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EFFECT OF *TELFAIRIA OCCIDENTALIS* ON THE PHARMACOKINETIC PARAMETERS AND EFFICACY OF DIHYDROARTEMISININ AND AMODIAQUINE COMBINATION THERAPY

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ABSTRACT

Vegetables are rich sources of bioactive compounds which often than not are capable of altering the pharmacokinetic parameters of drugs. The aim of this study was to evaluate the effect of *Telfairia occidentalis* on the pharmacokinetic parameters and efficacy of dihydroartemisinin (DHA) and amodiaquine (AQ) combination therapy. Rats were used in the study and blood samples were obtained through cardiac puncture at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours and 12 hours post administration, respectively. The serum was separated by centrifugation at 2,000 rpm for 15 minutes. Liquid-liquid extraction was used to isolate drugs from serum and simulated intestinal fluid was used to reconstitute extracted drugs and at the same time derivatised DHA for UV analysis. The result revealed that *T. occidentalis* extract (Ext) altered all the pharmacokinetic parameters of both DHA and AQ. The $t_{½}$ of DHA was extended between 1.70 % and 40.34 % while the $t_{½}$ of AQ was altered between 1.09 % and 25.61%. It was also observed that the extract increased the C_{max} in all treatment groups except in the group administered with AQ + DHA + Ext 250mg/kg in which the C_{max} was reduced. The two-way ANOVA revealed a very significant ($p < 0.05$) difference in the pharmacokinetic parameters among the test groups with respect to the groups administered with only DHA and AQ. The effect of aqueous extract of *T. occidentalis* leaf increased the total exposure and efficacy of AQ and DHA.

KEYWORDS: Pharmacokinetic, *Telfairia occidentalis*, amodiaquine, dihydroartemisinin.

1.0 INTRODUCTION

Most foods that people consume in Nigeria are made up of appreciable percentage of vegetables and these vegetables are very rich in phytochemicals. *Telfairia occidentalis*, the most commonly available and consumed vegetable in Nigeria particularly in the Southern Nigeria, is rich in minerals such as iron, potassium, phosphorus, calcium and magnesium.[1] Antioxidants and phytochemical compounds such as phenols, cucubitacine, anthocynins, flavonoids, tannins, β-carotene, lycopene, vitamins A, C and E[2] are also present in *T. occidentalis*. These chemicals can influence the pharmacological activities of drugs by modifying their absorption characteristics through interaction with drug transporters as well as drug-metabolizing enzymes thereby altering drug concentration at the target sites.[3,4] Such interactions are likely to occur where phytochemicals occur in large concentrations, such as in the intestine and liver. Modification of cytochrome P_{450} and other metabolizing enzyme activities can seriously affect the fate of drugs.[5,6,7] Factors affecting the activity of drug-metabolizing enzymes also affect the bioavailability and efficacy of the drugs. Such factors include age, organs, immunological aspect and nutrition.

A 70 % methanol extract of *T. occidentalis* is reported to affect all the pharmacokinetic parameters of dihydroartemisinin and chloroquine, a 4-aminoquinoline just like amodiaquine, thereby altering the systemic bioavailability of the drugs.[8,9] The aqueous extract of the leaves of *T. occidentalis* has been reported to possess synergistic activity on artesunate when co-administered.[10] However, the pharmacokinetic activity of the leaves of *T. occidentalis* has not been reported in any of the studies so far. In the present study, the effect of *T. occidentalis* on the pharmacokinetic parameters and efficacy of dihydroartemisinin and amodiaquine combination therapy in rat was assessed.

