EFFECT OF SELECTED CHALCONES ON RBCS MEMBRANE OF BOTH INFECTED AND UNINFECTED RBCS

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Abstract - Chalcones are diaryl keton derivatives with broad distribution in botanical products and diverse biological activities. Their hydrophobicity may entitle them to produce their action through compromising the functional properties of membranous structures. In this experiment, the potential of six chalcone derivatives; namely; chalcone, 2',4'-dihydroxychalcone, 2',4' dimethoxychalcone, 4'chlorochalcone, 4- isopropyl-4'-methylchalcone and 4'-methylthiochalcone to inhibit the plasmodial growth was screened using SYBRE green-1 based drug sensitivity assay In order to get some clues about their mode of action, their impact on sorbitol induced hemolysis of the parasitized cell (PRBCs) was screened. Furthermore, their antioxidant potential was tested in vitro and compared with its anti-plasmodium effect. In addition, their cytotoxicity against RBCs and mammalian cells were tested to exclude any potential toxicity against normal cells. Results show that all the mentioned chalcones have a limited action against Plasmodium falciparum K1. These actions were not related to their effect on RBCs membrane or to the parasite induced new permeation pathway. Further studies are required to elucidate their full mechanism of actions and if they can augment CQ action in vivo.

Keywords - Chalcone, Chloroquine, Hemolysis, Plasmodium Falciparum and SYBR Green-I.

I. INTRODUCTION

The intra-erythrocytic ubiquity of plasmodium induces remodeling in the RBCs membrane to improve the membrane permeability for nutrients and waste products. This remodeling is characterized by expression of unique protein channels, known as; New Permeation Pathways (NPP) which mediate entry of more nutrients to the PRBCs. This pathway was proposed previously as a target to anti malarial due to its ubiquity in the infected cells only [1] [2]. Chalcones are highly abundant naturally occurring phytochemicals with di-aryl ketone nucleus (figure 1). They have diverse pharmacological activities, such as; antibacterial, antifungal, anti-viral, anticancer, anti-inflammatory and organ protective[3].This encouraged the scientists to study its impact on the biological system. Previous studies had pointed out to the discrepancy in the anti- plasmodium effect of different chalcone structures and have highlighted the significance of acetophenone ring substitution (ring B) (figure 1) with some groups, viz; methoxy, alkoxy, prenyl or azole. Any change in RBCs morphology or membrane properties may affect invasiveness and growth of the parasite. Different compounds with lipophilic and surface-active properties may adversely affect the membrane functions through changing their permeability and the ATP dependent homeostatic mechanisms that maintain cell shape and volume [4]. In our experiment, six chalcone derivatives, namely; chalcone, 2', 4'-dihydroxychalcone, 2',4' dimethoxychalcone, 4'chlorochalcone, 4-isopropyl-4'-methylchalcone and 4'-methylthiochalcone were used to investigate their anti-plasmodium and their impact on RBCM of both Plasmodium falciparum infected and uninfected cells.

II. MATERIALS AND METHOD

A. Materials and Chemicals

Human O+ blood was donated by the first author. RPMI-1640 medium, albumax II, were procured from Gibco BRL (Grand Island, NY, USA). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), triton X-100, sorbitol, hypoxanthine, (100X) phosphate buffered saline (PBS), chloroquine diphosphate (CQ) were purchased form Sigma-Aldrich (St. Louis, MO, USA). Gentamicin was purchased from Jiangxi Dongxu Chemical Technology Co., Ltd. Six chalcone derivatives were procured from Indofine company Inc and used, namely; 2'4'-dimethoxychalcone, 2',4', dihydroxychalcone, 4'-chlorochalcone, 4-isopropyl -4'methylchlacone, 4-methyl-4'- methyl- thio-chalcone and the unsubstituted chalcone. They represent the unsubstituted, hydroxylated, methoxylated, halogenated, alkylated chalcones and the thiochalcone derivatives respectively. (figure 1).

