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Effect of Aqueous Extract of *Cryptolepis sanguinolenta* on Pharmacokinetics of Artesunate

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ABSTRACT
The aim of this study is to investigate the effect of aqueous extract of *Cryptolepis sanguinolenta* on the pharmacokinetics of the antimalarial; artesumate in male Sprague-Dawley rats. Reconstituted freeze dried *Cryptolepis sanguinolenta* was administered in drinking water at the therapeutic dose of 36 g/kg body weight for two (2) weeks, followed by a single oral dose of artesunate (150 mg/kg body weight). The Concentration - Time course; Maximum Whole Blood Concentration, C_{max}; Time for the Maximum Concentration, T_{max}; Bioavailability; Elimination Rate Constant; Half- life; Clearance; Volume of Distribution and Absorption Rate Constant were measured. Results indicated that about 80% of dihydroartemisinin was recovered during extraction suggesting that the extraction procedure was effective. The elimination rate constant and clearance of dihydroartemisinin the most potent metabolite of artesunate increased by 233.3% and 62.1% respectively in rats which received concurrent administration of *Cryptolepis sanguinolenta* compared to control (artesunate only). This resulted in significant decrease ($p < 0.05$) in the Bioavailability (40.1%), Volume of Distribution (68.1%) and Half-life (52.1%) of dihydroartemesinin. The findings therefore, showed that *Cryptolepis sanguinolenta* could cause sub-therapeutic blood levels of dihydroartemisinin of artesunate leading to decreased effectiveness and possibly drug resistance as a result of herb-drug interactions. Thus, in clinical practice, patients should be advised on the serious implication of using *Cryptolepis sanguinolenta* and artesunate together.

Keywords: *Cryptolepis sanguinolenta,* Pharmacokinetics, Artesunate, herb-drug interaction.

INTRODUCTION

As the patronage of herbal medicines increases across the world, herbs have been increasingly used with other drugs rather than in place of drugs, [1] raising the concern about the interaction of herbs and drugs which may be adverse. [2] Regarding therapy, some patients may consume some drugs including herbal medicine before reporting to allopathic health facilities. Many of them, however, do not disclose this information to their physicians thus increasing the chances of possible herb – drug interactions. This practice is common with disease conditions, in which self-medication is mostly possible or where combination therapy is the approach recommended. [3-5]

Malaria, which ranks second to HIV/AIDS as the leading health problem in sub-saharan Africa, [6] causing about 1-3 million deaths of mostly children under five (5) years of age and pregnant women annually, [7] is a typical example of such diseases in which self-medication is possible or combined therapy is recommended.

Artemisinin and its derivatives are currently the first-line drugs in use. [8] However, the prohibitive cost of these drugs and the adherence to the rigid dosage regimen results in noncompliance by patients. As a result of the scourging effect of malaria disease, coupled with the incidence of resistance to affordable antimalarials and noncompliance to expensive antimalarials, the practice of patients taking herbal antimalarials and artemisinin derivatives concomitantly is on the rise and in Ghana, the practice of concurrent administration of antimalarial preparations, most of which contain *Cryptolepis sanguinolenta* and some orthodox antimalarials has been observed (unpublished observation).

In herb-drug interactions, some herbs are reported to interact with the drugs in a beneficial way, such as reducing the toxicity or other adverse reactions that might be experienced. Some herbs also increase or decrease the metabolism of the drug thus affecting the drug availability. [9] For instance, ginger is reported to cause increase in the bioavailability and half-life, and decrease in the clearance and elimination rate constant of metronidazole per oral administration. [10] This

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may pose a negative implication in clinical practice as toxicity of metronidazole may easily be reached especially during multiple dosing because of the possibility of drug accumulation. [10] Ang-Lee *et al*. reported and recommended discontinuation of herbal or other products before proceeding for surgery and anaesthesia to avoid undesirable and unexpected side effects, complications and delayed outcome. [11] For instance, Garlic and ginseng intake are discontinued at least seven days before surgery because both herbs have been reported to cause aggressive bleeding. [11]

Unfortunately not much is known about herb-drug interaction. This is because research on herbal therapies is not nearly as advanced as research on pharmaceutical drugs. [12] In addition, studies on prescription drugs rarely consider potential interactions with herbs. Also, most healthcare practitioners do not have clinical experience combining herbs and drugs. [13-14]

The present study was therefore aimed at determining the effect of *Cryptolepis sanguinolenta* administration on the following pharmacokinetic parameters of artesunate; Concentration - time course: Maximum whole blood concentration and Time for the maximum concentration, Bioavailability, Elimination rate constant, Half-life, Clearance, Volume of distribution and Absorption rate constant. The outcome would provide information on the effect(s) of possible herb-drug interaction on the therapeutic levels of artesunate, and the clinical significance in the treatment of malaria.

