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Cetirizine and liposomal Inhibitory Effect on *Plasmodium berghei* P-Glycoproteins and Chloroquine Induced Pruritus

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Abstract

Chloroquine been the mainstay of malaria chemotherapy in the world for decades but lost its efficacy against resistant strains of *Plasmodium* and also individual discontinuation of therapy due to associated chloroquine-induced pruritic side effects. This increasing resistance by *Plasmodium* parasite against antimalarial drugs led to the search for new antimalarial drugs with cost-effective approach. The interaction of chloroquine with cetirizine as chloroquine resistance modulator was examined and also the drugs were encapsulated in liposome for co-delivery in enhancing antimalarial activity. The drugs were encapsulated in liposomal vesicle by active loading technique (ion trapping method).

In vivo model study was carried out to evaluate the antimalarial activities against chloroquine resistance strains of *P. berghei* (ANKA) and antipruritic effects against chloroquine-induced pruritus. Cetirizine proved to either possess intrinsic anti-malarial activity or restoring the activity of chloroquine against the *Plasmodium* resistance strain by binding competitively to parasite p-glycoproteins to inhibit chloroquine efflux. Cetirizine as H1 tricyclic anti-histamine have the tendency of binding to the alkyl amino side chain of chloroquine to increase its lipophilicity and prevent efflux. Cetirizine and liposome had shown to be protective against *Plasmodium* parasites induced anaemia, and chloroquine-induced pruritus when used together with chloroquine in treatment of malaria. Liposome nanoparticle drug delivery vesicle proved to be effective as site targeting dosage form and addition of stealth (PEG) improved tissues penetration.

This investigation proved cetirizine as an adjunct in modify the efficacy of chloroquine as a first line antimalarial drug in use and in pregnancy. This combination was seen to be protective against *Plasmodium* parasites induced anaemia, chloroquine-induced pruritus and can be promising in resolving cerebral malaria of severe *P. falciparum* complication with more benefit achieved in a liposomal vesicle.

Keywords: Chloroquine, Resistance, Plasmodium, Cetirizine, Liposome, Anti-malaria

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1. Introduction

Malaria is an important parasitic disease in terms of human suffering. About 3.3 billion people of the world's population are at risk of malaria and accounts for 20% of all childhood deaths every 45 seconds [1]. The control of malaria is threatened by inadequate resources and drug resistance of which Africa carries the greatest burden of Plasmodium falciparum malaria mortality and morbidity worldwide [2-3].

Chloroquine (CQ) of family quinolines; member of the drug class 4-aminoquinoline discovered in 1934 [4] as malaria chemotherapy used in African countries for decades have emerged with therapeutic failure due to the development of many resistant strain of Plasmodium falciparum in the past twenty years and disruption of treatment associated with pruritic side effect [5-6]. Artemisinin-based combination therapies (ACTs) have been used to address the resistant problem associated with chloroquine and other old therapies [7]. These ACTs have proved very successful in clinical use but have serious economic implications to the third world countries where malaria was seen as poverty-related disease (PRD) and have raised questions regarding long term sustainability in the absence of international aid [3].

The search for effective antimalarial agents is ongoing and lead to the need of design and development of effective and affordable antimalarial agents or chloroquine resistance modulators for chemotherapy and chemoprophylaxis from compounds which have the ability to restore the anti-neoplastic effect in multi-drugs resistant (MDR) cancer cells [8-9].

In different clinical trials conducted with chemo-sensitizing modulators, verapamil, phenothiazine, and tricyclic anti-depressants were found to be unsafe for use [10] at the concentrations required. Some tricyclic anti-histamines like chlorpheniramine, promethazine, astemizole, and desmethylastemizole were found to be potent inhibitors of chloroquine efflux [11-13]. The H1 tricyclic anti-histamines can alter the pH of the acidic food vacuole of Plasmodium falciparum and also compete for the CQ binding site in PfCRT to inhibit CQ efflux [14]. Second-generation H1 tricyclic anti-histamines as P-gp substrate may also bind to PfMDR of Plasmodium falciparum to inhibit CQ efflux [15].

Liposome can be used to encapsulate the drugs for easy diffusion of drugs across biological membranes and for targeting [16-19]. Sterols (ergosterol and cholesterol) are also incorporated in liposome to increases the rigidity, stability and decrease toxicity of liposomes but may decrease activity due to tight drug-sterol binding which impedes the exchange of the drug with target membranes [20]. Due to liposomes are easily detected by the body's immune system; specifically, the cells of reticuloendothelial system (RES), further advance researches were constructed with PEG (Polyethylene Glycol) studding the outside of the membrane known as "stealth liposomes" [21-22]. The stealth which is inert in the body, allows for longer circulatory life for the drug delivery mechanism and most stealth liposomes also have some sort of biological species attached as a ligand to the liposome, to enable binding via a specific expression on the targeted drug delivery site [23].

