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EFFECT OF AQUEOUS EXTRACT OF *CRYPTOLEPIS SANGUINOLENTA* ADMINISTRATION ON THE METABOLISM OF CHLOROQUINE *VIA* CYTOCHROME P450 ISOZYMES

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Keywords:

Concurrent administration, Chloroquine, *Cryptolepis sanguinolenta*, Cytochrome P450 isozymes, Metabolism

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ABSTRACT: Concurrent administration of herbal medicines and conventional drugs is a common practice globally, especially as the patronage of medicinal plants increases across the world. This study aimed to determine the effect of *Cryptolepis sanguinolenta* administration on the metabolism of chloroquine by evaluating specific Cytochrome P450 isozymes. Reconstituted freeze dried *Cryptolepis sanguinolenta* was administered to male Sprague-Dawley rats in drinking water for 2 weeks at a dose of 36mg/kg, followed by a single dose of chloroquine (15mg/kg). All the animals were sacrificed by cervical dislocation after 24 h of final drug administration, and the liver was excised for hepatic microsomes preparation. Using the microsomes, the PNPH, AmD and NOD activities were determined as a measure of CYP2E1, CYP2B1 and CYP1A activities, respectively. The study showed that concurrent administration of chloroquine and *Cryptolepis sanguinolenta* impaired the CYP-mediated metabolism of chloroquine due to competitive inhibition of CYP2B1 by *C. sanguinolenta.* However, concurrent administration of chloroquine and *Cryptolepissanguinolenta* showed no significant effect on CYP2E1 and CYP1A. Patients should be advised on the clinical consequence of concurrent administering *Cryptolepis sanguinolenta*-based herbal products and chloroquine.

INTRODUCTION: Chloroquine is a synthetic 4 – aminoquinoline, used for many decades in the prophylaxis and treatment of malaria caused by *Plasmodium vivax (P. vivax), P. ovale*, *P. malaria* and the sensitive strains of *P. falciparum* [1]. Despite resistant *P. falciparum* strains, chloroquine remains a key antimalarial drug used alone or in combination with other drugs as the first-line treatment for malaria due to its low cost, tolerance, and availability [2].

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In addition to its anti-plasmodial activity, chloroquine is also used in the treatment of amoebiasis, rheumatoid arthritis, discoid lupus erythematosus, and photosensitive diseases [3, 4]. In the wake of the coronavirus disease 2019 (COVID-19), especially in the early days of the pandemic, hydroxychloroquine (a derivative of chloroquine) became the life-saving drug of the time, providing relief to many patients scourged with the disease [5].

This implies that chloroquine holds future promises in treating several diseases, including emerging conditions. Apart from these therapeutic indications, the potential for chloroquine to modulate drug metabolism and cause drug interactions especially when used with other drugs, has also been reported [6].

Chloroquine is metabolized to *N*-desethyl-chloroquine (DCQ) and *N*-bis-desethylchloroquine (BDCQ) in the liver by cytochrome P450 (CYP) enzymes. Therefore, drugs that share similar CYP isozymes with chloroquine may cause drug interactions leading to toxicity as a result of accumulated doses or development of resistance or loss of efficacy due to subtherapeutic levels – these call for major concern. *Cryptolepis sanguinolenta* (Lindl.) Schltr. is a thin-stemmed twining and scrambling shrub indigenous to Africa. It is mostly used for the treatment of malaria although it has other pharmacological properties. They include anti-bacterial [7], anti-viral, anti-inflammatory [8], anti-diabetic and hypotensive effects [9-11]. Recently, alkaloids from *C. sanguinolenta* have been reported to represent a promising class of compounds that could serve as lead compounds in the search for a cure for coronavirus disease. Again, six months of treatment with *Nibima*, a product of *C. sanguinolenta*, showed 99% reduction in a patient's hepatitis B virus (HBV) load [12].

