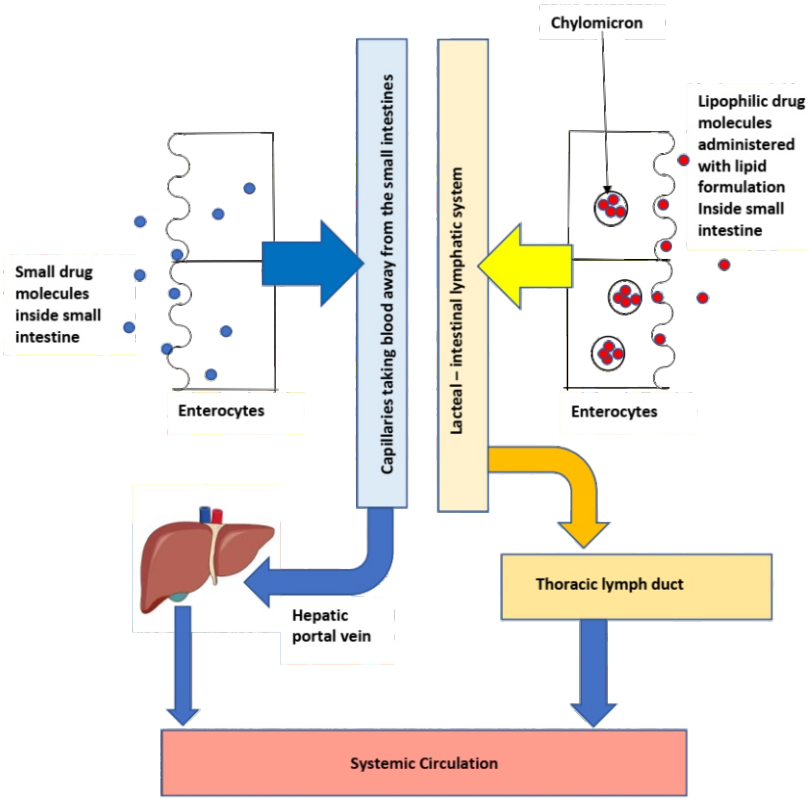

Figure 3: Diagrammatic representation of the different routes that drug molecules take to get into the systemic circulation from the small intestine. Lipid formulations trigger the formation of chylomicrons which encapsulate the lipophilic drugs and take them into the lacteal and eventually into the main circulation and bypasses the liver.

METHODOLOGY

The Parasite

Chloroquine-sensitive *Plasmodium berghei* NK-65 was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, University of Ibadan. The parasite was preserved by intra-peritoneal passaging in mice.

Experimental Mice

Adult albino mice (100) of either sex and of weight range 18-23 g were obtained from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The mice were acclimatized for five days to laboratory conditions prior to the experiment. They were allowed free access to food and water *ad libitum* throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces from cages daily. The mice were maintained and cared for according to the international guidelines for the use and maintenance of experimental animals (OECD, 2002).

Antimalarial Assays

Inoculum Preparation:

The inoculums of blood from donor with about 20-32 % parasitaemia was got by first anaesthesising the mice with chloroform, and through the cardiac puncture, using sterile syringe, blood was collected into sterile disposable syringes. The red blood cells per unit volume was calculated for the inoculums size. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final (0.2 mL) for each mouse would contain the required number of red blood cells ($1.0x10^7$ parasitised red blood cells) [Iwalewa *et al.*, 2008].

Suppressive assay (4-day schizonticidal test)

Albino mice (25) were divided into groups of five. They were all inoculated intraperitoneally with 0.2 mL of parasitized blood containing 10^7 parasites. The various doses of caffeine (dissolved in olive oil) were then administered orally to the animals: the test groups received 5, 10 and 20 mg/kg, the negative control group received 0.2 mL of the vehicle (olive oil) while the positive control group received 10 mg/kg of chloroquine diphosphate (dissolved in distilled water). The treatment continued for 4 days. **[**Knight and Peters, 1980**]**Thin smear of each mouse was done by taking a drop of blood from a tiny cut to the tail of the animal onto a slide and spreading it thinly with the aid of another slide. The smear was allowed to air-dry, then fixed with methanol. The smear was then stained with prepared Giemsa stain (diluted 1:20 with phosphate buffered saline). The slides were then individually placed on the light microscope and viewed under the ×100 objective with the aid of immersion oil. The number of parasitized red blood cells were counted against the total number of red blood cells in ten fields. This gives the % parasitaemia in each field. The average values were calculated and further the % chemo suppression.