Chloroquine-induced pruritus

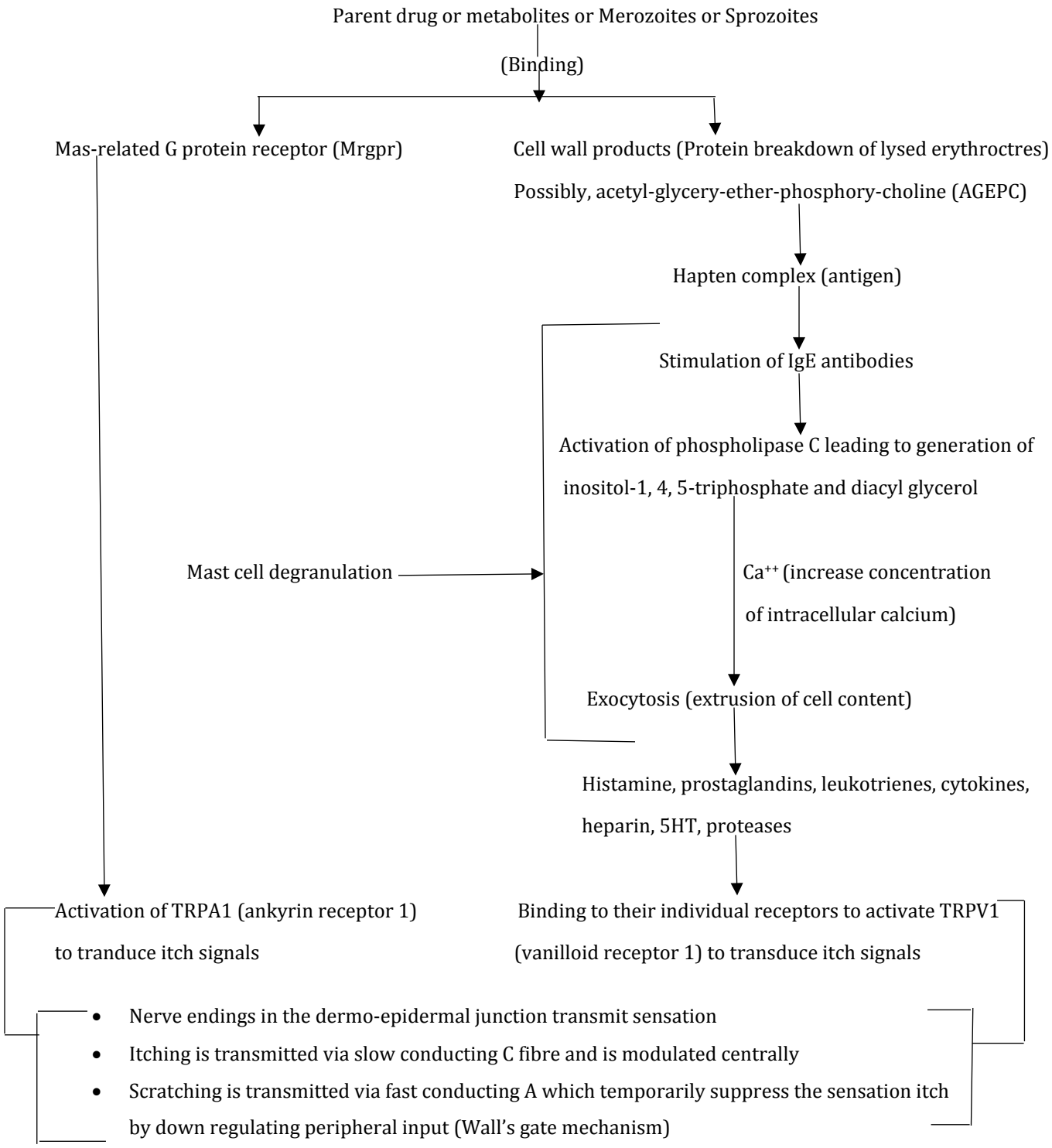
Chloroquine-induced pruritus described as a ‘biting’ or stinging sensation which is also experienced with halofantrine and amodiaquine with less intensity remains one of the most common side-effects in the use of chloroquine in the prophylaxis and treatment of uncomplicated malaria before the advent of artemisinin-based combination therapies [24]. It has been reported in 50 % of dark skinned Africans, Caucasians and African albinos with numbers of melanocytes but opposed to keratinocytes varying from a tolerable to intolerable intensity. Chloroquine-induced pruritus may result to disruption of treatment and development of chloroquine resistant strains of Plasmodium falciparum, thus leading to therapeutic failures. Chloroquine-induced pruritus is not associated with skin lesions or systemic manifestation and may resolve spontaneously within several days after the drug is discontinued [6]. The transient receptor potential channels (TRPs) which respond to chemical irritation and temperature is involved in chloroquine-induced pruritus. Chloroquine activates TRPV1 (vanilloid receptor 1) which responds to histamine induced itch (35 % chloroquine-induced pruritus) and TRPA1 (ankyrin receptor 1) which responds to histamine independent itch (65 % chloroquine-induced pruritus).

Chloroquine-induced pruritus takes different complex mechanisms that can be at play alone or together in a susceptible individual due to roles played by some factors responsible for the induction as well as exacerbation of pruritus. These factors includes: hereditary factors such as glucose-6-phosphate dehydrogenase enzyme deficiency and reduced frequency of the sickle cell trait [24,25-28], age [27,29], racial and skin pigment factors [6, 30-39], density of parasitaemia [6,38,40], chloroquine metabolites [41-42], mediators such as bradykinin, prostaglandins, and various

neurotrophins and proteases[43-45], dosage form of chloroquine [46-47], species of plasmodium[6,38] and impurities and excipients in commercial preparation.

Chloroquine or its metabolite (monodesethylchloroquine) acts as haptens binders to break down products of parasitized erythrocytes [40] or possibly interacting with acetyl-glyceryl-ether-phosphoryl-choline (AGEPC), a new class of phospholipids implicated in many aspects of allergy and inflammation [41]. This resulting complex act as an antigen, triggering reagenic antibodies (IgE) that bind to the surface of mast cells and basophils. These IgE molecules interacts with signal transduction system TRPV1 in the membrane of sensitized cell [48]. Upon subsequent exposure to TRPV1, the antigen bridges the IgE molecules and causes activation of phospholipase C, leading to the generation of inositol-1, 4, 5- triphosphate and diacylglycerol and elevation of intercellular calcium (Ca2+). These final events trigger the extrusion of the content of the mast cell granules (degranulation) by exocytosis. The mechanism by which the rise in Ca2+ leads to fusion of secretory granules with plasma membrane is not fully elucidated but it likely to involve activation of Ca2+/calmodulin-dependent protein kinase and protein kinase C [43]. Several mediators such as histamine, kallikrein, bradykinin, substance P, prostaglandins and various proteolytic enzymes have been implicated in itching [44-45]. These mediators induce or aggravate itch by direct or indirect actions on the sensory nerves. A wide variety of mediators released during allergic response may explain the ineffectiveness of a single drug therapy to inhibit multiple mediators.