MATERIALS AND METHODS

Animals

A total of twelve (12) male Sprague - Dawley rats weighing between 298 – 343 g were obtained from the Animal Unit of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong Akuapem, Ghana and standard laboratory chow diet was obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana.

Plant material

Powdered dried roots of *Cryptolepis sanguinolenta* was obtained from CSRPM, Mampong Akuapem, Ghana.

Preparation of Plant Extract

Powdered dried roots of *Cryptolepis sanguinolenta* (360 g) was boiled in 6 liters of water for 45 min and cooled. The resultant extract was filtered through a cotton wool, pre-freezed and lyophilized into powder using a freeze dryer (EYELA, Tokyo Rikakikai Co LTD, Japan). The dry powdered extract was weighed to determine the yield (5.92%) and stored in a desiccator at room temperature. The powder was reconstituted in sterilized distilled water before administering to the animals.

Experimental design

The rats were randomly divided into two (2) 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: Received artesunate, as a single dose (150 mg/kg; based on the recommended dosage in humans) after two weeks served as control.

Group II: Served as concurrent administration group received reconstituted freeze dried *Cryptolepis sanguinolenta* in their drinking water at the therapeutic dose (36 g/kg) (CSRPM, unpublished data) for two (2) weeks and single oral dose of artesunate at the end of the two weeks.

Samples

Whole blood samples (1 ml) of animals in each group were then aseptically withdrawn by tail bleeding at 0.5, 1, 2, 6 and 24 h after administration of the single dose of artesunate. Blood was collected into tubes containing trisodium citrate as an anticoagulant, and stored at –80°C for pharmacokinetic studies.

Extraction of dihydroartemisinin in whole blood

Dihydroartemisinin was extracted from whole blood using the method by Ortelli *et al* with some modifications. [15] Briefly, whole blood sample (200µl) was pipetted into a Pyrex tube and dihydroartemisinin extracted with 5 ml hexane – ethyl acetate (4:1 v/v) by gently mixing for at least 15 min. The mixture was then centrifuged at 3000 g for 5 min. The upper organic phase was transferred to a clean glass tube and dried under a stream of nitrogen at room temperature. The residue was reconstituted with 1 ml of the mobile phase and 10µl was injected onto the HPLC column. The efficiency of the extraction procedure was assessed by determination of percentage recovery. Whole blood (200µl) was spiked with 50µg/ml dihydroartemisinin standard followed by the extraction procedures outlined above. Equal mass of the drug dissolved in the mobile phase was injected onto the column. Percentage recovery was determined by comparing the peak area obtained after extraction of the known amount of drug from whole blood with that obtained after injecting equal mass of the drug dissolved in mobile phase directly onto the column.

High Performance Liquid Chromatography analysis of Dihydroartemisinin

A calibration curve of dihydroartemisinin concentration versus peak area in the range of 10µg/ml to 100µg/ml in methanol was prepared as follows: Drug-free whole blood was taken through extraction process of dihydroartemisinin, and spiked with stock solutions of dihydroartemisinin to make a calibration curve in the range of 10µg/ml to 100µg/ml. Reference standard (50µg/ml of dihydroartemisinin) was used to check the system suitability, due to difficulty in obtaining appropriate internal standard and was also run before each sample. Peak area ratio (drug/reference standard) was plotted against concentrations and linearity was accepted for $r^2 \geq 99$ %.

Chromatography was run between 25°C-30°C using the High Performance Liquid Chromatography equipment, Thermo Finnigan with Spectra SYTEMSP4000 isocratic pump and a Thermo Finnigan Spectra SERIES autosampler AS 3000 fitted with a 100µl injection loop. Column used was a reverse-phase HYPER PEP C4 column 250 mm × 4.6 mm, 8µm particle sizes. The column effluent was monitored by a variable wavelength Thermo Finnigan Spectra SYTEMS UV1000 detector and peak areas and retention times were calculated using a Thermo Finnigan Chrome Quest integrator. The mobile phase was acetonitrile and deionized water (80: 20 % v/v), adjusted to a flow rate of 1ml/min, with the UV detector set at 210 nm.