B. Blood washing

Once it was donated, the blood was centrifuged at 3000 RPM for 5 min and washed thrice with an incomplete RPMI-1640 (RPMI-1640, 20µg/ml gentamicin and 25 mM HEPES). Then, the washed RBCs was kept in the fridge overnight and used within not more than one week after its preparation [5].

C. Parasite Culturing, Maintenance

The parasites were cultured in the washed blood at the standard conditions for 48 hrs. They were synchronized at the ring stage using the well-known

sorbitol induced synchronization technique. The cultures were loaded into a 96 well plate featured serial dilution of each drug (1-250 nm).The test was done in triplicate. After incubation, and the plates were freeze-thawed and 100 µL of SYBR green-I lysis buffer ((20 mM Tris, 5 mM EDTA, 0.008% saponin and 0.008% triton-X-100) was loaded to each well. Then the plates were incubated at room temperature for 1 hr. and finally fluorescence was measured twice after 15 seconds of plate agitation using Victor Plate reader (Perkin Elmer, Salem, MA) at an excitation/ emission wavelength of 485/535 nm. Each the geometric mean of the first and second pass was used to exclude any measurement error [6]. We were restricted to 250 nM as it was the maximum concentration at which some of our lipophilic chalcones; viz, chalcone, were miscible with cMCM. Although, the hydroxylated and alkoxylated ones were miscible at higher concentrations.

D. Estimation of IC_{50} and IC_{90}

Both IC_{50} and IC_{90} of each test drug against Plasmodium falciparum K1 were determined using Microsoft excel 2007 software according to the recommended protocol of percentage of parasite inhibition versus log [drug concentration].

Effect of the phytochemicals on uninfected RBCs

Different concentrations of each chalcone (1 nM -250µM) were incubated with O +ve human RBCs in an Incomplete Culture Medium iCM (RPMI-1640, 25 mM HEPES and 20 µg/ml gentamicin) at 37 ºC for 48 hrs using 24 well plate (1 ml/cell). After incubation, 500 µl from each well was transferred to Eppendrof tubes after through mixing, centrifuged at 500 g for 5 min, 200 µl of the supernatants were loaded into a flat bottomed 96 well plate and the released hemoglobin was measured at 540 nm (VersaMaxtm).

Results were compared with both negative and positive controls wherein the RBCs were incubated with drug free media and a medium containing 1% Tween 20; which produces 100% hemolysis. RBCs hemolysis at each concentration was calculated as following.

E. Selectivity index

Selectivity index was calculated through finding the ratio of the drug IC_{50} against plasmodium to that against RBCs and Vero cell line. It is calculated to find the degree of selectivity of the drug toward the parasite as compared to two types of mammalian cells; RBCs, where the plasmodium thrives.

F. Effect of on merozoites invasiveness

Effect on merozoite invasion was performed as previously described (Mary Luz López 2009). Briefly, non-infected RBCs were treated at 37 ºC for 2 hrs. with different concentrations of the drug (1 µM-1 mM) dissolved in the incomplete malaria culture medium (RPMI-1640, HEPES-tris buffer 25 mM and gentamicin 20µg/ml). Then the exuberant drug was washed twice and to each 300 µl of the treated RBCs, 100 µl of PRBCs at parasitemia of 4% and rich in the schizont stage (after >35 hrs. of parasite synchronization) was added and the final Hct was adjusted at 1%. The mixture was incubated at the mentioned incubation conditions for 20 hrs.; the time point at which microscopic determination of parasitemia was done. In this test, the amount of merozoites that could have invaded the treated RBCs was compared to the positive control that contains only non-treated RBCs. The percentage of parasite duplication inhibition at each concentration was calculated using the following equation Wherein Pa and Pbx represents the parasitemia of the positive control and that of the culture containing RBCs treated with x concentration of the test drug respectively. Percentage of parasitemia at each concentration was plotted versus log concentration to determine the dose response curve.