Another possible mechanism is binding of chloroquine or its metabolite to the Mas-related G protein receptor (Mrgpr) at the region of dermo-epidermal junction, mainly expressed in sensory neurons to activate TRPA1 an irritant sensor highly expressed on nociceptive neurons which transmit itching sensation via slow conducting ‘C’ fibres centrally. Scratching is transmitted via fast conducting ‘A’ fibers which temporally suppress the sensation peripheral input (wall’s gate mechanism) [26]. MrgprA3 is the CQ receptor in mice and MrgprX1 in human orthologue [49].



Schematic I: Cascade of events showing possible mechanism of chloroquine-induced pruritus

The effectiveness of anti-pruritus agent has been difficult due to these complex physiologic mechanisms involved in chloroquine induced pruritus. Efforts to ameliorate this burden have necessitated the use of drugs of diverse pharmacological classes either alone or as a combination. These drugs includes: anti-histamines, corticosteroids [50], Opioid antagonists like naloxone and naltrexone [51-53], Anxiolytics and sedatives like diazepam [54], Dapsone [35], TRP antagonists like TRPV1 antagonist which includes urea, thiourea, or amide groups typical of the classic TRPV1 ligands and the imidazole derivatives and TRPA1 antagonist includes alcohols, amino ketones, prolines and aminoacid derivatives, pyrimidinedione/ xanthines based compounds, decalins, oximes, thioureas and various other structures such as small molecules [55-56].

Cetirizine as an antihistamine (second generation H1-inverse agonists) with less or no sedative side-effect is a piperazine derivative (diphenylmethyl-piperazine group)/ carboxylic acid metabolite with a racemic mixture of R and S enantiomers, synthesized from hydroxyzine with carboxyl group and also a synthesis of alpha-aminoacid derived chiral components such as aldehyde and isocyanoacetate [57-58]. Long-term use of cetirizine by children with atopic dermatitis have no impact on their behavioural, cognitive and psychomotor development [59].

Liposomes an amphipathic molecule that have a hydrophilic head group and a hydrophobic tail group undergo several studies with the aim of decreasing drug toxicity and unwanted side effects by targeting specific cells [60-62]. Toxic drug side effects and the development of resistance to current drug regimens had been limited by encapsulation within liposomes to alter the dose and distribution of drugs within the body especially when so called “stealth” liposomes are used [63-64]. Liposome intracellular delivery bypasses chloroquine transponder, pass through cell membrane by alternative mechanisms such as membrane fusion or entrapment of chloroquine in pH-sensitive targets and minimize interaction with the sensory receptors [65]. Prolonged use of chloroquine competitively inhibits breaking down and removal of waste and damaged or excess lipids from cells by lysosome containing a particular protein (saposin B) that have affinity for both natural lipids and chloroquine [66]. There is possibility of these damaged lipids and waste from the cell to form hapten complex (antigen) with chloroquine which bridges the IgE molecules (antibodies) to activate mediators in transduction of chloroquine-induced itching.

2. Material and methods

Collection of materials

Chloroquine phosphate (99.5 % pure) was donated by Juhel Nigeria Ltd, Enugu, Nigeria. Cetirizine hydrochloride (99.5 % pure) was donated by Pharmatex Nigeria Ltd, Lagos, Nigeria. Promethazine (99.0 % pure) was donated by Rico Phamaceuticals Ltd, Onitsha, Anambra State, Nigeria. Artemether and Lumefantrine were donated by Alben Pharmaceutical ltd, Ogidi, Anambra State Nigeria.

Excipients and solvents

Soybeans phosphatidylcholine (Sigma Aldrich, UK) and Cholesterol (Keshi, Xindu, China), Acetone (Guangdong Guanghua Sci-Tech Co., Ltd, China), Distilled water, Normal saline, 60 % citrated buffer acetone solution (pH 3.6) Sigma-Aldrich (sigma-854), Ammonia-ammonium chloride buffer solution (pH 8.5).

Animals

About 4 - 8 weeks old Swiss albino mice (13 – 34 g) and 7 - 10 weeks old Swiss albino rat (100 – 165 g) were obtained from the Laboratory Animal House of the Department of Pharmacology, University of Nigeria, Nsukka. They were housed in standard conditions and were maintained on a standard pelleted feed and water *ad libitum*.

Equipment

Water distiller (Bhanu Scientific Instrument, Bangalore), Rotary evapourator (Barloworld Scientific Ltd, UK), Ultrasonic water bath sonicator (Grant Instruments Ltd, Cambridge, England), Centrifuge (Canfort Laboratory and Education Suppliers Co., Ltd, China), Lyophillizer (York Scientific Industries Pvt Ltd, India).

Ethical approval

The proposed thesis was reviewed and approved by the appropriate ethics committee of Chukwuemeka Odumegbu Ojukwu University Teaching Hospital Amaku (COOUTH), P.M.B; 5022, Awka, Anambra State, Nigeria with reference number COOUTH/AA/VOL.I.031.

Liposomes loading of chloroquine and cetirizine using active loading technique (ion trapping method)

Different batches of chloroquine and cetirizine loaded liposomes were formulated using both active loading technique with the formula below.

Table 1: Formula for the liposome batches.