Pharmacokinetic analysis

The Pharmacokinetics analysis was done as described by Shargel and Yu, and Rath *et al.* [16-17]

Concentration-time profile: Concentration-time profiles were analyzed using non-compartmental methods. The concentration-time profile was generated from the chromatographic analysis and the maximum whole blood

concentration (C_{max}) and time to C_{max} (T_{max}) was derived directly from the profile.

The elimination rate constant (K_{el}): Was first estimated from log-linear regression of the straight terminal part of the curve; in all instances this was made up of the last three or four concentration –time points.

The K_{el} was calculated from slope of the equation;

$$\log C_p = -kt / 2.3 + \log C_{po}$$

Where Cp is concentration at any time (t), k is the elimination rate constant; C_{po} is the intercept on the Y- axis, Therefore, the slope = -k / 2.3.

Elimination half-life ($t_{1/2}$): This was calculated using the equation;

$$t_{1/2} = In2/K_{el}.$$

Area under the concentration-time curve (AUC): This is a measure of the bioavailability and was calculated by the trapezoidal rule as shown below;

$$AUC = [C_{n-1} + C_n][\ t_n - t_{n-1}] / 2$$

Where t_n is the time of observation of drug concentration C_n , and t_{n-1} is the time of the prior observation of drug concentration corresponding to C_{n-1}.

Clearance rate (Cl): This was calculated as dose/AUC

Volume of distribution (V_d): This was calculated as Cl * K_{el}

Absorption rate constant (K_a): From a concentration-time semi-log graph, K_a was estimated from the intercept of the equation below;

$$\log C_p = \text{Log}\ [(FK_aD_o)/Vd(K_a-K_{el})] - kt / 2.3,$$

Where C_p is the concentration at time, t, K_{el} is the elimation rate constant, V_d is the volume of distribution, D_o is the dose and F is the fraction of the drug absorbed given as peak concentration per dose.

Statistical Analysis

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

RESULTS

Extraction and Measurement of Dihydroartemisinin Level in Whole Blood

The efficiency of the extraction process was determined by measuring the percentage of the total amount of dihydroartemisinin recovered during extraction. Whole blood was spiked with 50µg/ml dihydroartemisinin standard and taken through extraction process. The amount of dihydroartemisinin extracted was determined by measurement of peak area and expressed as a percentage of the peak area of 50µg/ml of dihydroartemisinin. About 80% of the dihydroartemisinin (78.34 ± 1.58) was recovered for three independent extractions.

The sensitivity of HPLC condition and the possible interference of extracts of whole blood with dihydroartemisinin were determined by comparing the chromatogram of extracted whole blood (blank) with extracted dihydroartemisinin containing whole blood. DHA showed twin peaks with retention times at about 3.2 min and 3.5 min (Fig. 1B). The extracts in the whole blood only absorbed minimally at about 2.3 min (Fig. 1A & B) and did not interfere with the peaks of dihydroartemisinin (Fig. 1B).

Effect of aqueous extract of *C. sanguinoleta* on Pharmacokinetics of Dihydroartemisinin

Fig. 2 shows the whole blood concentration-time profile of DHA in presence and absence of the aqueous extract of *C. sanguinoleta*. Even though the co-administration of the extract did not significantly alter the peak or maximum whole blood concentration (C_{max}) of DHA and the time to reach maximum blood concentration (T_{max}), it did alter the overall concentration-time profile of DHA (Fig. 2 and Table 1) and consequently, significantly changed ($p < 0.05$) the other pharmacokinetic parameters of dihydroartemisinin (Table 1).