Percentage parasitaemia in each field was calculated as follows:

$$\%P = \frac{\sum PRBC}{\sum RBC} \times 100$$

Where *P* = Parasitaemia, *PRBC* = parasitized red blood cells & *RBC* = red blood cells.

Calculation of average percentage chemosuppression or reduction in parasitaemia:

$$\%CM = \frac{\%P_{Negativecontrol} - \%P_{Testgroup}}{\%P_{Negativecontrol}} \times 100$$

Where *CM* = chemosuppression & *P* = parasitaemia

Assessment of Curative activity (Established infection)

The curative assay was performed as follows; The animals were grouped into five per group, inoculated with the parasites on first day and then left without treatment for 72 h to allow the infection to develop. Treatment with the caffeine-olive oil suspension commenced after 72 h for 5 days. **[**Ryley & Peters, 1970**]** Parasitaemia was taken every other day from the first day of treatment with caffeine till when treatment stopped.

Repeat Chemosuppression and Curative Assays with twice daily drug administration

The suppressive and curative tests were repeated as performed above (sections 3.3.2 & 3.3.3) but with twice daily administration of caffeine and

controls. The twice daily was achieved by dosing every 12 h after the time of the first dose in the experiments.

Survival index

The survival index was estimated based on the observed day of death of the animals in each test group including the negative control. The animals were observed for 28 days post infection and the day of death recorded for each animal. The formula below was used to calculate the survival index.

$$SI = \frac{D_{Test} - D_{Negativecontrol}}{D_{Max} - D_{Negativecontrol}} \times 100$$

Where SI = Survival index, D_{Test} = average day of Death for test group, D_{Max} = Maximum observation day which is 28 for this study & $D_{Negative\ control}$ = average day of Death for negative control group

Statistical analysis

The results were expressed as Mean values SEM. The analysis will be done using an Analysis of variance followed by an ad hoc test, Student's Newman Keull's test.

RESULTS AND DISCUSSION

In the baseline experiment done to check if any antimalarial activity will be observed for caffeine, it was discovered that caffeine had significantly higher chemosuppression relative to the reference drug chloroquine. Caffeine showed chemosuppression between 79 % - 82 % (within the three doses tested, 5 mg/kg, 10 mg/kg and 20 mg/kg) whereas chloroquine had a chemosuppression of 70 % (Table 1, Figure 4). This made it obvious that caffeine has potential as an antimalarial agent and that the delivery via a lipid-based drug delivery system must have enhanced its bioavailability which could have contributed to the observed high chemosuppression.

Table 1: Summary of Suppressive Assay (Once daily administration)

Test agent	Mean % Parasitaemia	% Chemosuppression
Vehicle	4.93 ± 0.83	0.00
CQ (10 mg/kg)	1.46 ± 0.63	70.39
Caffeine (5 mg/kg)	1.04 ± 0.45	78.90
Caffeine (10 mg/kg)	0.95 ± 0.20	80.73
Caffeine (20 mg/kg)	0.89 ± 0.16	81.95