Ingredients	Liposomes			Stealth liposomes		
Batches	A1	B1	C1	A2	B2	C2
SPC	6000	6000	6000	6000	6000	6000
Cholesterol	1200	1200	1200	1200	1200	1200
PEG-6000	-	-	-	600	600	600
Chloroquine phosphate	200	200	-	200	200	-
Cetirizine Hcl	3	-	3	3	-	3

Soybeans phosphatidylcholine and cholesterol were dissolved in 50 ml citrate buffer acetone solution and 50 ml acetone in the ratio of 5:1. The dispersion was sonicated for 20 min to convert to w/o emulsion at room temperature and acetone gently evapourated with the aid of rotary evapourator 40 ^{0}C for 10 – 15 min to get multi-lamellar vesicle (MLV). The drugs were dissolved in 50 ml of distilled water and 50 ml of ammonia-ammonium chloride buffer which was used to hydrate the dispersion. The dispersion was further hydrated with 200 ml distilled water and placed in water bath sonicator at 40 ^{0}C for 30 min which trapped the drugs in the aqueous solution at the hydrophobic core of the phospholipids. The dispersion was further sonicated for 15 min for size reduction and placed in refrigerator at 4 ^{0}C overnight to mature. The dispersion volume was noted, centrifuged at 2000 rpm for 30 min and decanted to remove the unentrapped drugs. The noted volume was made up with distilled water, centrifuged and decanted which was repeated up to 3 times.

Determination of particle size and zeta potential

The mean hydrodynamic diameter and polydispersity of liposomes were determined by the dynamic light scattering technique (DLS) in a Malvern Zetasizer Nano ZS equipped with a 633 nm laser source. The analysis were performed at room temperature (26 ℃) and a scattering angle of 90° after the appropriate dilution of 1 ml liposome dispersion with 10 ml water and filtered with double 0.1 µm filters. Each value given will be the average of three measurements.

In vivo* anti-malarial treatment against *Plasmodium berghei

Parasite inoculation and treatment oral route of administration:

The blood schizontocidal action of different formulations of chloroquine and cetirizine against *P. berghei* was performed using a 4-day curative standard test [67-68]. The mice were randomly divided into eleven groups (1 - 11) of five (5) mice each. They were all infected intraperitoneally with 1 x 10^7 *P. berghei* –infected blood cells in a volume of 0.1 ml diluted in phosphate buffer saline (pH 7.2) [69]. Group 1(negative control) received just distilled water and pellets. Groups 2-11 were treated with A1, A2, B1, B2, C1, C2, CQ, CTZ, CQ+CTZ and ART. Treatments were performed daily for 4 consecutive days. The animals were observed at day 5 after treatment and thin blood smears were prepared from each mouse, fixed with methanol and stained with Giemsa stain [70-71]. The parasitemia count was determined by microscopic examination with the percentage count of the effected cell against the normal.

Collection of blood and analysis of haematological parameters.

Blood samples was collected at the 3rd day after the inoculation of the *P. berghei* –infected blood and after the treatment with different formulations. The blood samples were obtained from the optical plexus of the mice using a heparinized (plain) haematocrit capillary and the determination of the packed cell volume, haemoglobin concentration, red blood cell count, was done according to [72].

1.Packed cell volume: Haematocrit capillary tube was filled with blood by placing one of the open ends of the tube in the blood bottle and tilting it at an angle about 30o. One end of the filled capillary tube was sealed with a plastercine and the tube centrifuged for 20 minutes at 300 rpm in a haematocrit centrifuge. A haematocrit reader was used to read off the length of the packed cell in percentage

2.Haemoglobin concentration: Sahli haemoglobin metre was used for the determination of haemoglobin concentration. Up to the ten (10) mark of the sahli tube was 0.1N Hcl placed. With the Sahli blood pipette, 20µl of blood was placed into the Sahli tube and was sucked up and down. The mixture was allowed to stand for 5minutes for the formation of acid haematin. The dark mixture formed was diluted gradually with distilled water till the colour when compared with that in the haemoglobin metre is slightly darker than the standard. The dilution continued till the colour turns exactly and slightly paler than the standard. The volumes of the noted colour change were taken and the average of the of the slightly darker and paler were compared and was ensure that the variation is not more than ±5 for the average value to be adopted. The average value was applied in the formula below:

$X(Y)/100$. Where X is the 14g Hb in 100ml of blood, y is the average value of dilutions.

3.Red blood cell count:
The following equipment were used for the experiment; the microscope, haemocytometer (counting chamber), red cell pipette, ringer solution, cover slip, the apparatus was set up and procedure according to Evans David [73] were followed strictly. Using the dilution pipette with RED mixer from haemocytometer kit, blood was drawn up to the 0.5 mark. Continuing to hold the pipette as horizontal as possible, draw Ringer's solution diluent up to the 101 mark. (Dilution of 1 to 200. The tip of the pipette was seal with the finger and agitated well to mix. Half of the content of the pipette was emptied into a waste container and a small amount of the diluted blood was placed into one chamber of the haemocytometer. The preparation was allowed to sit for a minute (for cells to settle). The centre of the grid was focused with 100x objective, and was counted with 400x objective. The count of each five fields (each with 16 smallest squares) with a clicker (fields: top R & L, bottom R & L, centre) was noted. Include in the count all cells touching left and bottom sides, ignore cells touching top and right sides. The RBCs was calculated by adding the cells in the 5 groups and multiplying by 10,000 (i.e., add four zeros).

***In vivo* anti-pruritic treatment against chloroquine-induced pruritus**

Male albino rats (7 weeks old) were weighed and recorded, followed by shaving of the neck region and proper removal of hairs from the rostral back of the rats using depilatory cream (Veet cream) which was later wiped with water. The drugs (CQ, CQ+CZ, CQ+PMZ, B1, B1+C1, B1+CZ) were dissolved/suspended in normal saline and appropriate dose injected subcutaneously at the shaved neck on the back side. The rats were individually placed in an observation glass chamber mounted with video camera on top for recording over a span of 30 min. Scratching bouts which was defined as repetitive fast movement of the hind paws, rubbing the neck or area of injection, were counted manually from the video recording for period of 30 min. Scratching on other body region/ facial rubbing was disregarded. Promethazine (PMZ) was used as the reference standard.

Statistical analysis

Analysis of Variance (one-way ANOVA) was performed using graph pad prism to determine the statistical significance ($p \leq 0.05$ and $P \leq 0.01$) for activities of the drugs ($n = 5$) followed by post hoc test under various experimental conditions.