G. Effect of each chalcone on sorbitol induced hemolysis of PRBCS

Chalcones Effect on parasite induced permeability pathway was investigated as previously described (Go ML 2004). Briefly, PRBCs predominated with trophozoites (after 30 hrs of synchronization) at 15 % parasitemia were washed and 100 µl of the washed cells were loaded into 24 well plates, featured two folds serial dilution of each chalcone or frusemide; a well-known inhibitor of plasmodium induced NPPs, with a concentration range of (500 nM-250 µM) for each at 1 ml /well. The dilutions were done using sorbitol buffer solutions (300 mM sorbitol in 10 nM HEPES-Tris (pH 7.4)). After 30 min incubation at 37 ºC, 500 µl was aliquoted from each suspension after through mixing, centrifuged at (500 g for 5 min) and 200 µl of the supernatants were loaded to a flat bottomed 96 well plate. The released hemoglobin was measured at 540 nm (Spectramax plus 384) and the percentage of hemolysis in each sample was compared to the positive control (wells containing the drug free sorbitol buffer solution) [7]. Finally, IC_{50} (drug concentration required to inhibit 50%e of parasite induced permeability pathway) was obtained by linear regression analysis using Microsoft excel 2007 software. Frusemide, a well-known inhibitor of NPP, was used as a negative control at 100 µM [7].

H. Effect of chalcones on RBCs osmotic fragility (O.F)

RBC fragility test was performed as previously described. Briefly, washed RBCs were suspended in series of solutions containing isotonic PBS diluted serially using D.W so that tonicity ratio would be kept in the range of 0-0.9% noticing that 0% tonicity gives 100 % hemolysis. Then sets of the mentioned dilutions were prepared and each chalcone at two different

concentrations (1µM and 250 µM) were served. Finally, percentage of hemolysis vs. tonicity curve was extrapolated for each drug concentration and was compared to that of the drug free set.

I. Physiochemical properties calculation and bioactivity prediction

Both of the physiochemical properties and the predicted bioactivity parameters for each chalcone were determined using chemi-informatic software known Molinspiration (http://www.molinspiration.com). It performs a fragment based virtual screening of the following, viz; CLOGP (logarithm of octanol/water partition coefficient), PSA (molecular polar surface area), nON (number of non-hydrogen atoms), nOHNH (number of hydrogen donating bonds) and nrotb (number of rotatable bonds). Furthermore, the software gives predictive drug-likeness score toward the following intracellular targets; GPCR, kinase, nuclear factors, ion channels and protease enzyme.

II. RESULTS

A. Drug sensitivity assay

Plasmodium falciparum K1 was resistant to CQ (IC_{50}= 265 ± 3.3 µM, IC90= 433 nM). It was poorly responsive to all the mentioned chalcones with IC50 values within the micro molar range as seen in (Table 2).

B. Effect on nRBCs stability

All the used chalcones were cytotoxic to nRBCs as seen in table 2. Only 2',4'dimethoxychalcone and 2',4' dihydroxy-chalcone showed good selectivity index SI toward the plasmodium as compared to nRBCs (SI>10). Others, like; chalcone and 4 methoxy-4'thiomethyl chalcone were partially selective with SI value <10 & > 4 while the rest; 4'chlorochalcone and 4-isopropyl-4'methyl chalcone were totally non selective to the plasmodium as their SI values were < 4 (table 2). Due to their higher RBCs hemolytic effect and poor selectivity to the plasmodium, merozoite invasion assay and the effect on RBCs fragility test were not performed for each of 4'chlorochalcone, 4'-methy-4-isopropylchalcone and 4-methyl-4'methylthiochalcone. The change in RBCs indices (MCV (mean corpuscular volume) and MCH (mean corpuscular hemoglobin) as detected using Coulter hemocytometer are demonstrated in table 1

1) Merozoite invasion inhibition assay

Results of the potential of merozoites to invade the RBCs treated with chalcone, 2',4'- dimethoxy chalcone or 2',4' dihydroxy chalcone are illustrated in figure 2. Effect of the others was not tested due to their detrimental impact on RBCs at low concentrations. Figure 3 illustrates the percentage of parasite duplication inhibition in the cultures containing RBCs treated with test chalcone as compared with that of the cultures containing the untreated RBCs only. Their IC_{50} as calculated by curve fitting of the percentage of parasitemia versus log (drug concentration) curve was 1.106, 1.846 and 15.021 mM respectively (figures 3A and 3 B).