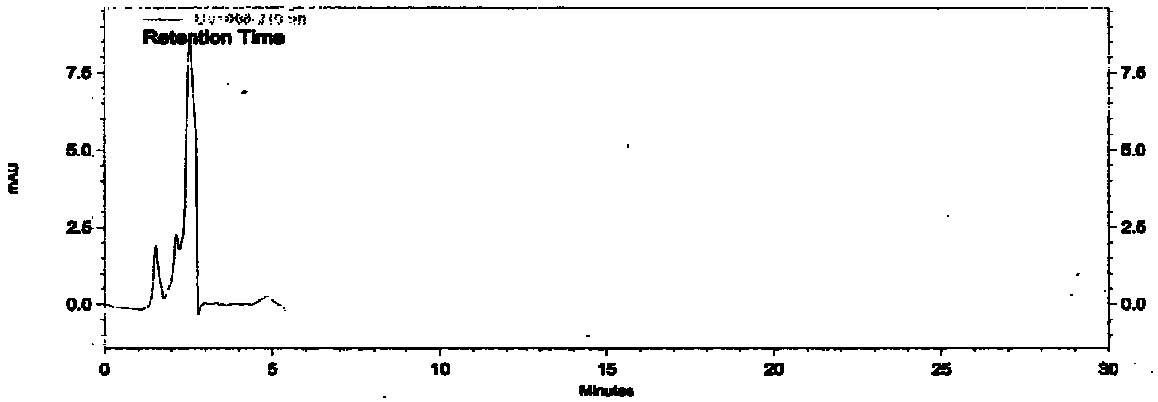
The peak whole blood levels of both control group (artesunate treated group) and test group (*C. sanguinolenta* and artesunate treated group) were 9000.00 ± 363.72 ng/ml and 8400.00 ± 514.74 ng/ml respectively and the time for maximum concentration was about 0.5 h. However, the DHA levels of the test group (*C. sanguinolenta* and artesunate treated group) decreased more rapidly to undetectable levels after 6 h, whilst the level in the control group decreased gradually and only reached undetectable levels after about 22 h.

Whilst the area under the curve (AUC), elimination half-life ($T_{1/2}$) and volume of distribution (V_d) of dihydroartemisinin significantly decreased ($p < 0.05$) by 40.1%, 52.1% and 68.1% respectively, the C_{max} of dihydroartemisinin decreased insignificantly by 6.7% on administration of artesunate in the presence of *C. sanguinolenta.* The elimination rate constant (K_{el}), clearance (Cl), and absorption rate constant (K_a) of dihydroartemisinin increased significantly by 233.3%, 62.1% and 358.8% respectively (Table 1).

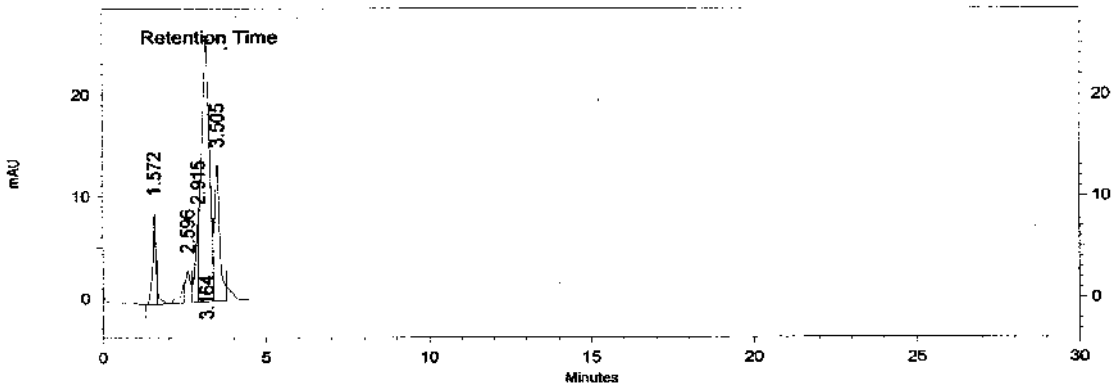
DISCUSSION

As the patronage of herbal medicines increases across the world, herbs have been increasingly used with other drugs rather than in place of drugs. [1] Patients tend to take other xenobiotics such as drugs and herbs together to facilitate or hasten their recovery. The consequences of such practice, which are often overlooked, could lead to drug interactions that may be clinically significant. [18-19] Artemisinin and its derivatives are the most effective and recommended first-line drugs in most countries. It is against this background that the effect of the aqueous extract of *Cryptolepis sanguinolenta* on the pharmacokinetics of artesunate has been investigated to ascertain any possible herb–drug interactions. The study sought to ascertain possible drug interactions that could lead to drug resistance, and hence to draw attention of the health authorities to address the issue of concurrent usage of *C. sanguinolenta* and artemisinin derivatives.

Every drug has its own drug profile; the half-life, absorption rate, elimination, peak serum concentration, volume of distribution and serum clearance rate. [20] However, all these properties (the pharmacokinetic parameters) can easily be affected by other compounds (drugs, food, herbs, etc). [18] The present study provides evidence that concurrent usage of *C. sanguinolenta* and artesunate altered all the pharmacokinetic parameters; the area under the curve (AUC), maximum whole blood concentration (C_{max}), elimination half-life ($T_{1/2}$), elimination rate constant (K_{el}), clearance (Cl), volume of distribution (V_d) and absorption rate constant (K_a), except the T_{max} (time for maximum concentration to be attained) of dihydroartemisinin (DHA), the more potent metabolite of artesunate. Apart from the C_{max}, all the alterations in the other parameters were significant ($p < 0.05$).