2.0 MATERIALS AND METHODS

Sample collection, extract preparation, qualitative and quantitative analyses on phytochemical constituents of aqueous extract of *T. occidentalis* have already been reported,[1]

2.1 Animals Grouping and Administration of Extract and Drugs

The rats were separated into male and female and maintained in standard laboratory conditions. The animals were fed with pelletized grower mash and water *ad libitum*. They

were randomly divided into two main groups (A and B) to be administered with 250mg/kg and 500mg/kg extract, respectively. Each of the main groups was further divided into 8 subgroups (I to VIII) (each containing 35 rats) which were treated with DHA alone, AQ alone, DHA + Ext 250mg/kg, AQ + Ext 250mg/kg, DHA + Ext 500mg/kg, AQ + Ext 500 mg/kg, DHA + AQ + Ext 250mg/kg and DHA + AQ + Ext 500mg/kg, respectively. In each of the 8 subgroups, blood samples were obtained from 5 rats each at 15minutes, 30minutes, 1 hour, 2hours, 4hours, 8hours and 12hours post administration, respectively.

2.2 Blood Collection and Serum Preparation

The terminal method of blood collection was adopted. The animals were anaesthetized with chloroform and blood collected from the heart through cardiac puncture using a 5mL syringe.[11] The blood was immediately transferred into a labeled serum separator tube and its stopper tightly replaced. The blood was allowed to stand vertically at room temperature for 30minutes after which the tubes were placed in a centrifuge and spun at 2,000 rpm for 15minutes. The serum which separated as the upper layer was aspirated into a plain sample bottle and labeled accordingly.

2.3 Extraction of Drug from Serum

Liquid-liquid extraction method was adopted in the extraction of dihydroartemisinin (DHA) and amodiaquine (AQ) from the serum. Two milliliters (2mL) of the serum was transferred into a plain specimen bottle and equal volume of acetonitrile was added to precipitate proteins. The tubes were tightly corked and centrifuged at 4,000 rpm for 20minutes and the upper clear layer aspirated into another plain specimen bottle and labeled accordingly. Three milliliters (3ml) of hexane : ethyl acetate (60:40 v/v)[12] and three milliliters (3ml) of 100 % diethyl ether[13] were added to DHA and AQ samples, respectively and vigorously vortexed for 10 minutes. The upper layers in both cases were again aspirated into another set of plain specimen bottles that were previously labeled and the aqueous layers re-extracted with another 2 mL each of the respective extracting solvents. The pooled organic phases were dried with a current of air.

2.4 Preparation of Simulated Intestinal Fluid (SIF) Test Solution

The simulated intestinal fluid (SIF) was prepared without pancreatin. Exactly 13.61g and 1.80g of KH_2PO_4 and NaOH, respectively were dissolved separately in distilled water in 500mL beakers and later decanted into 2.0L volumetric flask. The beakers were rinsed severally into the volumetric flask and swirled for complete homogenization. The pH was

adjusted to 6.80 ± 0.01 with HCl and NaOH solutions, and the volume was finally made up to 2.0 L mark with distilled water. This was used in the dissolution of the formulated drugs (DHA and AQ) for calibration curve as well as reconstitution of the extracted drug from the serum for UV-spectrophotometric drug concentration determination.[14][15]

2.5 Reconstitution of Recovered Drugs and Derivatisation of DHA for UV-Vis Analysis and Estimation of Drug Concentration

Both DHA and AQ were reconstituted with SIF. Four milliliters (4mL) of SIF was added to each specimen bottle containing the dry sample of the recovered drugs and shaken vigorously for 10minutes to ensure homoginisation. The SIF also help in derivatising dihydroartemisinin so that it can absorb at a higher wave length of 290nm.[15] The concentrations of both DHA and AQ recovered from the serum were determined directly from their respective calibration graphs by extrapolation.