2) Effect of chalcones on RBCs O.F

Effect of each of chalcone, 2',4'-dimethoxychalcone, 2',4'-dihydroxychalcone on RBCs O.F is depicted in figure 4. The figure shows the threshold ionic strength wherein maximum RBCs hemolysis commences was elevated when the mentioned chalcones were used at a concentration of 250 µM. Meanwhile, at 1 µM, no any noticeable action was observed (figure 4).

3) Physiochemical properties and bioactivity prediction

Results of Molinspriation software (www.molinspiraton.com) are illustrated in (table 4) wherein the impact of the substitutions on both the hydro-lipophilicity and the polarity of the molecule. Both ClogP and PSA were obviously increased in the chalcones substituted with lipophilic groups. The upmost increment was obtained for 4'-methyl-4-isopropyl chalcone. Meanwhile it was less in 4'- methyl-thio-chalcone and 4-chlorochalcone and it was negligible in the methoxylated chalcone. On the other hand, Chalcone hydroxylation at ring B could have reduced the lipophilicity.

The simulation software shows that all the substitutions confer for higher predictive bioactivity against the main intracellular targets; GPCR, kinase, protease and nuclear receptors as represented by drug-likeliness score. The predictive improvement was higher for both the hydroxylated and methoxylated chalcones and less or absent for the halogenated chalcone and its thio-methyl derivative.

III. DISCUSSION

Previous studies had pointed out to the role of natural products in eradicating malaria parasite. Few of them were worth to be used in the clinical field, such as; Artimisia annua. Research is going on to investigate the plausible effect of the others. Although lots of them were not as powerful as artemisinin [8]. In this study, six chalcone derivatives were used. They represent the unsubstituted, hydroxylated, methoxylated, halogenated, alkylated and thio-chalcone derivatives. They were chosen based on the previous literatures that highlighted the significance of ring B substitution of the chalcone nucleus (figure 1) [9]. This study investigated their selectivity to plasmodium as compared to RBCs and their action against the RBCM of both infected and uninfected RBCs.

Formerly, it had been suggested that their pharmacological activity is retained within its carbonyl group which acts as a nucleophile attaching