Therefore, *C. sanguinolenta* and its products have gained popularity as a cheaper source of therapy for malaria and other infections such as Hepatitis and coronavirus disease, despite the scanty clinical data. Meanwhile, the concurrent administration of *C. sanguinolenta* and its products with orthodox drugs is not uncommon, with little attention to the clinical consequence. In a previous study, for instance, concurrent administration of aqueous extract of *C. sanguinolenta* and chloroquine resulted in changes to the pharmacokinetics of chloroquine; that is, an increase in the bioavailability, half-life, and decrease in the absorption rate constant, maximum whole blood concentration, volume of distribution, clearance and elimination rate constant of chloroquine diphosphate [13]. However, the role of metabolism in such herb-drug interaction, *viz*-a-*viz*, the CYP isozymes involved, was not reported. Therefore, the aim of this study was to determine the effect of *C. sanguinolenta* administration on the metabolism of chloroquine by evaluating specific CYP isozymes.

MATERIALS AND METHODS:

2.1 Materials:

2.1.1 Plant Materials: Powdered dried roots of *Cryptolepis sanguinolenta* were obtained from the Centre for Plant Medicine Research (CPMR), Mampong-Akuapem, Ghana. It was authenticated by the Plant Development Department (PDD) of CPMR, and a sample kept at the department's herbarium with the voucher number CPMR 5018-Crypto.

2.1.2 Experimental Animals: A total of twenty-four (24) male Sprague - Dawley rats weighing between 298 – 343 g were obtained from the Animal Unit of CPMR, Mampong-Akuapem, Ghana and standard laboratory chow diet was obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana.

2.1.3 Chemicals and Reagents: Chloroquine diphosphate tablets were obtained from Letap Pharmaceuticals Ltd, Ghana, Accra. Trichloroacetic acid was obtained from Timstar Laboratory Suppliers Ltd, UK. Sodium hydroxide, calcium chloride and Magnesium chloride were obtained from Fluka AG Buchs, Switzerland. Sucrose, n-hexane, ethyl acetate and hydrochloric acid were obtained from BDH Laboratory Supplies, England. All other chemicals and reagents were obtained from Sigma Aldrich, USA, and UK.

2.2 Methodology:

2.2.1 extract Preparation: Powdered dried roots of *Cryptolepis sanguinolenta* (360 g) were boiled in 6 liters of water for 45 minutes, cooled, and filtered through a filter paper. The resulting filtrate was lyophilized into powder using a freeze dryer (EYELA, Tokyo Rikakikai Co LTD, Japan). The dry powder extract was weighed (yield 5.9%) and stored in a desiccator at room temperature. The powder was reconstituted in sterilized distilled water before administering to the animals.

2.2.2 Experimental Design: The rats were randomly divided into four (4) groups of six (6) animals each, fed on the standard laboratory chow and water *ad libitum* and acclimatized for a week before the commencement of the treatment.

Group I (No Drug): Animals in this group received only distilled water throughout the study (No drug) serving as normal control

Group II (Cs ext): This group received reconstituted freeze-dried *C. sanguinolenta* in their drinking water at the therapeutic dose (36 mg/kg)

(CPMR, unpublished data) for two (2) weeks and a single oral dose of the extract at the end of the two weeks(Cs ext) was administered

Group III (CQ): This group received distilled water for two weeks and a single dose of chloroquine (15 mg/kg based on the recommended dosage in humans) after two weeks

Group IV (Cs ext + CQ): This group received the reconstituted extract for two weeks and a single oral dose of chloroquine at the end of the two weeks. All rats were treated in accordance with the National Institute of Health Guidelines for the care and use of laboratory animals [14]. The Ethics Committee approved the research protocol of CPMR. The animals were sacrificed by cervical dislocation after 24 h of final drug administration, and liver was excised and weighed into sucrose solution for hepatic microsomes preparation.