2.6 Preparation of stock solutions of DHA and AQ

Ten tablets of DHA (Codisin®) were weighed and average weight calculated. The tablets were crushed and one hundred milligrams (100mg) of the powder was accurately weighed and dissolved in 50 mL of SIF. The flask was agitated for about 30 minutes for complete dissolution of the drug. The solution was filtered into a 100mL volumetric flask and made up to volume with fresh SIF. This gives a stock solution concentration of 1 mg/mL. The same procedure was repeated for the preparation of AQ (Camasonate®) stock solution.[15]

2.7 Preparation of Working Solutions and Calibration Graph of Derivatised DHA and AQ with SIF

The working solutions of 2.5, 5, 10, 20, 40, 60, 80, 100 mg % were prepared accordingly from the stock using SIF to make up the volume where necessary. Then 5 mL of the different dilutions were collected separately and their triplicate absorbance recorded at the established λmax of 290nm and 343nm for DHA and AQ, respectively. From the results obtained, Beer's plot was generated.[15]

3.0 Data and Statistical Analyses

Data are reported as the mean ± SEM of triplicate determinations. Statistical analysis was performed using Graph Pad Prism version 7.03 for Windows. Two-way ANOVA was used to test for variation in pharmacokinetic parameters among the treatment groups. Post hoc

comparison of means was performed by Bonferroni's multiple comparison, and $p < 0.05$ was considered to represent a statistically significant difference between test groups.[16]

4.0 RESULTS

4.1 Pharmacokinetics Studies

The calibration graphs of derivatised DHA and AQ in SIF are represented in Figures 1 and 2, respectively. The results of pharmacokinetics study of the effect of aqueous leaf extract of *T. occidentalis* on the pharmacokinetic parameters of DHA and AQ administered singly and in various combinations are shown in Tables 2 and 3. The comparison of the concentration-time graph of DHA and AQ alone with other combinations are shown in Figures 3 and 4, respectively.

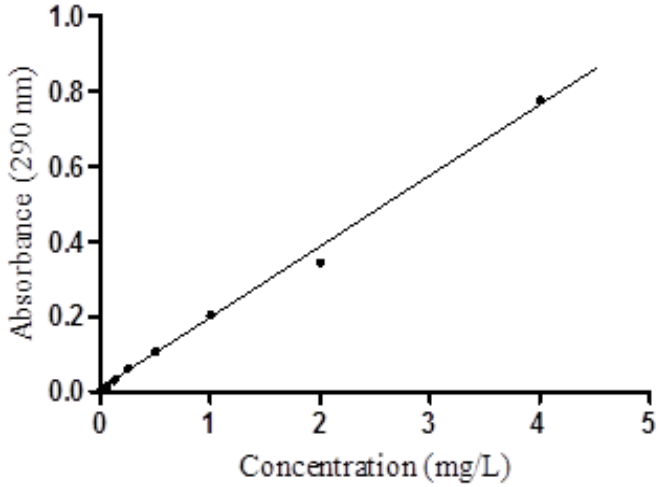

Figure 1: Standard curve of derivatised DHA.

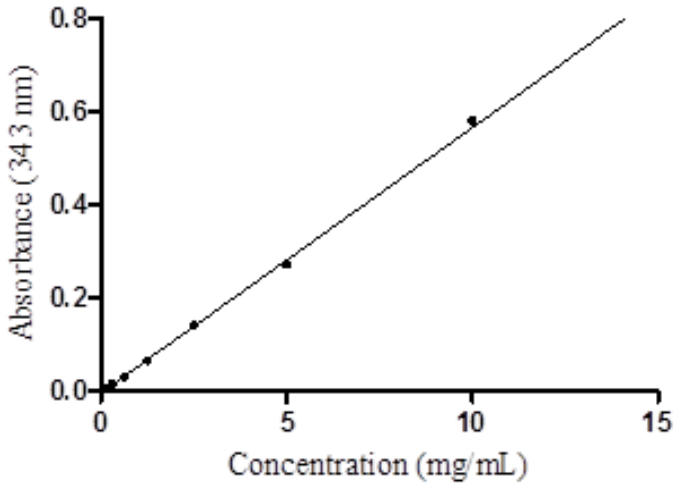

Figure 2: Standard curve of amodiaquine in SIF.