different intracellular targets (figure 1). The other substitution alters the physiochemical properties, like; the polarity (table 4) which changes its permeability to the intracellular targets. Furthermore, the structural changes may alter the nucleophilic attack to the active sites of the intracellular targets [9]. On the other hand, they may add an antioxidant power [10]; a character to which the potential of the compounds to mop out the free radicals. Antioxidants act as double edged sword weapons for the cells. Form one side; they halt flow of the deleterious free radicals but from the other side, they may turn into pro-oxidants and release more free radicals at higher concentrations. The concentration threshold is different between different cells and it is not sure if there is a discrepancy in this threshold between plasmodia and human cells [11]. Previous studies had pointed out to the significance of such discrepancy in eradicating the undeveloped cells [12]. In our study, all the test chalcones produced an anti-plasmodium effect with IC_{50}s within the micro-molar range which is higher than that of any conventional anti-malarial. Hydroxylation of the B ring at both para and ortho positions did not intensify the anti-plasmodial effect of the chalcone although it augmented its anti-oxidant potential. This excludes any potential for the compound to produce any pro-oxidant effect against Plasmodium falciparum. Some previous studies have pointed out to the significance of ring B hydroxylation in potentiating the anti-tuberculosis effect [3]. But according to our results, this modification is not advantageous for the anti-plasmodial effect of chalcones. Furthermore, the study shows that the lipophilic chalcones have better tendency to limit the parasite growth as compared to the less lipophilic ones as IC_{50} values of both the methoxylated and hydroxylated chalcones were lower as compared to others [3] has pointed out to the significance of the lipophilic groups to confer an anti-microbial effect. The highest effect was obtained for the thiochalcone derivative positing a role for sulfur to enhance the anti-plasmodium effect. Nevertheless, none of them has succeeded to inhibit plasmodium growth like any potential anti-plasmodium drug. It is noteworthy that these results came in contrary to what was predicted by Molinspiration chemi-informatic software. The software predicts higher activity for the hydroxylated and methoxylated chalcones and less for the thiochalcone derivative. This suggests either presence of other intra-cellular mechanisms through which the latter produces its action or may be its permeation is higher than other chalcones. The results show also that in spite of its higher lipophilicity, 4'methyl-4-isopropyl chalcone has lower antiplasmodial action in comparison to the thiochalcone derivative. This suggests that the former's lipophilicity is higher than the optimum threshold required for the drug penetration into its intracellular target. Their effect on RBCM was exclusively higher for those which possess high ClogP and low PSA (table 2 &4). This suggests that such chalcones have the ability to accumulate in the double layered membrane of the RBCs resulting in disruption of their integrity and enhancing their hemolysis. This cytotoxic effect was prominent at concentrations approaching their anti-plasmodial IC_{50} for each of 4'-chlorochalcone, 4'methyl-4-isopropylchlacone and 4-methyl-4methylthiochalcone. At such concentrations, all of them produced a pronounced effect on RBCs morphology characterized by echinocytosis and spherocytosis (figure 5). This suggests that their anti-plasmodium effect may be due to their impact RBCM rather than the plasmodium itself. Spherocytosis is characterized by changing the morphology of RBCs from the normal discoid biconcave shape; with a central pallor in the middle area, into darker smaller spherocytes. Meanwhile, echinocytosis is characterized by round protrusions, speckles or crenations on the RBCs surface. The crenations result from the imbalance in the bilayers of the RBCs membrane and changing the area between them. In contrast to echinocytosis, stomatocytosis is associated with changes in the inner leaflet of the RBCs membrane resulting in its invagination into the intracellular compartments and creation of stomata like structures [13]. O.F test was performed only on the chalcones that did not deteriorate the RBCs stability significantly (chalcone, 2',4' dihydroxy chalcone and 2'4' dimethoxy chalcone). All of them have shifted the % of RBCs hemolysis versus ionic strength curve into upward direction indicating higher sensitivity of the chalcones treated RBCs to the hypotonicity induced stress. The increment was higher and more significant for the unsubstituted and the methoxylated chalcones. This may be attributed to their higher lipophilicity which augments their impact on RBCs membrane function. It is well known that when RBCs encounter the hypotonicity stress, they tend to swell and then hemolyze Furthermore, It has been also suggested that chalcones may affect cell membrane function through targeting protein channels present on the cell membrane or affecting the membrane stability due to their hydrophobic nature that entitle them to accumulate in the cell membrane [14]. During the intraerythrocytic stage, Plasmodium falciparum, triggers remodeling in the RBCs membrane resulting in changing its function for the favor of the parasite. Infected RBCs expresses specific protein channels called (New Permeation pathway-NPP) which regulate entry of the nutrients and exodus of the waste products. PRBCs start expressing NNPs after 10-15 hours post invasion and reach the plateau after 30 hrs. Consequently, we opted to start our experiment with trophozoites grown after 30 hrs. of synchronization [2]. They have broad specificity to various agents so they allow passage of different types of species, like; monosaccharides, polyols, amino acids, nucleotides, mono carboxylates, quaternary