2.2.3 Preparation of Hepatic Microsomes: Microsomes were prepared as described by Lake [15, 16]. Rat liver was excised, weighed, and placed immediately in a 0.25 M ice-cold sucrose solution. The liver (9g) was blot dried and homogenized using a Teflon pestle and a glass homogenizer tube with a drilling machine to yield a 20 % w/v liver homogenate. The homogenate was centrifuged at 12,500 g for 15 min using a high-speed refrigerated centrifuge (Hitachi 20RR- 52D) to intact pellet cells, cell debris, nuclei, and mitochondria. The resultant post-mitochondrial fraction was then decanted. To aliquots of 1 ml post- mitochondrial supernatant fraction, 0.1 ml of 88 mM $CaCl_2$ was added and the mixture was left on ice with intermittent gentle swirling for 5 min. The mixture was then centrifuged at 27,000 g for 15 min using the refrigerated centrifuge. The supernatant was discarded, and the pellet was washed in a 0.1M Tris buffer, pH 7.4 containing 0.15 M KCl, and centrifuged at 27,000 g for 15 min. The washing was repeated, and the pellet was resuspended in a solution of storage buffer of 0.1 M Tris HCl, pH 7.4 containing 0.154 M KCl and 20 % v/v glycerol. The prepared microsome was stored in aliquots of 1 ml at – 40 ˚C for further studies.

2.2.4 Determination of Microsomal Protein Concentration: The microsomal protein concentration of the hepatic microsomes was determined using the Bio-Rad Protein Assay based on the Bradford's method [17]. A working Bio-Rad reagent (1.0 ml) was prepared by adding 1 part of stock solution (100 mg Coomassie Brillant Blue G-250, 50 ml methanol, and 150 ml of 85 % phosphoric acid) to 4 parts of distilled water. Microsomal samples were diluted 1 in 100 for use in the protein determination. Each diluted sample was added to 1 ml of the working solutions, mixed and incubated for 5 min at room temperature. The absorbance was read at 595 nm. The protein concentration was extrapolated from a standard curve generated from the protein standard, bovine serum albumin (BSA), over 0 – 0.2 mg/ml.

2.2.5 Determination of Cytochrome c Reductase Activity: To 250 µl of 5 mg/ml cytochrome c was added 631 µl of a 4 mg/ml microsomal sample and made up to 2.275 ml with 0.1 M Tris-HCl buffer, pH 7.4. The reaction was initiated by the addition of 250 µl of 2 % (w/v) NADPH solution to the test solution. The final microsomal concentration was 1 mg/ml in the 2.525 ml reaction mixture. A reference blanks with similar composition as the reaction mixture, except that an equal volume of 0.1 M replaced the NADPH Tris buffer pH 7.4 was prepared for each sample. After thorough mixing, the absorbance at 550 nm was recorded every minute for 3 min against the reference blank.

The NADPH – cytochrome c reductase activity was determined from the equation below [17];

$$\text{Specific activity} = \Delta A / \epsilon\ (mM^{-1}cm^{-1})\ Pr\ (mg/ml)\ \Delta T\ (min) \times \text{mol/min/mg protein}$$

ΔA - Absorbance change for the linear portion of the curve over 3 min. Є - Molar Absorptivity of reduced cytochrome c, given as 19.6 $mM^{-1}cm^{-1}$ Pr - Concentration of microsomal protein in the reaction (1 mg/ml), ΔT - Average change in time.

2.2.6 Determination of para-Nitrophenol Hydroxylase (PNPH) Activity: PNPH activity was determined as a measure of CYP2E1 activity by the method of Reinke and Meyer [18]. To 10 µl of 1.396 mg/ml para-Nitrophenol (PNP) added 5 µl of 5 mM MgCl2, 776µl of a 4 mg/ml microsomal sample topped up to 1.025 ml with 50 mM Tris buffer, pH 7.4. The reaction mixture was incubated at 37 ˚C for 3 min, and 10 µl of 50 mM NADPH was added to initiate the reaction. The 1.035 ml

reaction mixture contained 3 mg/ml microsomes and was incubated for a further 10 min at 37 ˚C, after which 0.5 ml of 0.6 M $HClO_4$ was added to stop the reactions. A blank was prepared to contain 0.975 ml of 50 mM Tris buffer, pH 7.4, 10 µl of 50 mM NADPH, 776 µl of distilled water, 5 µl of 5 mM $MgCl_2$ and 10 µl of 1.396 mg/ml PNP, incubated for 3 min at 37 °C.