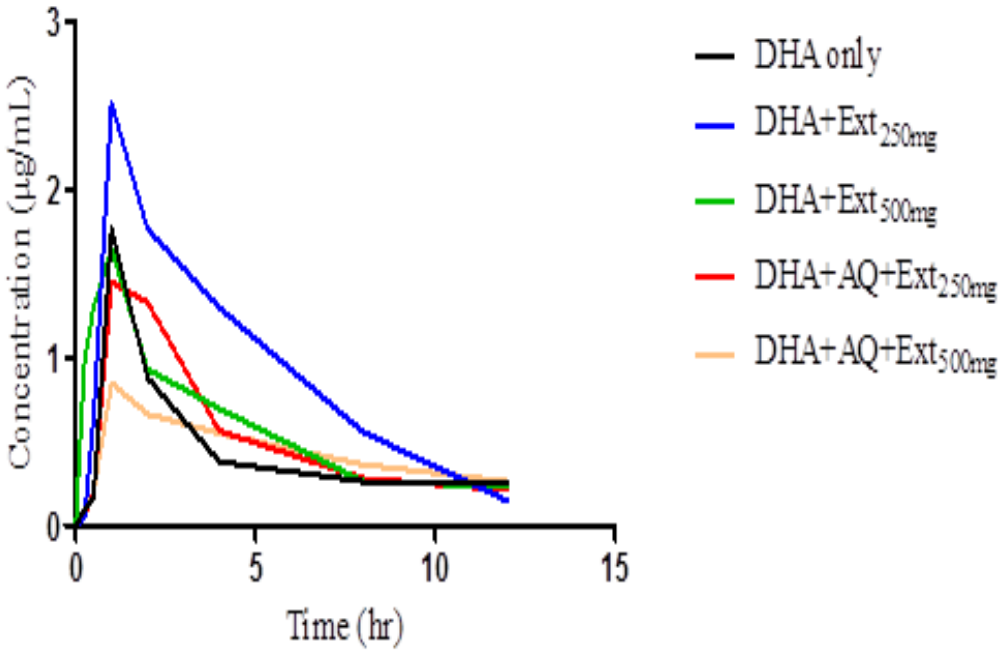

Figure 3: Comparison of the concentration-time graph of DHA alone and DHA plus other combinations.

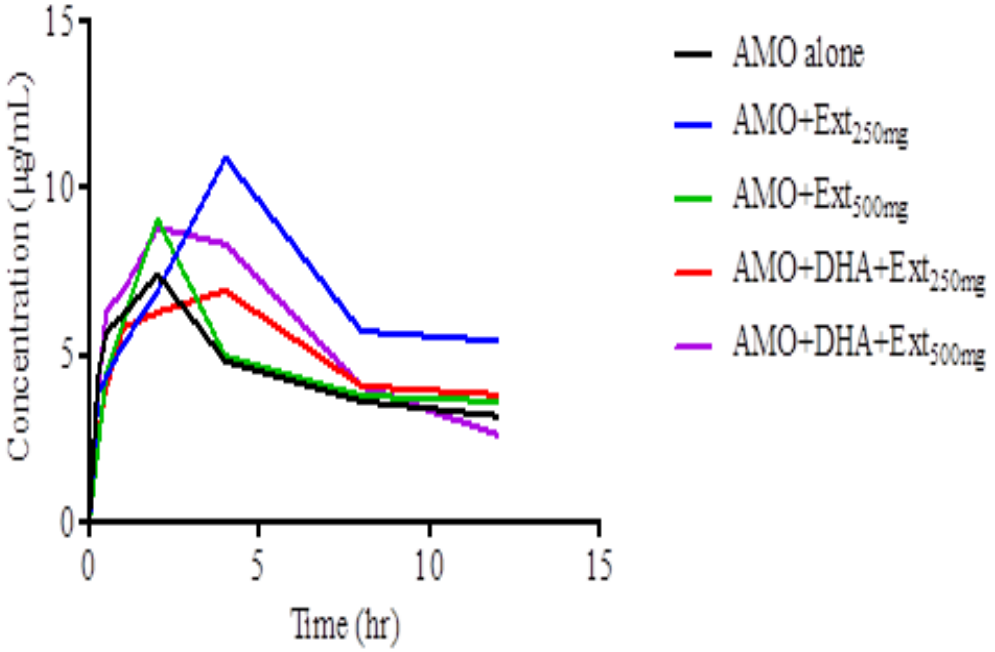

Figure 4: Comparison of the concentration-time graph of AQ and AQ plus other combinations.