ammonium compounds and monovalent inorganic cations and anions, viz; calcium ion. Furthermore, they are permeable to large molecules, such as; glutathione. But they have low permeability to sucrose. PRBCs hemolyze when they are incubated with an isotonic solution of any of these species due to solute influx along with water into the intracellular compartment. The most famous solutes that have ever been used in malaria research to synchronize the plasmodium stages and to study effects of drugs on NPPs are sorbitol and alanine. Frusemide was used as a positive control drug for NNPs inhibition as it compromises entry of these species into the intracellular compartment resulting in less PRBCs hemolysis [2]. It is noteworthy that some chalcones are used conventionally as model drugs against the plasmodium induced new permeation pathways on PRBCs surface, viz phlorizin. Results of the sorbitol induced hemolysis inhibitory assay shows that phenolic substitutions on B ring (hydroxylated and methoxylated chalcones) increased the potential of the chalcone to inhibit NPPs function. Presence of the lipophilic groups did not produce any improvement in this regard. This suggests that as chalcone derivatives, they can attack the NPP pathway resulting in inhibition of nutrients influx. Furthermore, their impact on NPP may be due to their tendency to bind to the NPPs rather than accumulation on RBCs membrane and affecting RBCs fluidity. The chemi-informatic data obtained from Molinspiration software suggest that the hydroxylated chalcones have the highest impact on the ion channels as compared to the others (table 3). This observation was also seen during the NPP activity assessment. This may be due to the discrepancy in the nature of such channels with that of the NPPs. Direct inhibitory effect of drugs with amphiphile characters was proposed previously as NPPs are made up of two chains; nonionic hydrophobic and cationic chain. The former mediates ingress of non-ions while the latter facilitates passage of the anionic substrates which are driven by the electrochemical gradient while the cationic ones are repulsed by the cationic chain. Previously, it was found that NPPs play a role in cellular development and growth. As an inwardly rectified channel, NPPs can regulate the membrane potential through increasing the ion conductance which in turn raises the of cell membrane linked adenyl cyclase enzyme that activates protein kinase A and trigger cellular growth [2]. Their impact on merozoite invasion was screened through merozoite inhibition assay. Three of the tested chalcones; chalcone, 2',4'-dihydroxychalcone and 2',4'-dimethoxychalcone, have reduced the capacity of the merozoites to invade the treated RBCs in different extent (figure 3). This suggests that they can inhibit merozoite invasion in a manner dependent on their lipophilicity and their ability to accumulate in the RBCs membrane. Merozoites invasion is a multi-steps process starts with RBCs recognition by the parasite, re-orientation of the merozoites on RBCs surface and junction formation, inward motion driven by the merozoites apical complex actinomyosine then finally pinching of the junction and entry of the parasite into RBCs where the parasitphorous vacuolar membrane evolution starts. Merozoites carry some specific surface antigens that bind to RBCs in sialic acid dependent manner, viz; MSP-1 (merozoite surface protein -1), gp195 (GPI-Glycosyl phosphotidyl inositol) and EBA 175 (Erythrocyte binding antigen-175). Sailic acid is a part of glycophorin molecules whose ubiquity in RBCs membrane relies on its integrity. Our results confirm the capacity of the abovementioned chalcones to compromise RBCM function and integrity and sialic acid ubiquity which is crucial for the invasion process. The intra-membranous accumulation of these chalcones may compromise the potential of RBCM invagination; which is a crucial preliminary step for the parasite re-orientation and the subsequent entry into the intra-erythrocytic space. Such action has come in coincidence with results of the osmotic fragility test that showed a prominent loss of the aptitude of the RBCs treated with the abovementioned chalcones; chalcone, 2',4'-dihydroxychalcone and 2',4'-dimethoxychalcone , to resist the hypotonicity induced spherocytosis [13]-[15]. The Molinspiration software shows that both the methoxylated and the hydroxylated chalcones have a comparable predictive activity against the mentioned cellular targets and their activity was higher than that of the unsubstituted one. This excludes the notion that the claimed action is attributed to their potential to attack the intracellular targets. On the other hand, in spite of their comparable predictive effect in, the impact of the former on the plasmodium is more prominent and was comparable to that of the un-substituted chalcone.