The reaction mixture was centrifuged at 3000 rpm for 5 min to remove precipitated proteins. An aliquot of the supernatant fraction (1 ml) from each reaction mixture was added to an equal volume of 10 M NaOH. The absorbance of the resulting solutions was quickly read at 546 nm against the auto blank. The PNPH activity was determined as product formed (4-nitrocatechol) per min per mg protein, using 10.28 $mM^{-1}cm^{-1}$ as the molar absorptivity of 4-nitrocatechol.

The PNPH activity was determined from the equation below:

$$\text{Specific activity} = A / Є \times Pr\ (mg/ml) \times T\ (min) \times \text{nmol/min/mg protein}$$

Pr - Total protein in incubation mixture, T - Incubation time, A - Absorbance at 546 nm, Є - Molar absorptivity of 4-nitrocatechol = 10.28 $mM^{-1}cm^{-1}$.

The dilution factor was taken into consideration.

2.2.7 Determination of Aminopyrine – N - Demethylase (AmD) Activity: Aminopyrine - N - Demethylase (AmD) activity was measured by determining the production of formaldehyde [19]. To 0.2 ml of 50 mM semicarbazide HCl, 0.8 ml of 2.5 mM NADPH and 0.5 ml 20 mM aminopyrine were added, and the mixture was incubated at 37 ˚C for 2 min, in a shaking water bath. The enzyme reaction was initiated by adding 0.5 ml of diluted microsomal fraction (containing 4 mg/ml protein) to yield a final 1 mg/ml protein concentration in the reaction mixture. The incubation was continued at 37 °C for a further 30 min. Aminopyrine was substituted for an equal volume of distilled water in the blank, which contained other constituents of the reaction mixture. The blank was subjected to the same treatment as the reaction mixture. The reaction was stopped by adding 0.5 ml of 25% w/v $ZnSO_4$, thoroughly mixed, and kept on ice for 5 min. 0.5 ml of saturated Ba(OH)2 was added to the mixture and centrifuged for 5 min at maximum speed using the Denly bench centrifuge (BS 400, England), after a second round of mixing and cooling on ice. To 1 ml of the supernatant, 2 ml of Nash reagent (prepared from a mixture of 30 g ammonium acetate, 0.4 ml acetylacetone, and then made up to 100 ml with distilled water) was added and incubated at 60 ˚C for 30 min after tightly capping the test tubes. The tubes were extensively cooled, and the absorbance read at 415 nm against the blank. A standard curve over 0 – 0.1 mM formaldehyde was prepared using distilled water. The standard was subjected to the same treatment with the Nash reagent as the supernatant.

The specific activity was determined as the formaldehyde formed per incubation time per total protein (in the reaction mixture) in nmol/min/mg.

2.2.8 Determination of 4- Nitroanisole – O – Demethylase (NOD) Activity: The 4- Nitroanisole – O – Demethylase (NOD) activity was determined by measuring the 4- nitrophenol produced [20]. A mixture of 1 ml of 2 mM NADPH and an equal volume of microsomal dilution (containing a 4 mg/ml protein) was prepared and incubated at 37 ˚C for 2 min in a shaking water bath. The enzyme reaction was initiated with 10 µl of 500 mM 4 – Nitroanisole and incubated again for 15 min.