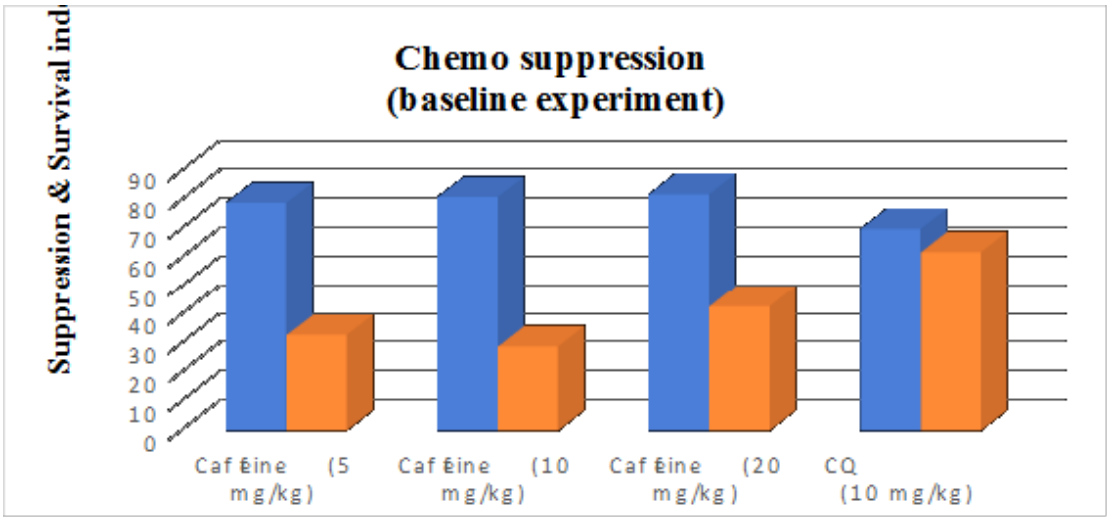

Figure 4: Chart for Chemosuppression and Survival Index from baseline experiment (the blue bar is for chemosuppression while the orange bar is for survival index.

However, the survival index observed for caffeine relative to chloroquine after monitoring the animals for 30 days shows that whatever effect the caffeine had in terms of antimalarial activity was short-lived due to the low survival index relative to that of chloroquine (Table 2 & Figure 4).

Table 2: Estimate of survival index for suppressive assay (once daily administration)

	Recorded Day of Death from Suppressive Experiment					
Dose Group	Test Animal 1	Test Animal 2	Test Animal 3	Test Animal 4	Test Animal 5	Survival Index %
Negative Control	7	7	8	7	7	-
Chloroquine	0*	0*	0*	9	9	62
Caffeine (5 mg/kg)	11	12	15	15	17	33
Caffeine (10 mg/kg)	10	7	16	16	17	29
Caffeine (20 mg/kg)	10	18	17	18	18	43

**** "0" implies animal did not die but the max number of days i.e., 28 was used to calculate the average for the test group***

Sequel to the initial chemosuppressive test with a once daily administration experiment, the curative test showed the same trend in which the observed chemosuppression for caffeine was comparable to that of chloroquine. But in the end the animals administered chloroquin had a higher survival index (100 %) compared to caffeine.

The chemosuppression observed at day 3 for 5 mg/kg and 10 mg/kg dose of caffeine was 100% (Table 3) and the same for chloroquine at 10 mg/kg.

Table 3: Summary of curative assay (once daily administration): Day 3

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	0.42 ± 0.10	0.00
CQ (10 mg/kg)	0.00 ± 0.00	100.00
Caffeine (5 mg/kg)	0.00 ± 0.00	100.00
Caffeine (10 mg/kg)	0.00 ± 0.00	100.00
Caffeine (20 mg/kg)	0.04 ± 0.02	90.48

Chemosuppression of 90 % was observed for caffeine at 20 mg/kg which was quite lower than the other two lower doses (Table 4 & Figure 5). But by day 7, caffeine at 20 mg/kg had a much higher chemosuppression (94 %) than was observed at 5 mg/kg and 10 mg/kg and comparable to that of chloroquin at day 7 (97 %). This observation shows that the 20 mg/kg dose for caffeine is the effective dose from the study (Table 5 & Figure 5). However, taking the survival index into account, approximately half of the animals in the 20 mg/kg group for caffeine died (survival index of 53 %) unlike those that were administered chloroquine in which 100 % survival index was recorded (Table 6 & Figure 5). This shows that ultimately chloroquine has a higher therapeutic index relative to caffeine (Figure 5).