A. Figures and Tables

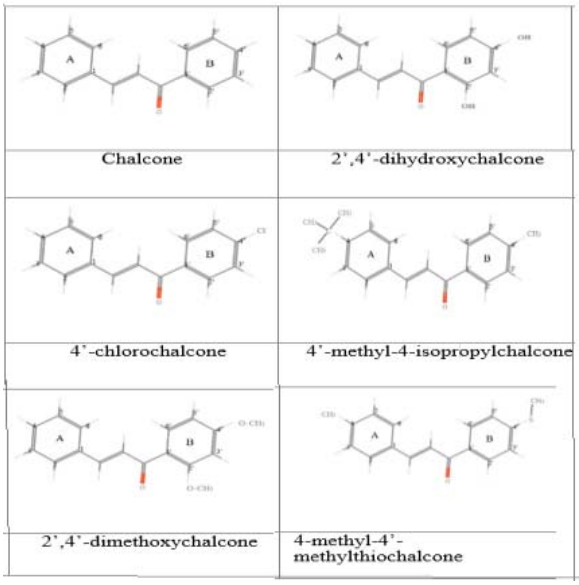


Figure 1:-Chemical structures of the chalcone and the chalcone derivatives that was used in the study. The structure in the upper left corner represents the structure of the basic chalcone nucleus that is made of a di-aryl ketone structure (1,3-Diphenyl-2-propen-1-one). It contains two rings;A & B. Any substitution on ring B is tagged by "". Substitutions on ring B affect the electron density on the carbonyl group resulting in changing their ability to attack nucleophilic groups. The other structures represent the substituted chalcones that has been used in the study.

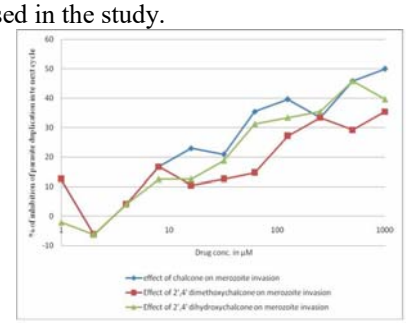


Figure 2:-Percentage of inhibition of parasite duplication in the next cycle as a parameter of merozoite invasion versus drug concentration. Figure B shows the curve fitting of the log dose response curve.

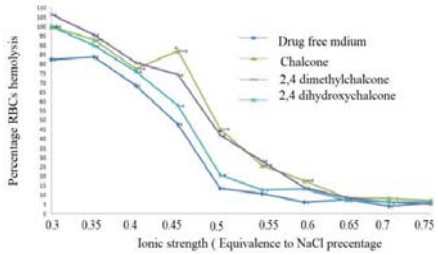


Figure 3:-RBCs osmotic fragility curve wherein the ionic strength of the medium was represented as equivalence to NaCl concentration was plotted against percentage of RBCs hemolysis. The curve was plotted both in chalcones containing and free media. Each chalcone was at a concentration of 250 µM. The effect of each chalcone on the threshold ionic strength below which RBCs hemolysis is augmented was depicted. * refers to the statistical significance ($P<0.05$) of the hemolysis in the presence of the chalcone as compared to that in its absence.

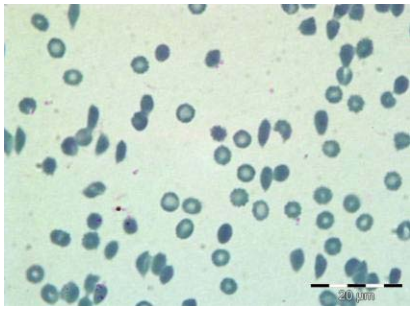


Figure 4:-RBCs morphology after exposure to the lipophilic chalcones. The figure shows some RBCs are transformed to echinocytes or spherocytes after exposure to chalcone