A.



B.

Fig. 1: HPLC analyses of dihydroartemisinin: Chromatograms showing peaks after extraction of (A) drug free whole blood as blank and (B) DHA contained whole blood. Retention times (t_{Rs}) in min are shown adjacent to the peaks.

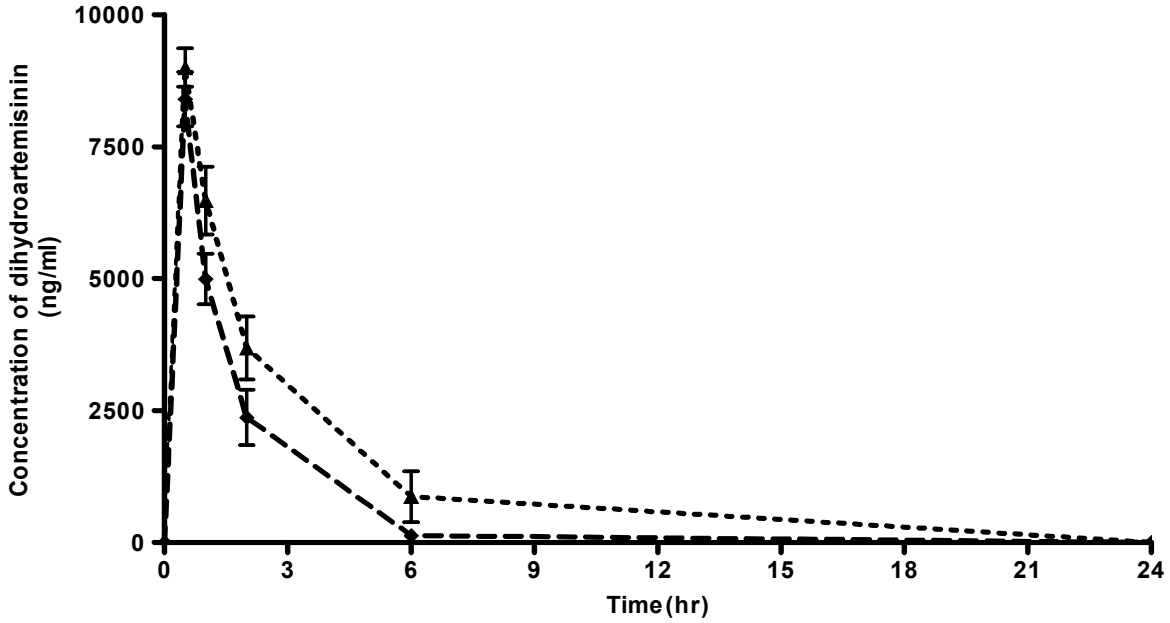


Fig. 2: Effect of *C. sanguinolenta* on whole blood concentration of dihydroartemisinin
Aqueous extract of *Cryptolepis sanguinolenta* aqueous at concentration of 36 g/kg body weight was administered for two weeks after which a single dose of artesunate (150 mg/kg body weight) was administered (test group) (broken lines with square bars). Control group (dotted lines with triangle bars) were given only the single dose of artesunate. Whole blood dihydroartemisinin was extracted and analysed. Values are **Mean ± SEM** for six (n=6) samples.

Table 1: Pharmacokinetic parameters of dihydroartemisinin after administration of a single oral dose of artesunate in the absence and presence of *C. sanguinolenta*

Pharmacokinetic Parameter	Artesunate	*C. sanguinolenta* + Artesunate	Percentage Change / comment	p-value
AUC ($ng/ml^{-1}h^{-1}$)	23.98 ± 2.31^{a}	14.37 ± 0.29^{a}	40.1% / decrease	0.017
C_{max} (ng/ml)	9000.00 ± 363.73	8400.00 ± 514.75	6.7% / decrease	0.364
T_{max} (h)	0.5	0.5	no change	-
$T_{1/2}$ (h)	1.94 ± 0.05^{b}	0.93 ± 0.31^{b}	52.1 %/ decrease	0.009
K_{el} (h^{-1})	0.36 ± 0.01^{c}	1.20 ± 0.28^{c}	233.3% /increase	0.014
Clearance (Lh^{-1})	2.06 ± 0.20^{d}	3.34 ± 0.07^{d}	62.1% / increase	0.003
V_d (L/kg)	5.87 ± 0.75^{e}	1.87 ± 0.06^{e}	68.1% / decrease	0.006
K_a (h^{-1})	0.34 ± 0.01^{f}	1.56 ± 0.21^{f}	358.8%/ increase	0.004