The reaction mixture contained 2 mg/ml of microsomal proteins. A blank was prepared as the reaction mixture, except that the microsomal proteins were denatured at 100 ˚C. The enzyme reaction was terminated with 1 ml of ice-cold 20 % w/v TCA and allowed to stand on ice for 5 min. It was centrifuged to a clear supernatant for 5 min at maximum speed using the Denly bench centrifuge (BS 400, England). To 1 ml of the supernatant, 40 µl of NaOH was added to the pH of approximately 10 – 11. Distilled water was added to yield a final volume of 1.5 ml, and the absorbance read at 400 nm. A standard curve over 0 – 0.1 mM of 4-Nitrophenol was prepared using 6 % w/v TCA solution. The standard was treated in the same way as the supernatant collected from the reaction mixture. The specific activity was determined as 4-nitrophenol formed per incubation time per total protein in the reaction mixture in nmol/min/mg.

2.3 Statistical Analysis: The results were expressed as mean ± standard error of the mean (SEM). The significance of the difference between the control and test values was evaluated using analysis of variance (ANOVA). This was done using the computer programme 'Statistical Package for Social Sciences (SPSS), version *16.0.* P – Value less than 0.05 ($p < 0.05$) was taken as significant.

RESULTS:

3.1 Microsomal Protein Concentration: The effect of concurrent administration of *C. sanguinolenta* and chloroquine on rat hepatic microsomal protein content is shown in **Fig. 1**. All the treatment groups' hepatic microsomal protein content decreased significantly compared to the normal control group (no drugs). Concurrent administration of *C. sanguinolenta* and chloroquine resulted in a further decrease (63.8%, p = 0.003) in the microsomal protein content compared to the normal control (no drug).

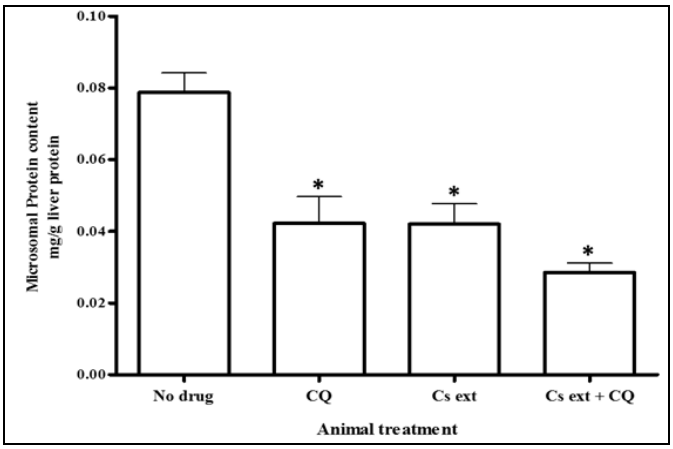

FIG. 1: EFFECT OF CONCURRENT ADMINISTRATION OF *C. SANGUINOLENTA* AND CHLOROQUINE ON MICROSOMAL PROTEIN CONTENT IN RATS. *Cryptolepis sanguinolenta* (Cs ext) was administered to rats for 2 weeks at a dose of 36 mg/kg, followed by a single dose of chloroquine, CQ (15m g/kg). The values are given as Mean ± SEM; n = 6 * Value significantly different (p < 0.05) from the normal control group (No drug).

3.2 Cytochrome c Reductase Activity: Fig. 2 shows the effect of concurrent administration of *C. sanguinolenta* and chloroquine on rat hepatic microsomal cytochrome c reductase activity. Apart from the chloroquine treated group, which showed a significant increase in the cytochrome reductase activity, all the other treatments showed a slight insignificant decrease (p > 0.05) in the enzyme activity relative to the normal control (no drug).