B. List of tables

Compound	IC_{50} against *Plasmodium falciparum*	IC50 against nRBCs stability	SI to plasmodium as compared to RBCs	IC_{50} of drug induced inhibition of New Permeation Pathway
Chloroquine	255 nM	N.A		N.A
Chalcone	38 µM	280 µM	7.6	26 µM
2'4'-dimethoxychalcone	44 µM	667 µM	15	18 µM
2',4' dihydroxychalcone	86 µM	941 µM	11	11 µM
4' chlorochalcone	28.2 µM	82 µM	2.9	84µM
4-isopropyl4'methylchlacone	33 µM	43µM	1.3	39 µM
4-methyl-4'-methylthiochalcone	12 µM	62µM	5.12	59 µM

Table 1:- In vitro cytotoxic effect of each test compound on RBCs and plasmodium along with the selectivity index toward the plasmodium as compared to the both abovementioned cells. The last column represents the IC_{50} values for the inhibitory action of the tested chalcones on NPPs

	4-methoxyl-4'-methylthiochalcone	4-isoprpyl-4'methylchalcone	Chalcone	2',4'-dihydroxychalcone	2',4'-dimethoxychalcone	4-chlorochalcone
Physiochemical properties						
ClogP	4.1	5.772	3.2	2.7	3.306	4.5
PSA	17.1	17.07	177	57.5	35.54	17
Natoms	18.0	20.0	15.0	17.0	19.0	17
MW (g/mole)	256.3	264.3	196.2	228.2	256.3	242.7
nON	1	1	1	3	3	1
nOHNH	0	0	0	2	0	0
Nrotb	4	4	3	3	5	3
Predictive bioactivity						
GPCR ligand		-0.21	-0.43	-0.17	-0.15	-0.35
Ion channel	-0.30	-0.21	-0.18	-0.09	-0.21	-0.16
Kinase inhibitor	-0.61	-0.43	-0.66	-0.42	-0.40	-0.58
Nuclear receptor	-0.32	-0.16	-0.51	-0.05	-0.13	-0.45
Protease inhibitor	-0.33	-0.37	-0.60	-0.29	-0.26	-0.57
Enzyme inhibitor	-0.17	-0.08	-0.12	0.06	-0.05	-0.13

Table 2:- Physiochemical properties and the predictive bioactivity of the tested chalcones against four main intracellular targets; GPCR, ion channels, kinase, protease and nuclear receptors. The physiochemical parameters include ClogP, PSA, nON, nOHNH and nrotb. ClogP represents a global measure of the molecules hydrolipophilicity. PSA (polar surface area) is a measure of the surface area where the polarity is high. natoms is the total number

of atoms within the molecule, MW represents the molecular weight in gm/mole, nON is the total number of non hydrogen attached groups, nOHNH total number of hydrogen donating groups and nrotb is the total number of rotatable bond. The last is a measure of the molecules flexibility and is represented by single non ring bonds which are bounded to non terminal heavy atom (all except hydrogen). On the other hand, the predictive bioactivity parameters include the drug likeliness score which is a measure of the coincidence of each compound with the standard inhibitors of each of GPCR, kinase and protease enzymes and the nuclear receptors as well as to the standard ion channels modulator.

IV. CONCLUSION

Overall, in spite of the limited anti plasmodium effect of chalcones, their use in malaria chemotherapy is not applicable as they had deleterious impacts on RBCs stability at concentrations comparable to their IC_{50s} against the plasmodia. These results suggest that lipophilicity controls the anti-plasmodium activity of the chalcone. Furthermore, the anti-plasmodium effect is not closely associated with the impact of the compound on the RBCs stability or activity of the NPPs. Further studies are recommended to study the impact of other substitutions on the anti-plasmodium effect of the compound and further in vivo studies are recommended to see if there is effect on the CQ pharmacokinetic.

CONFLICT OF INTEREST

All authors declare that there is no any conflict of interest regarding publication of this article

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