Table 4: Summary of curative assay (once daily administration): Day 5

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	1.10 ± 0.46	0.00
CQ (10 mg/kg)	0.06 ± 0.04	94.55
Caffeine (5 mg/kg)	0.23 ± 0.08	79.09
Caffeine (10 mg/kg)	0.18 ± 0.08	83.64
Caffeine (20 mg/kg)	0.14 ± 0.11	87.27

Table 5: Summary of curative assay (once daily administration): Day 7.

Test agent	Mean % Parasitaemia	% Chemosuppression
Vehicle	3.84 ± 1.08	0.00
CQ (10 mg/kg)	0.11 ± 0.01	97.14
Caffeine (5 mg/kg)	0.50 ± 0.06	86.98
Caffeine (10 mg/kg)	0.53 ± 0.05	86.20
Caffeine (20 mg/kg)	0.23 ± 0.07	94.01

Table 6: Estimate of survival index, curative assay (once daily administration).

	Recorded Day of Death from Suppressive Experiment					
Dose Group	Test Animal 1	Test Animal 2	Test Animal 3	Test Animal 4	Test Animal 5	Survival Index %
Negative Control	10	10	11	11	11	-
Chloroquine	0*	0*	0*	0*	0*	100
Caffeine (5 mg/kg)	16	14	14	17	12	24
Caffeine (10 mg/kg)	18	19	19	13	14	35
Caffeine (20 mg/kg)	19	15	20	20	23	47

**** "0" implies animal did not die but the max number of days i.e., 28 was used to calculate the average for the test group.***

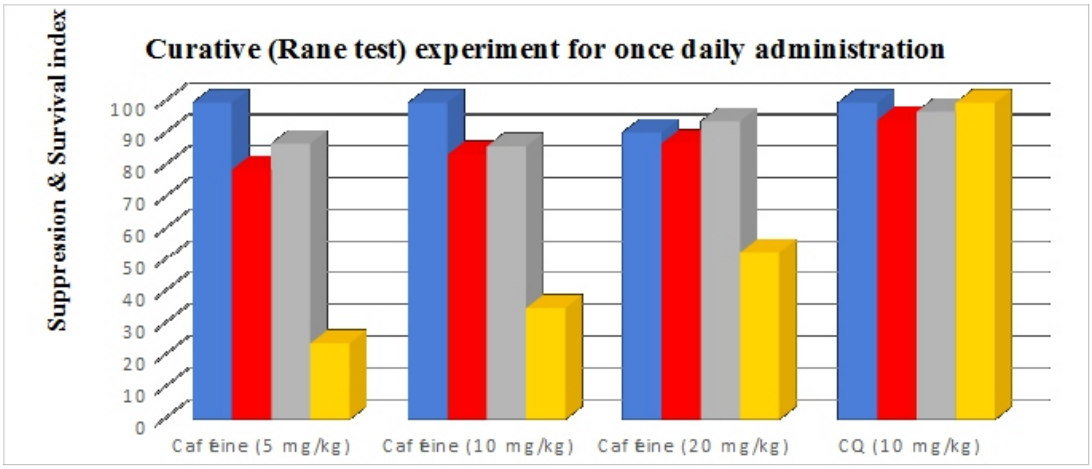

Figure 5: The Chemosupression and Survival Index from the Curative experiment in which compounds were administered once daily (the yellow bar is for survival index, while the blue, red and grey bars are the chemosuppression at day 3, 5 & 7 respectively).

Although, caffeine, administered by a lipid-based drug delivery system (in this study), appears to have high chemosuppression at 20 mg/kg and shows good potential as an antimalarial agent. A follow-up study was conducted in which the caffeine was administered twice daily (12 h interval) with the hope that if a high blood concentration of the caffeine is sustained over the 4 days dosage regime, that a better therapeutic index (relative to chloroquine) will be observed. Hence, a 4-day suppressive test with twice daily administration was conducted using chloroquine as the reference drug. The 20 mg/kg dose for caffeine was used based on the observations from the last two experiments in which caffeine had optimum activity at 20 mg/kg. The 4-day suppressive test (with twice daily administration) showed that caffeine at 20 mg/kg had similar antiplasmodial activity with chloroquine at 10 mg/kg (Table 7). Both had chemosuppression of 53.6 % and 54.36 % respectively and a survival index of 100 % was recorded for both compounds (Table 8 & Figure 6). The curative experiment that followed (also with twice daily administration) further showed that caffeine compares favourably with chloroquine.