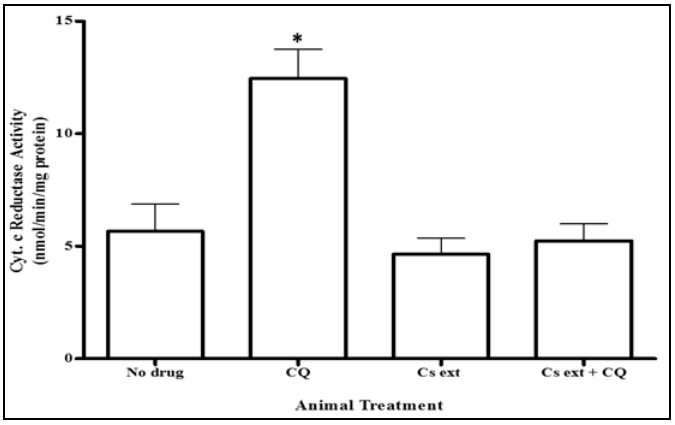

FIG. 2: EFFECT OF CONCURRENT ADMINISTRATION OF *C. SANGUINOLENTA* AND CHLOROQUINE ON THE HEPATIC MICROSOMAL CYTOCHROME C REDUCTASE ACTIVITY IN RATS. *Cryptolepis sanguinolenta* (Cs ext) was administered to rats for 2 weeks at a dose of 36 mg/kg, followed by a single dose of chloroquine, CQ (15m g/kg). The values are given as Mean ± SEM; n = 6 * Value significantly different (p < 0.05) from the normal control group (No drug)

3.3 Para- Nitrophenol Hydroxylase (PNPH) Activity: Administration of *C. sanguinolenta* and chloroquine separately and concurrently did not show significant difference (p > 0.05) in the enzyme activity **Fig. 3** although chloroquine treated and *C. sanguinolenta*-chloroquine treated groups slightly increased the PNPH activity to about the same degree.

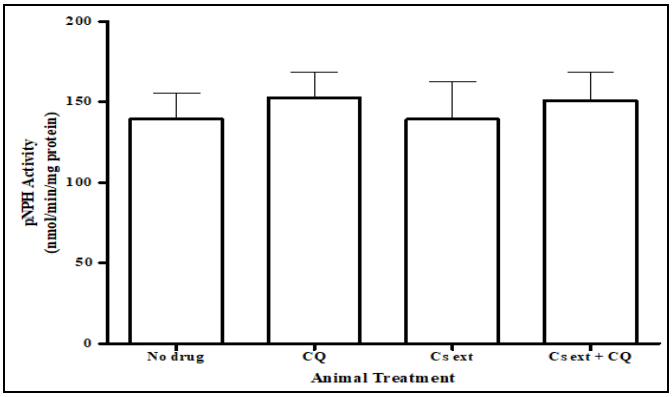

FIG. 3: EFFECT OF CONCURRENT ADMINISTRATION OF *C. SANGUINOLENTA* AND CHLOROQUINE ON THE HEPATIC MICROSOMAL PNPH ACTIVITY IN RATS. *Cryptolepis sanguinolenta* (Cs ext) was administered to rats for 2 weeks at a dose of 36 mg/kg, followed by a single dose of chloroquine, CQ (15m g/kg). The values are given as Mean ± SEM; n = 6

3.4 Aminopyrine - N - Demethylase (AmD) Activity: Fig. 4 shows the concurrent administration of *C. sanguinolenta* and chloroquine on rat hepatic microsomal Aminopyrine N-Demethylase (AmD) activity.

Treatment with *C. sanguinolenta* and chloroquine administered separately and concurrently all increased the enzyme activity significantly ($p < 0.05$), although the chloroquine treated group showed the highest activity (87.1% increase, $p = 0.001$) among the three treatment groups.