3. Results

The results and the experimental analysis carried out are represented in figures and tables below:

Table 2: Particle Size and Zeta Potential of Lyophilized Liposome using zetasizer machine (Malvern Panalytical, UK) at Room Temperature 26 ℃

Batches	Z average size (d.nm)	Polydispersity index
A1	907 ± 3.56	0.822
A2	824 ± 6.57	0.96
B1	1201 ± 6.7	0.483
B2	1039 ± 5.18	0.68
C1	1030 ± 0.56	0.805
C2	940 ± 3.04	0.822

***In vivo* anti-malarial treatment against *Plasmodium berghei* ANKA**

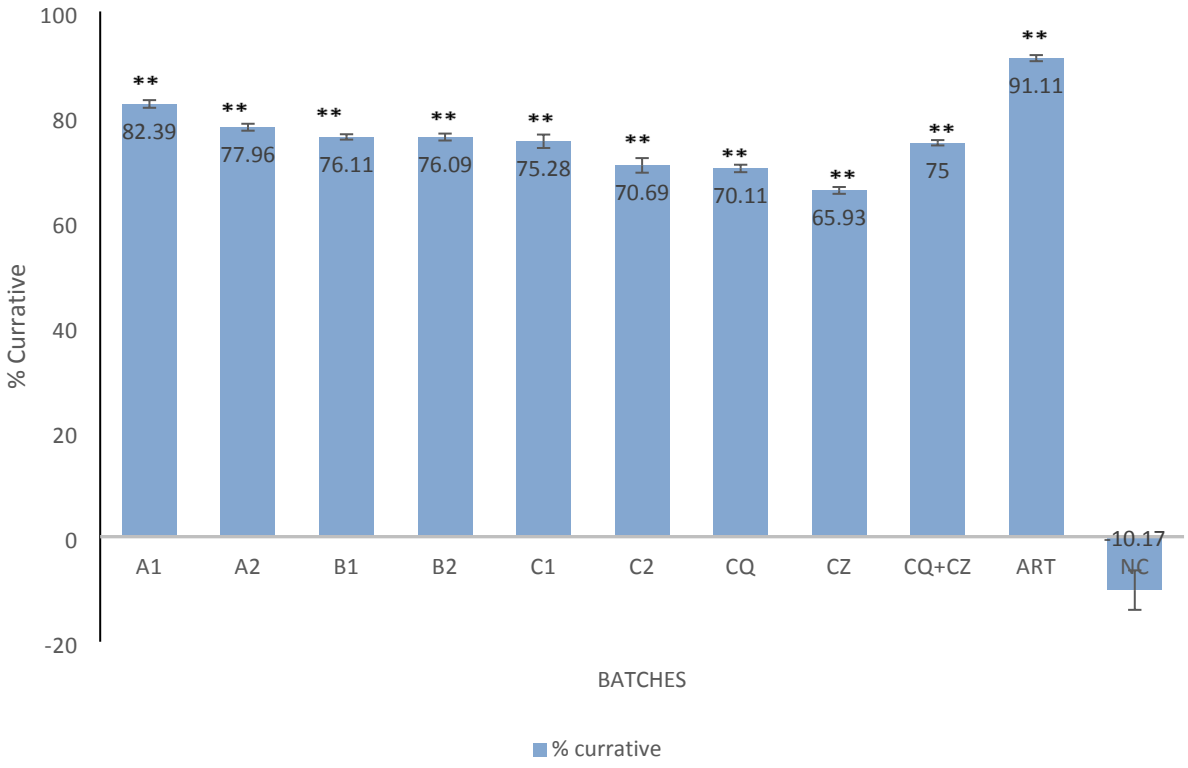


Fig 1: Anti-malaria activity of drug formulations on *P. berghei* ANKA

Results were presented as mean ± SEM, n= *P ≤ 0.05, **P ≤ 0.01: Significantly difference from negative control (NC).