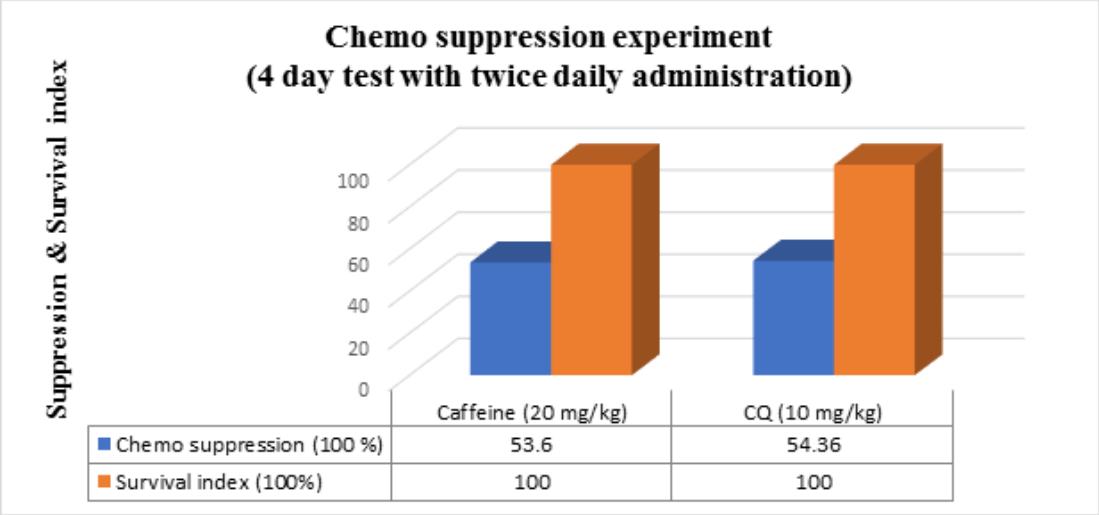

Figure 6: Chemosuppression and Survival index from chemusppressive assay in which compounds were administered twice daily (the blue bar is for chemosuppression while the orange bar is for survival index).

Table 7: Summary of suppressive assay (2 doses per day regimen at 20 mg/kg)

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	15.82 ± 1.41	0.00
CQ (10 mg/kg)	7.22 ± 0.26	54.36
Caffeine (20 mg/kg)	7.34 ± 1.29	53.6

Table 8: Estimate of survival index, suppressive assay (2 doses per day regimen at 20 mg/kg)

	Recorded Day of Death from Suppressive Experiment					
Dose Group	Test Animal 1	Test Animal 2	Test Animal 3	Test Animal 4	Test Animal 5	Survival Index %
Negative Control	8	8	10	16	18	-
Chloroquine	0*	0*	0*	0*	0*	100%
Caffeine	0*	0*	0*	0*	0*	100%

**** "0" implies animal did not die but the max number of days i.e., 28 was used to calculate the average for the test group***

Caffeine appears to have higher and better chemosuppression relative to chloroquine in day 3, 5 and 7 (Tables 9, 10 & 11). The chemosuppression recorded for caffeine was 45.92 % (day 3), 72 % (day 5) and 69.87 % (day 7) while chemosuppression for chloroquine was recorded as 20.97 %, 65.64 %, 60.95 % respectively. Caffeine's survival index was very high and much better than what was observed in the once daily administration experiment (Table 12). A survival index of 93 % was observed in the twice daily administration curative experiment against the 53 % survival index observed in the once daily curative experiment (Table 12 & Figure 7). The results obtained for caffeine at 20 mg/kg and twice daily administration shows that caffeine indeed has potential as a putatuve antimalarial agent. Especially when administered with a lipid-based drug delivery system or perhaps in a postprandial state (after meals) or with fatty food.