Table 2: Effect of concurrent administration of the leaf extract of *T. occidentalis* on the pharmacokinetic parameters of DHA.

Parameter	DHA only	DHA+ Ext 250mg/kg	DHA+ Ext 500mg/kg	DHA+ AQ + Ext 250mg/kg	DHA+ AQ + Ext 500mg/kg
AUC (µg/h/mL)	5.27 ± 0.01	11.22 ± 0.01	7.01 ± 0.01	6.53 ± 0.01	5.39 ± 0.01
C_{max} (µg/mL)	1.77 ± 0.01	2.52 ± 0.01	1.65 ± 0.01	1.45 ± 0.01	0.85 ± 0.01
T_{max} (h)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
$t_{½}$ (h)	1.76 ± 0.02	2.40 ± 0.04	2.23 ± 0.02	2.47 ± 0.03	1.79 ± 0.01
K_a (h)	0.19 ± 0.01	0.22 ± 0.01	1.49 ± 0.01	0.02 ± 0.01	0.19 ± 0.01
K_{el} (h)	0.39 ± 0.01	0.29 ± 0.01	0.31± 0.01	0.28 ± 0.01	0.39 ± 0.01
CL (mL/h/kg)	1.10 ± 0.01	0.51± 0.01	0.82± 0.01	0.88 ± 0.01	1.07± 0.01
V_d (L/h)	2.79 ± 0.05	1.78 ± 0.03	2.65 ± 0.04	3.16 ± 0.02	2.78 ± 0.02
F (%)	65.00 ± 2.00	86.00 ± 2.00	73.00 ± 1.00	67.00 ± 2.00	66.00 ± 2.00

Table 3: Effect of concurrent administration of the leaf extract of *T. occidentalis* on the pharmacokinetic parameters of AQ.

Parameter	AQ only	AQ + Ext 250mg/kg	AQ + Ext 500mg/kg	AQ + DHA + Ext 250mg/kg	AQ + DHA + Ext 500mg/kg
AUC (µg/h/mL)	54.08 ± 0.01	83.22 ± 0.01	57.36 ± 0.01	60.38 ± 0.01	68.03 ± 0.01
C_{max} (µg/mL)	7.40 ± 0.01	10.90 ± 0.01	9.05 ± 0.01	6.90 ± 0.01	8.80 ± 0.01
T_{max} (h)	2.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00
$t_{½}$ (h)	3.67 ± 0.05	2.73 ± 0.03	3.79 ± 0.04	3.49 ± 0.05	3.71 ± 0.03
K_a (h)	2.51 ± 0.01	3.94 ± 0.01	0.65 ± 0.01	1.55 ± 0.01	1.72 ± 0.01
K_{el} (h)	0.19 ± 0.01	0.25 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
CL (mL/h/kg)	0.97 ± 0.01	0.63 ± 0.01	0.92 ± 0.01	0.87 ± 0.01	0.77 ± 0.01
V_d (L/h)	5.15 ± 0.06	2.49 ± 0.05	5.01 ± 0.08	4.38 ± 0.02	4.14 ± 0.03
F (%)	91.00 ± 2.00	97.00 ± 1.00	92.00 ± 2.00	93.00 ± 2.00	94.00 ± 1.00