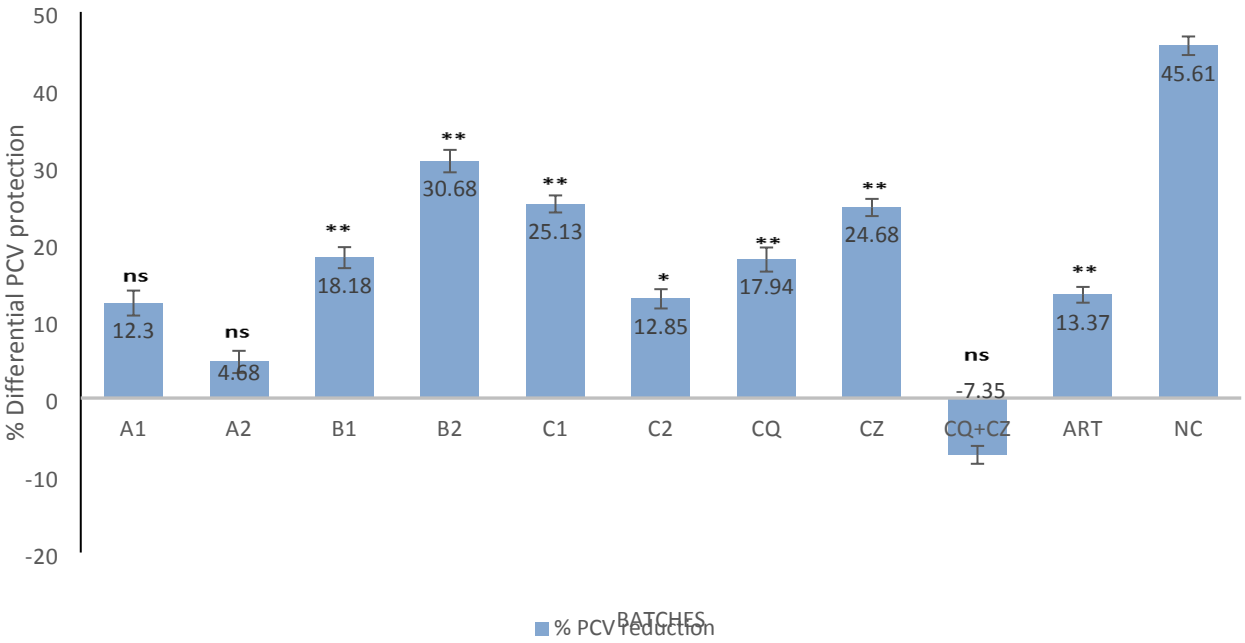


Fig. 2: Platelet cell volume count after treatment against *P. berghei* ANKA

Results were presented as mean ± SEM, n= *P ≤ 0.05, **P ≤ 0.01: Significantly difference from negative control (NC).

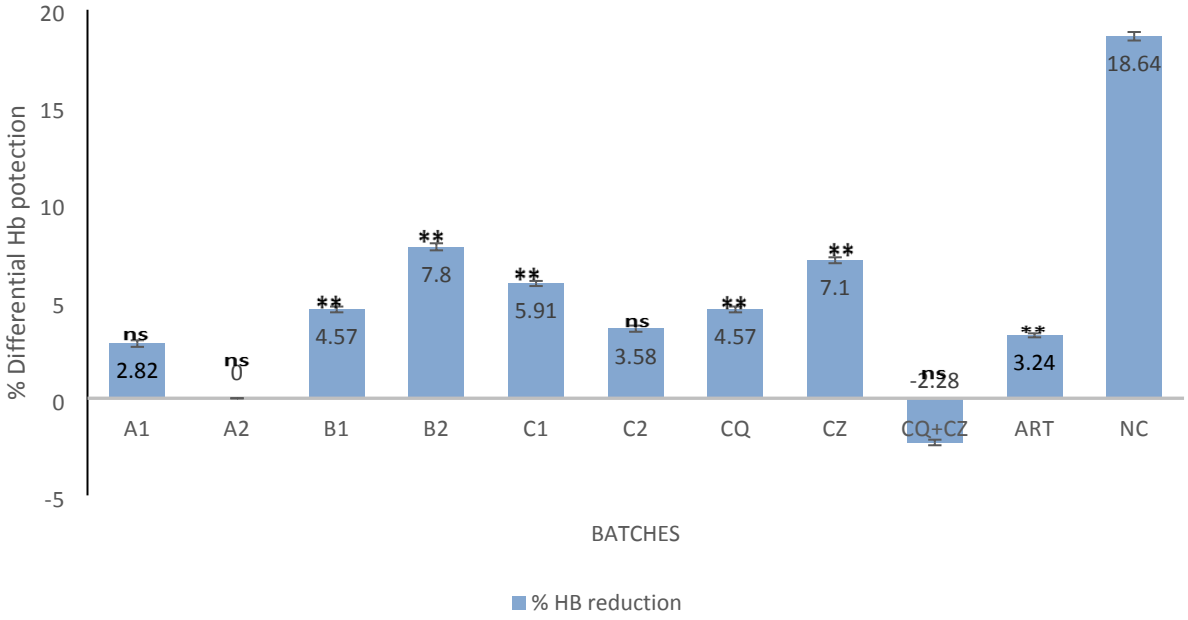


Fig. 3: Hemoglobin count after treatment against *P. berghei* ANKA

Results were presented as mean ± SEM, n= *P ≤ 0.05, **P ≤ 0.01: Significantly difference from negative control (NC).

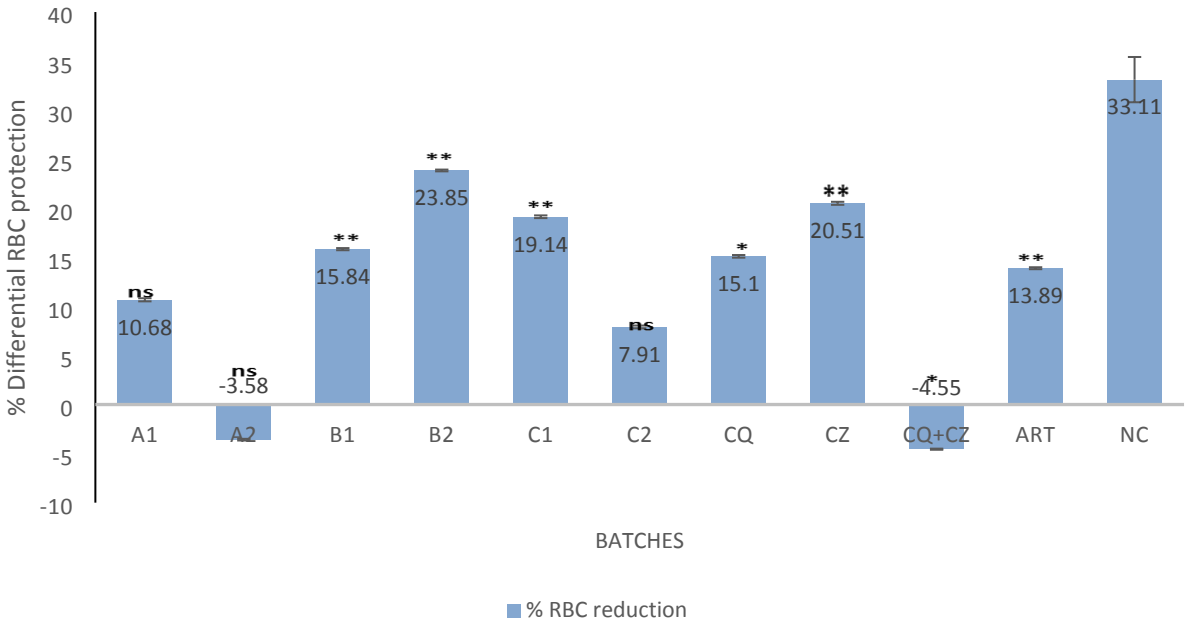


Fig. 4: Red blood cell count after treatment against *P. berghei* ANKA

Results were presented as mean ± SEM, n= *P ≤ 0.05, **P ≤ 0.01: Significantly difference from negative control (NC).