Table 9: Summary of curative assay: Day 3 (2 doses per day regimen at 20 mg/kg)

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	20.12 ± 2.17	0.00
CQ (10 mg/kg)	15.9 ± 0.82	20.97
Caffeine (20 mg/kg)	10.88 ± 1.50	45.92

Table 10: Summary of curative assay: Day 5 (2 doses per day regimen at 20 mg/kg)

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	16.82 ± 1.83	0.00
CQ (10 mg/kg)	5.78 ± 1.48	65.64
Caffeine (20 mg/kg)	4.71 ± 0.82	72.00

Table 11: Summary of curative assay: day 7 (2 doses per day regimen at 20 mg/kg)

Test agent	**Mean % Parasitaemia**	**% Chemosuppression**
Vehicle	13.11 ± 0.69	0.00
CQ (10 mg/kg)	5.12 ± 1.26	60.95
Caffeine (20 mg/kg)	3.95 ± 1.55	69.87

Table 12: Estimate of survival index, curative assay (2 doses per day regimen at 20 mg/kg)

	Recorded Day of Death from Suppressive Experiment					
Dose Group	Test Animal 1	Test Animal 2	Test Animal 3	Test Animal 4	Test Animal 5	Survival Index %
Negative Control	10	12	12	16	14	-
Chloroquine	0*	0*	0*	0*	0*	100
Caffeine	0*	24	0*	0*	26	93.33

**** "0" implies animal did not die but the max number of days i.e., 28 was used to calculate the average for the test group***

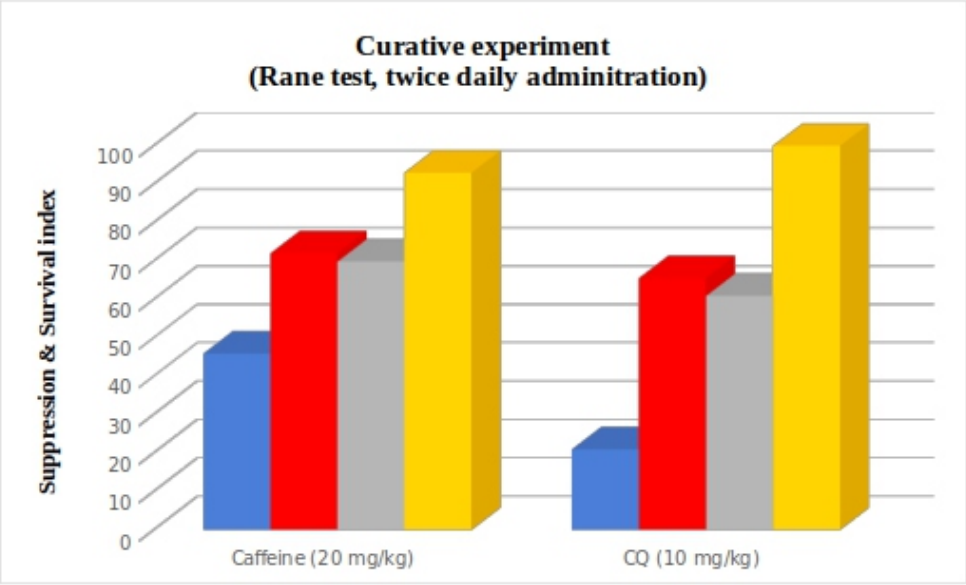

Figure 7: Chemosuppression and Survival Index from curative experiment in which compounds were administered twice daily (the yellow bar is for survival index, while the blue, red and grey bars are the chemosuppression at day 3, 5 & 7 respectively)