Values are Mean ± SEM for six (n=6) samples. $^{a-f}$ statistically significant difference ($p < 0.05$) between the group with *C. sanguinolenta* aqueous extract and the group without extract

Even though, the absorption rate, K_a increased from 0.34± 0.01 to 1.56±0.2 representing an increase of 358.8% ($p = 0.004$), C_{max} decreased slightly (6.7%) whilst T_{max} was not altered in presence of the extract (Fig. 2; Table 1). Artesunate is absorbed rapidly from the gut and biotransformed into its more active metabolite DHA attaining relative bioavailability of 82 - 85% whereas artesunate, the pro-drug reaches low or undetectable concentrations. [21] The biotransformation of artesunate to DHA is mainly via blood esterases. [22] This means that the conversion of artesunate to DHA primarily takes place after absorption, implying that the observed change in K_a was not a true reflection of the absorption of artesunate from the gut; suggesting that *C. sanguinolenta* did not alter the absorption of artesunate.

Consequently, the bioavailability of DHA after concurrent administration of *C. sanguinolenta* and artesunate was expected not to change significantly since the absorption after the treatment was not altered. Such observation was expected because, according to Thomson, [23] the major effect of decreased absorption of a drug is a corresponding decrease in the bioavailability of the drug in the systemic circulation. On the contrary, the bioavailability (AUC) of DHA was significantly decreased after concurrent administration of *C. sanguinolenta* and artesunate although the absorption was probably not affected.

From the present study, in addition to decreasing the AUC of DHA, the elimination half-life ($T_{1/2}$) of DHA significantly decreased ($p < 0.05$) by 52.1% while its elimination rate constant (K_{el}) and clearance significantly increased by 233.3% and 62.1% respectively after administration of artesunate in the presence of *C. sanguinolenta* as shown in Table 1. These observations suggest that the metabolism of DHA was probably enhanced in the presence of *C. sanguinolenta*.

Artemisinin and its derivatives (artemether, artesunate, arteether, deoxyartemisinin and dihydroartemisinin) have been reported to undergo hepatic metabolism [24] and are known to affect several principal cytochrome P450 (CYP) isozymes. Although the metabolic changes are usually moderate, in several cases such effects are shared by all five endoperoxides suggesting a class effect. [25] For instance, artemether and dihydroartemisinin (DHA) have been reported to be metabolized *in vitro* by CYP1A, CYP2B6, CYP2C19 and CYP3A4. [22]

Pharmacokinetic studies of a drug are necessary in assessing its safety and efficacy. The major areas considered are the absorption, distribution and elimination or metabolism. From the present study, concurrent administration of *C. sanguinolenta* and artesunate did not affect the absorption of artesunate but decreased the bioavailability and distribution of DHA though the C_{max} was not significantly affected. The elimination of DHA was significantly enhanced after concurrent administration of *C. sanguinolenta* and artesunate. This observation suggests that the effectiveness of artesunate after concurrent administration of *C. sanguinolenta* and artesunate may be affected as a result of rapid flush out of the drug leading to sub therapeutic levels, a major factor for drug resistance.

The results of the present study revealed that the aqueous extract of *Cryptolepis sanguinolenta* caused decrease in the bioavailability, maximum whole blood concentration, volume of distribution, half-life, and increase in the absorption rate constant, clearance and elimination rate constant of artesunate administered orally. The findings suggest that *Cryptolepis sanguinolenta* could cause sub therapeutic blood levels of dihydroartemisinin of artesunate leading to decreased effectiveness and possibly resistance as a result of herb-drug interactions. Further studies are therefore required to determine the effect of *C. sanguinolenta* on the metabolism of artesunate via the Cytochrome P450 monooxygenase system, and also investigate the outcome of the herb-drug combination on malaria parasites *in vivo*. Meanwhile, in clinical practice, patients should be advised on the serious implication of using *Cryptolepis sanguinolenta* and artesunate together.

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