3.1 *In vivo* anti-pruritic treatment against chloroquine- induced pruritus

The onset of itch and scratch count observed with the drugs are presented in fig. 2 and fig. 3 respectively.

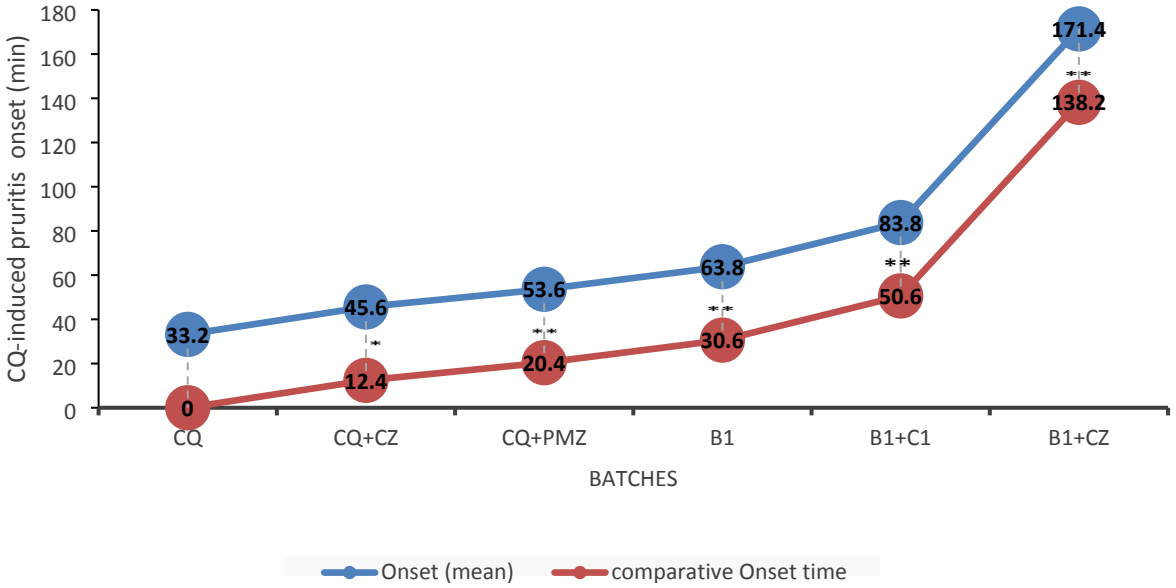


Fig. 5: Chloroquine-induced pruritus onset time

Results were presented as mean ± SEM, n= *P < 0.05, **P < 0.01: Significantly difference from chloroquine (CQ).

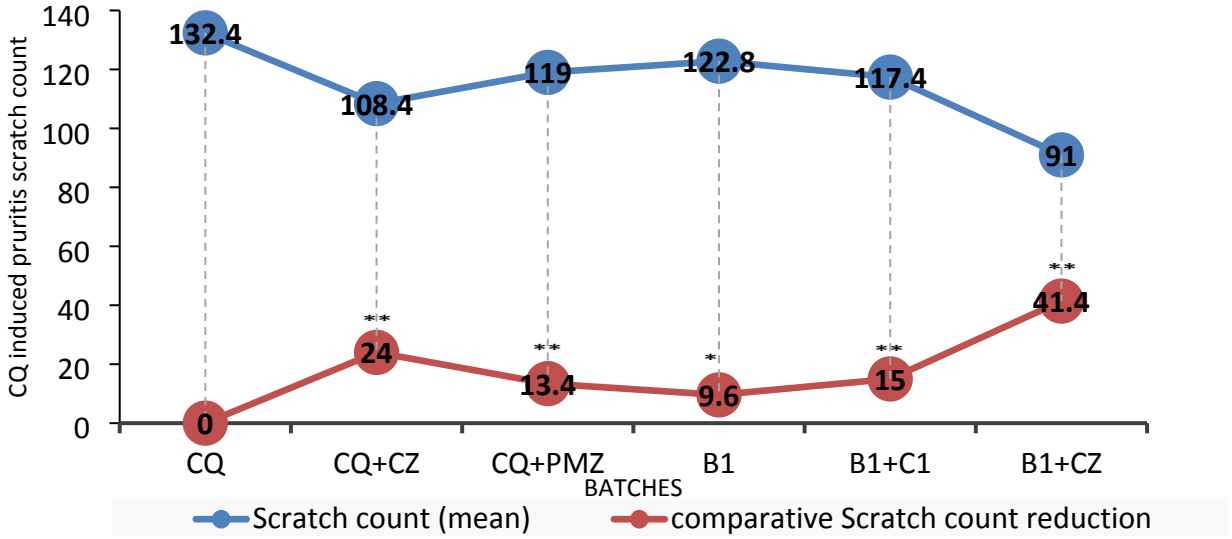


Fig. 6: Chloroquine-induced pruritus scratch count

Results were presented as mean ± SEM, n= *P < 0.05, **P < 0.01: Significantly difference from chloroquine (CQ).