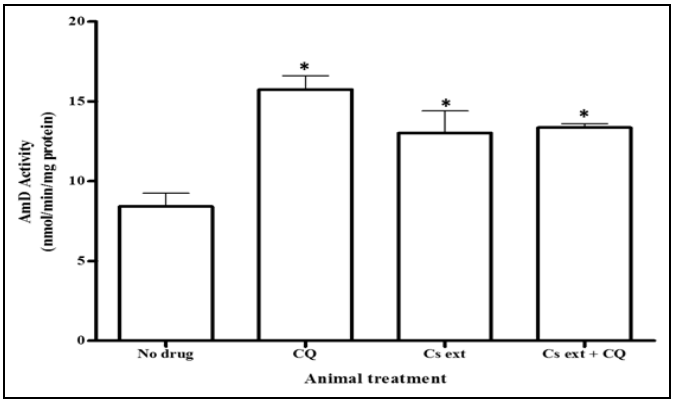

FIG. 4: EFFECT OF CONCURRENT ADMINISTRATION OF *C. SANGUINOLENTA* AND CHLOROQUINE ON RAT HEPATIC MICROSOMAL AMD ACTIVITY. *Cryptolepis sanguinolenta* (Cs ext) was administered to rats for 2 weeks at a dose of 36 mg/kg, followed by a single dose of chloroquine, CQ (15m g/kg). The values are given as Mean± SEM, n = 6. * Value significantly different ($p < 0.05$) from the normal control group (no drug).

3.5 4–Nitroanisole-O-Demethylase (NOD) Activity: Effects of concurrent administration of *C. sanguinolenta* and chloroquine on rat hepatic microsomal 4-Nitroanisole - O - Demethylase (NOD) activity is shown in **Fig. 5**.

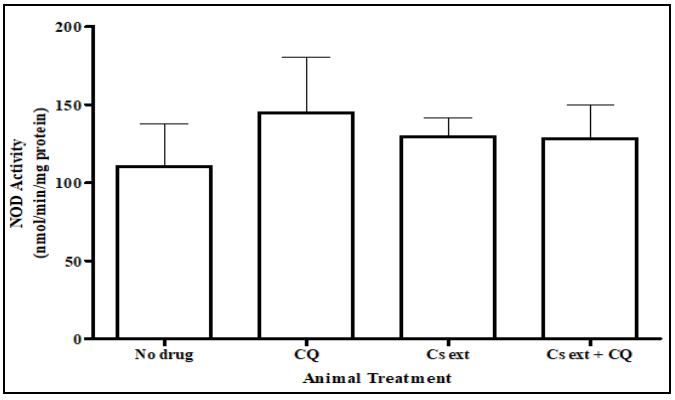

FIG. 5: EFFECT OF CONCURRENT ADMINISTRATION OF *C. SANGUIOLENTA* AND CHLOROQUINE ON RAT HEPATIC MICROSOMAL NOD ACTIVITY. *Cryptolepis sanguinolenta* (Cs ext) was administered to rats for 2 weeks at a dose of 36 mg/kg, followed by a single dose of chloroquine, CQ (15m g/kg). The values are given as Mean ± SEM; n = 6.

Treatment with *C. sanguinolenta* and chloroquine separately and concurrently showed a slight insignificant increase ($p > 0.05$) in the enzyme activity (17.4%, 31%, and 16%, respectively) relative to the normal control group.

DISCUSSION: Concurrent administration of herbal medicines and conventional drugs is a common practice globally, especially as the patronage of medicinal plants increases across the world. However, the clinical consequence of such practice has not gained much attention as expected, probably due to the dearth of literature concerning the utility of herbal medicines with orthodox drugs. In a previous study, Sakyiamah *et al.* reported that concurrent administration of aqueous extract of *Cryptolepis sanguinolenta* and chloroquine resulted in changes to the pharmacokinetics of chloroquine [13]. As a follow-up, the role of metabolism in such herb-drug interaction, that is, the effect of *C. sanguinolenta* administration on the CYP metabolism of chloroquine, was reported in this study.

Two major factors that contribute significantly to the bioavailability of a drug are the degree of first-pass metabolism of the drug in the liver and intestine and the extent of protein binding of the drug [21]. Chloroquine inhibits protein synthesis, with mean values for protein binding ranging between 58 – 64% [22-24]. From the present study, chloroquine decreased the microsomal protein content significantly. Contrary to the previous report by Ansah *et al*. that *C. sanguinolenta* did not cause any significant change in total protein [25], the present study indicated a significant decrease in the microsomal protein content **Fig. 1**. Concurrent administration of *C. sanguinolenta* and chloroquine further decreased the protein content, suggesting that both *C. sanguinolenta* and chloroquine inhibit protein synthesis and/or bind extensively to proteins, thus, affecting levels of the free proteins. Protein-bound drugs are not free to be detected in the blood and are also not available for metabolism reflecting in changes of the pharmacokinetics of targeted drugs, especially bioavailability, half-life and elimination [26]. Although administration of chloroquine and C. sanguinolenta separately and concurrently decreased the microsomal protein content, it did not affect the cytochrome c reductase's integrity, as shown in **Fig. 2**. Chloroquine administration, however, increased the activity of cytochrome c reductase, reiterating the