5.0 Discussion

5.1 Time to Reach Maximum Concentration and Maximum Serum Concentration (t_{max} and C_{max})

The results of the concentration-time graphs (Figures 3 and 4) show that there was a rapid absorption of DHA and AQ but in different rates. The serum concentration of DHA increased rapidly to a peak plasma concentration (C_{max}) within 1 hour post administration in all the test groups. The C_{max} of DHA was reduced in the groups administered with DHA + Ext 500mg/kg, DHA + AQ + Ext 250mg/kg, and DHA + AQ + Ext 500mg/kg but increased in the group that received DHA + Ext 250mg/kg. Similarly, the C_{max} and t_{max} of AQ were determined directly from Figures 3 and 4. The result showed that a c_{max} of AQ only, AQ + Ext 500mg/kg and AQ + DHA + Ext 500mg/kg were reached in 2 hours post dose, while those that received AQ + Ext 250mg/kg and AQ + DHA + Ext 250mg/kg, were delayed by 2

hours. It was observed that the extract increased the C_{max} in all treatment groups except in the group administered with AQ + DHA + Ext 250mg/kg in which the C_{max} was reduced.

The t_{max} and C_{max} of DHA in this study were in agreement with various reported t_{max} and C_{max} values.[17,18,19,20,21,22] Also the t_{max} and C_{max} of AQ are comparable to some reported values.[23,24] While the effect of the aqueous extract of *T. occidentalis* lowers the C_{max} of DHA in the co-administration groups except in DHA + Ext 250mg group, it increases those of AQ in the co-administration groups except in AQ + DHA + Ext 250mg group.

The C_{max} and t_{max} of orally administered drugs are dependent on the extent, and the rate of drug absorption and on the disposition profile of the drug.[25] Food and divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}), are among the factors that affect absorption of drugs.[26,27] Zn^{2+}in particular is known to cause microsomal protein induction in plasma. Protein induction brings about high plasma protein binding which can adversely affect drug absorption and distribution and hence the C_{max} and t_{max} of drugs. Food has been shown to delay the t_{max} of AQ and increases its c_{max}.[23] Co-administration of food was also found to result in both delayed and enhanced absorption of DHA resulting in an increase in c_{max}.[28] The volume of the initial emptying into the small intestine (known as adaptive phase) varies consistently with the type of food taken and it provides the first opportunity for drugs taken with food to be absorbed, leading to the first plasma drug peak.[29] The height of this peak corresponds to the amount of drug delivered into the duodenum with the food and depends on the gastrointestinal transit time.[30] A rapid transit decreases the peak and a slow one increases the peak. Since drugs are absorbed primarily from the upper part of the small intestine, oral absorption of drugs is often affected by the gastric emptying time and small intestinal motility, which vary considerably between individuals. Hence, the variation in the C_{max} and t_{max} of AQ and DHA co-administered groups in this study.

Also, during adaptive phase, various hormones are released (e.g cholecytokinin), which leads to the retention of materials in the stomach. Phytochemicals and some mineral elements are known to induce enzyme production. A number of P_{450} enzymes in human and/or rodent liver microsomes are inducible, including various members of the CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, CYP3A, and CYP4A subfamilies.[31] Enzyme induction enables some drugs to accelerate their own biotransformation (auto-induction) or the biotransformation and elimination of other drugs. Phytochemicals have the potential to elevate and suppress cytochrome P_{450} activity. Such effects are more likely to occur in the intestine, where high

concentrations of phytochemicals occur during or shortly after meals, and alteration in cytochrome P_{450} activity is likely to occur depending on the components of the food taken. The effect is more pronounced particularly in the fate of drugs that are subject to extensive first-pass metabolism as a result of intestinal cytochrome P_{450}-mediated biotransformation.[32] This also explains the relatively low peak heights of C_{max} in the study groups that received AQ and DHA concurrently with the aqueous extract of *T. occidentalis* leaf except in the groups that received DHA + Ext 250mg/kg and AQ + Ext 250mg/kg.