Discussion

The findings of this study demonstrated that the combination of chloroquine (CQ) and cetirizine (CZ) exhibited the highest schizonticidal activity against *Plasmodium berghei* ANKA. Parasite suppression rates exceeded 75 % by day 4 of treatment for CQ+CZ, compared with 70.11 % for chloroquine alone and 65.93 % for cetirizine alone **(Figure 1).** This indicated that cetirizine not only possessed intrinsic antimalarial activity but also potentiated the action of chloroquine, possibly by inhibiting parasite P-glycoprotein-mediated efflux and thereby restoring CQ sensitivity [11,13–15]. The enhanced parasite clearance observed with liposomal formulations supported the growing evidence that site-targeted nanocarrier delivery systems can significantly improve drug bioavailability and therapeutic activity [16,21]. Interestingly, stealth liposomes containing PEG exhibited relatively lower clearance (77.96 %) compared with non-PEGylated liposomes (82.39 %). This outcome was attributed to the altered bio-distribution and increased tissue penetration associated with PEGylation, which may have limited the drug's availability at the blood target sites [22,23].

Hematological indices further substantiated the protective roles of cetirizine and liposomal formulations. In untreated infected mice, packed cell volume (PCV) dropped significantly to 18.67 ± 1.2 % but treatment with CQ+CZ restored PCV to 43.8 ± 1.2 % **(Figure 2).** Similarly, hemoglobin concentration declined to 10.77 ± 0.32 g/dl in untreated controls but improved to 14.38 ± 0.12 g/dl in the CQ+CZ-treated groups **(Figure 3)**. Red blood cell counts also improved, rising from 2.02 ± 1.75 $\times 10^6$/µL in infected controls to 4.27 ± 0.05 $\times 10^6$/µL with CQ+CZ **(Figure 4).** These results indicated that cetirizine not only reduced parasitemia but also offered protection against erythrocyte hemolysis and oxidative damage associated with *Plasmodium* infection and antimalarial drugs [5,38,40]. Such hematological protection may be explained by cetirizine's histamine H1 receptor antagonism, which mitigated histamine-mediated vascular leakage, oxidative stress, and inflammatory cascades [43–45].

The study also addressed chloroquine-induced pruritus, a common adverse effect contributing to treatment discontinuation [6,24]. In untreated rats, the onset of pruritus occurred within 33.2 ± 1.2 sec, accompanied by a mean scratch count of 132.4 ± 2.5 bouts **(Figure 5 – 6).** Cetirizine delayed the onset of itching to 45.6 ± 1 sec and reduced scratching episodes to 108.4 ± 2.6 bouts. Promethazine provided a greater effect in delaying onset to 53.6 ± 1.4 sec with the scratching episodes up to 119 ± 1 bouts, even though its sedative side effects limited clinical utility [11,54]. Cetirizine's antipruritic effect extended beyond H1 antagonism by inhibiting additional mediators such as leukotrienes, substance P, and serotonin, which are implicated in TRPV1 activation [44,45]. Moreover, cetirizine was also shown to

antagonize TRPA1 pathways triggered by chloroquine–Mrgpr interactions, due to its structural similarity to amino and carboxylic acid (from parent prolines and amino acid derivatives) [49,55].

Remarkably, liposomal encapsulation of chloroquine further enhanced antipruritic effects. Rats treated with CQ-liposome formulations showed a delayed itch onset of 63.8 ± 3.4 sec and a reduced scratching count of 122.8 ± 1.5 bouts. Liposomes significantly delayed the antipruritic effect of cetirizine which indicated that the effects of liposomes were attributed to its ability to bypass direct chloroquine interaction with sensory receptors by employing mechanisms such as membrane fusion or sequestration in pH-sensitive compartments [65]. Additionally, liposomes prevented CQ from binding to damaged lipids and forming hapten complexes, which are known triggers of mast cell degranulation and pruritic responses [41,66,75]. Collectively, these results suggested that liposomal formulations offered dual therapeutic advantages: enhanced efficacy against resistant parasites and mitigation of chloroquine-associated pruritus.

Taken together, the study demonstrated that cetirizine acted as both an intrinsic antimalarial agent and a chemosensitizer for chloroquine, reversing resistance while alleviating CQ-induced pruritus. The combination therapy significantly improved hematological indices, enhanced parasite clearance, and reduced adverse effects, thereby conferring a multifaceted therapeutic benefit. Incorporation into liposomal carriers further amplified these outcomes, positioning liposome-based cetirizine–chloroquine co-delivery as a promising strategy for malaria treatment, particularly in resistant strains and patients susceptible to CQ-induced pruritus [16,21,63,74].

Conclusion

This study demonstrated that cetirizine possessed intrinsic antimalarial properties and enhanced chloroquine efficacy against resistant *P. berghei* strains, likely by modulating efflux mechanisms. The combination therapy significantly improved hematological indices and alleviated chloroquine-induced pruritus. Liposomal encapsulation further amplified these therapeutic benefits, delaying itch onset and reducing scratching frequency while enhancing parasite clearance. These findings highlighted cetirizine, especially when delivered via liposomal formulations, as a valuable adjunct to chloroquine therapy. This co-delivery strategy may represent a novel and effective approach to managing resistant malaria and minimizing adverse treatment outcomes.

Chloroquine use should have been accompanied by close monitoring for pruritus and other adverse reactions, particularly in endemic populations where prolonged exposure increased the risk of intolerance. Alternative therapies and adjunctive agents, including antihistamines or nanocarrier-based formulations, were suggested as viable strategies to minimize these effects and improve treatment adherence. Furthermore, future studies should investigate the genetic and biochemical mechanisms underlying chloroquine-induced pruritus in African populations, where host variability may influence clinical outcomes. Public health programs were also recommended to incorporate patient education and pharmacovigilance in order to promote safer and more rational use of chloroquine and related antimalarial. Finally, continued collaborative research efforts were suggested to focus on the development of innovative drug delivery systems capable of enhancing therapeutic efficacy while simultaneously reducing toxicity.

Compliance with ethical standards

Disclosure of conflict of interest

All the authors declare no conflict of interst

Contributions of the Authors

All the authors contributed in the conceptualization, planning and execution of the research

Ethical approval

Ethical approval for the animal studies was gotten from Chukwuemeka Odumegbu Ojukwu University Teaching Hospital Amaku (COOUTH), Ethics Committe with reference number COOUTH/AA/VOL.I.031.

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