potential for chloroquine to modulate drug metabolism. The other factor contributing significantly to the bioavailability of a drug is the degree of first-pass metabolism in the liver and the intestines. For instance, drugs with high first-pass metabolism (*e.g*., morphine, propanolol) have low oral bioavailability [27]. The liver mainly metabolizes chloroquine to its principal de-ethylated metabolite, desethylchloroquine [28]. From the study, administration of *C. sanguinolenta* and chloroquine separately and concurrently did not show a significant difference in the PNPH activity (a measure of CYP2E1 activity), although chloroquine treated and *C. sanguinolenta*-chloroquine treated groups slightly increased to about the same degree **Fig. 3**. This suggests that both *C. sanguinolenta* and chloroquine are probably not substrates of the CYP enzyme, CYP2E1.

Similarly, the results suggest that *C. sanguinolenta* and chloroquine are not substrates of CYP1A since the study showed that, the NOD activity (a measure of CYP1A activity) of chloroquine and *C. sanguinolenta* administered separately and concurrently were not significantly different from the control group **Fig. 5**. This finding is consistent with previous reports indicating that chloroquine has no effect on the activities of CYP1A2, CYP2C19, CYP2E1, CYP3A4 or *N*-acetyl-transferase [6, 29].

In the present study, both chloroquine and *C. sanguinolenta* appear to be substrates of CYP2B1 and possibly share the same active site of the enzyme. This is because the administration of *C. sanguinolenta* and chloroquine separately and concurrently all activated the AmD activity (a measure of CYP2B1 activity) significantly ($p < 0.05$), though administration of chloroquine only caused an enhanced activation (87.1% increase) of AmD activity **Fig. 4**. However, the AmD activity after concurrent administration of C. sanguinolenta and chloroquine was comparable to that of the *C. sanguinolenta* only treated group; 58.9% and 54.7%, respectively. This suggests that chloroquine, in the presence of *C. sanguinolenta*, is probably precluded access to the active site of CYP2B1, an indication of possible competitive enzyme inhibition. This implies that in the presence of *C. sanguinolenta*, the metabolism of chloroquine could be significantly reduced, thus leading to increased bioavailability of chloroquine and possibly its delayed elimination. This observation corroborates with earlier studies where concurrent administration of aqueous extract of *Cryptolepis sanguinolenta* and chloroquine increased the bioavailability, half-life, and decreased absorption rate constant, maximum whole blood concentration, the volume of distribution, clearance, and elimination rate constant of chloroquine diphosphate [13].

The clinical consequence of such herb-drug interaction may include possible toxicity as a result of drug accumulation. Furthermore, the extensive protein binding of chloroquine in the presence of *C. sanguinolenta* may lead to sub-therapeutic levels of chloroquine, culminating in loss of efficacy and/or development of resistance. In the midst of the CoVID-19 pandemic, several people, especially in the developing world, self-medicate and/or depend on herbal medicine to relieve or prevent the disease. In Ghana, most of these preparations contain *Cryptolepis sanguinolenta*. Therefore, patients should be advised on the clinical consequence of concurrent administering *Cryptolepis* sanguinolenta-based herbal products and chloroquine in the treatment of any disease, including COVID-19 disease.

CONCLUSION: The present study shows that concurrent administration of chloroquine and *Cryptolepis sanguinolenta* impaired the CYP-mediated metabolism of chloroquine due to competitive inhibition of CYP2B1 by *C. sanguinolenta*.

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CONFLICTS OF INTEREST: The author hereby declares that there is no conflict of interest

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