5.2 Area Under Curve (AUC)

The area under curve (AUC) of the concentration-time graphs (Figures 3 and 4) was determined by Graph Pad Prism version 7.03 and the values are shown in Tables 2 and 3. The AUC results revealed that the extract significantly increased the AUC of all treatment groups relative to those of DHA only and AQ only, respectively. The AUC value of DHA in this study is consistent with some published reports in this area.[33,34,35] Similarly, the AUC of AQ in this study is comparable to some documented AUC results of AQ.[23,36]

As a measure of the total exposure to DHA, the AUC of the combined DHA + AQ + Extract at 250 mg/kg and 500 mg/kg were analysed and contrary to the report of Orrell,[37] it was found that the AUC of DHA was higher following the combination therapy. This increase in AUC of DHA represents 112.90 % in DHA + Ext 250mg/kg group, 33.02 % in DHA + Ext 500mg/kg group, 23.91 % in DHA + AQ + Ext 250mg/kg group and 2.28 % in DHA + AQ + Ext 500mg/kg group. On the other hand, the comparison of the AUC of AQ administered alone to the combination therapy of AQ + DHA + Extract at the working dose of 250mg/kg and 500 mg/kg showed an increase in the AUC of AQ to the extent of 53.88 % in AQ + Ext 250mg/kg group, 6.07 % in AQ + Ext 500mg/kg group, 11.65 % in AQ + DHA + Ext 250mg/kg and 25.80 % in AQ + DHA + Ext 500mg/kg group. These levels of increases, particularly in the groups administered with DHA + Ext 250mg/kg and AQ + DHA + Ext 500mg/kg are capable of causing remarkable pharmacological changes. Currently, the most common co-treatment used with artesunate or dihydroartemisinin is amodiaquine or mefloquine, but there are no reports of any interaction. Thus, it is possible that bioactive components from the aqueous extract of *T. occidentalis* may have interacted with DHA and AQ or with the metabolizing enzymes to cause their prolonged stay in the animals.

It is suspected that increased number of conjugated double bonds in lycopene and chlorophyll a and b coupled with the opening of the β-ionone ring in lycopene could be responsible for

their increased chances of interaction thus making them potent bioactive compounds.[38] Phenolic compounds are also known to interact with drug molecules and alter their pharmacokinetic properties. The longer a drug resides in the body, the greater the exposure a patient has to a drug.

5.3 Elimination Half-life (t½)

The concomitant administration of DHA and AQ with the aqueous extract of *T. occidentalis* led to the alteration in the time that half of the drugs were eliminated from the general circulation. The $t_{½}$ of DHA was increased in all treatment groups relative to the group that received DHA only. A bidirectional trend in $t_{½}$ was noticed in AQ treatment groups. With reference to the group given AQ only, an increase in $t_{½}$ of AQ was observed in the groups that received AQ + DHA + Ext 500mg/kg, and AQ + Ext 500mg/kg, respectively while the $t_{½}$ in groups given AQ + DHA + Ext 250mg/kg and AQ + Ext 500mg/kg declined. It was observed that the effect of the extract brought about a concentration dependent reduction in the $t_{½}$ of DHA while it caused a concentration dependent increase in the $t_{½}$ of AQ.

The $t_{½}$ of DHA reported in this work is similar to various reported half-lives of DHA,[20,21,28,35] whereas the $t_{½}$ of AQ is slightly higher than some of the reported values but comparable toa few documented half-lives of AQ.[37,39] However, WHO[40] has reported a higher (28 hours) elimination half-life for AQ. Elimination $t_{½}$ shows how long the drug will have an effect in the body and when the next dose should be given. The elimination period of DHA is known to be short[41] and thus DHA and other derivatives of artemisinin are paired with a drug with longer half-life for sustained antiplasmodial activity. The concomitant administration of DHA with aqueous extract of *T. occidentalis* caused extension of t½ of DHA by 36.36 % in DHA + Ext 250mg/kg group, 26.70 % in DHA + Ext 500mg/kg group, 40.34 % in DHA + AQ +Ext 250mg/kg group and 1.70 % in DHA + AQ + Ext 500mg/kg group. Also, the co-administration of AQ with the same extract manifested in the extension of the $t_{½}$ of AQ by 3.27 % in the group gavaged with AQ + Ext 500mg/kg and 1.09 % in the group given AQ + DHA + Ext 500mg/kg while there was a shortening of the elimination half-life of AQ by 25.61 % in the AQ + DHA + Ext 250mg/kg group and 4.90 % in AQ + DHA + Ext 250mg/kg group. There was no change in the $t_{½}$ of AQ + Ext 250mg/kg and AQ + DHA + Ext 500mg/kg groups. Increased percentage in $t_{½}$ implies delayed absorption which can delay the commencement of pharmacological action of the drug.