It is worthy to note that previous studies on the pharmacokinetics of caffeine, particularly its absorption profile after oral ingestion shows that 99 % of ingested caffeine (mostly in the form of coffee and other preparations, taking into consideration that coffee beans is rich in lipids as well) is found in the blood roughly 45 mins later [Magkos & Kavouras, 2005, Eteng et al., 1997, Mumford et al., 1996, Liguori et al., 1997, Marks and Kelly, 1973, chard Blanand Sawers, 1983, Institute of Medicine (US) Committee on Military Nutrition Research, 2001]. This suggests strongly that caffeine does not pass through the liver to get to the main circulation since the liver first-pass effect is not observed [Institute of Medicine (US) Committee on Military Nutrition Research, 2001] which implies that caffeine prefers the lymphatic system for absorption in the gastrointestinal tract. Previous studies also point to the fact that, peak plasma concentrations may be influenced by route of administration, the form of administration, or other components of the diet.

It has also been established that the absorption rate constant of caffeine is influenced by the physicochemical properties of the dose formulation, including pH, volume, and composition [Bonati *et al*., 1982]. For example, caffeine absorption is faster from a gum than from a capsule, from a capsule than from coffee, cola, or chocolate and from coffee (and tea) than from cola [Mumford et al., 1996, Marks and Kelly, 1973, Bonati *et al*., 1982, Kamimori et al., 2002, Fredholm et al., 1999]. However, these differences are not always observed. Therefore, in a bid to ensure that the administered caffeine (in this study) gets into the main circulation in all experimental cases, the lipid-based drug delivery system was used. The lipid formulation involves using olive oil as the vehicle, being an 18-carbon triglyceride fatty acid, it is expected that this will trigger the formation of chylomicrons [Ahn and Park, 2016, Shrestha et al., 2014, Pund, 2021]. These chylomicrons are transported to the Golgi and exocytosed from the enterocyte. Finally, the chylomicrons enter the lymphatic route and circulate throughout the body [Ahn and Park, 2016]. This ability to use the lymphatic route by the drugs allows the drugs to escape first-pass metabolism which would allow for high bioavailability after oral administration. Chylomicron levels in the intestinal lymphatic duct increase substantially after a meal or feeding on fatty food. Fatty food enhances intestinal lipoprotein synthesis and the access of drugs to the intestinal lymphatic system. When lipophilic drugs are co-administered with fatty food, their lymphatic transport increases dramatically. Khoo *et al*., (2003) investigated the antimalarial drug halofantrine regarding intestinal lymphatic delivery in a canine model. The authors discovered that the long chain (C_{18}) glyceride lipids stimulated the intestinal lymphatic transport of halofanthrine. This study also used a C_{18} glyceride lipid (olive oil) for the formulation of Caffeine, building on existing knowledge as presented by Khoo *et al*., (2003), to ensure that the caffeine administered many cases for the administered caffeine relative to chloroquine (particularly on the first day of administration) and the improvement in the observed therapeutic index after twice daily administration experiments may not be far-fetched from is maximally absorbed/transfered into the lymphatic system via the intestines. High chemosuppression were observed in the lipid-based formulation used, in conjuction with the latent ability of the caffeine to inhibit the plasmodium in the blood stream, and consequently, have a curative effect similar to that of chloroquine in the murine model.

CONCLUSION

This study shows that caffeine has potential as an antimalaria agent. The chemosuppression observed for caffeine (administered with a lipid-based formulation for delivery) is quite high and comparable to that of chloroquin particularly when administered twice daily to sustain the plasma concentrations for longer and consequently, increase the exposure of the plasmodium to the caffeine in both suppressive and curative experiments. Caffeine may therefore be considered for further testing to determine its toxicity profile in the presence of the parasite and as a drug for treating malaria within the framework of drug repurposing and also as a compound that can be co-administered with other drugs for better results and in a bid to slow down the incidence of parasite resistance through combination therapy. The authors also plan a follow-up study in which synthetic derivatives of caffeine will be investigated for potential antimalaria activity.

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STATEMENT OF HUMAN AND ANIMAL RIGHTS

The Health Research Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife assessed the plan for use of experimental animals in this study and gave a clearance on ethics along with a certificate of clearance with the assigned number being IPHOAU/12/1463.

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