It is an established fact that food has the potentials to delay the absorption of DHA and AQ thus increasing their bioavailability.[23,36,42] Inhibition of the metabolizing enzyme by another drug or bioactive compound in a food can prolong the $t_{½}$ of a drug and sustain its activity in the body.[43] Flavonoids are a class of dietary phytochemicals that modulate various biological activities.[5] Chlorophyll a and b are metalloporphyrins with oxidative and chelating properties,[44] capable of altering the activity of gastrointestinal metabolizing enzymes, thereby affecting the unionized and unbound drug molecules available to be absorbed. These phytochemicals could have interacted with DHA and AQ and slow down their absorption process by either inhibition of metabolizing enzymes or interaction with the drug molecules. In either ways, delayed $t_{½}$ is likely to result. Generally, the duration of drug action is reflected by its plasma $t_{½}$. Short $t_{½}$ implies a large dose is required to maintain therapeutic concentration of drug in the body.

5.4 Absorption Rate Constant (Ka)

The result of the pharmacokinetics study showed a fluctuating k_a for both DHA (Table 2) and AQ (Table 3). The k_a values of DHA reported in this work are relatively lower than most of the documented values for DHA,[45] whereas the ka value of AQ reported in this work is comparatively higher but they are within the documented range.[39] The oral absorption of drugs is often approximated assuming linear kinetics, typically when given in solution, as was the practice in this study. Under these circumstances, and coupled with drug-food interaction, absorption is characterized by a variation in absorption rate constant and a corresponding absorption half-life. However, the absorption of many drugs does not exactly follow linear kinetics.

A saturable transport mechanism of the drug from the intestinal lumen to the portal circulation is one of the factors that may be responsible for nonlinear absorption. This is commonly associated with drug-food interaction in the gastrointestinal tract. It is becoming increasingly apparent that phytochemicals can influence the pharmacological activity of drugs by modifying their absorption characteristics through interaction with drug transporters.[32] The rate of absorption determines the required time for the administered drug to reach an effective plasma concentration and may thus affect the onset of the drug effect. This rate influences both the peak plasma concentration (C_{max}) and the time it takes to reach this peak (t_{max}). Variation in the rate of absorption can add to the pharmacokinetic variability, as evidenced in the result of this pharmacokinetics study.

5.5 Elimination Rate Constant (K_{el})

The elimination rate constant (K_{el}) of the unionized DHA and AQ showed a regular trend in this study. The k_{el} of DHA is within the documented range and that of AQ is comparable to the report of Bediako *et al*.[39] The variation in the k_{el} values in this study shows that the interaction of the bioactive compounds in the aqueous extract of *T. occidentalis* with DHA and AQ has really affected the duration of stay and activity of the drugs in the animals. While both the distribution and elimination half-lives contribute to the effects of the drug on its behaviour, it is usually the elimination half-life that is used to determine dosing schedules, to decide when it is safe to put patients on a new drug. Alteration of k_{el} is an indication of alteration in the plasma concentration and metabolic rate of drugs. Only free drugs are available for pharmacological activity and metabolism.[26] The result of the k_{el} of AQ and DHA correlated positively with C_{max}, t½ and bioavailability of the two drugs in this study.

5.6 Clearance (C_L)

A regular trend was observed in the clearance (C_L) pattern of DHA and AQ in the study. The volume of DHA and AQ eliminated from the general circulation was lower in all treatment groups with respect to the group that received DHA only and AQ only, respectively. However, the clearance pattern proved to be dose dependent in groups that received DHA with extract, and vice versa with groups that received AQ with extract.

Notwithstanding the fact that DHA is known to have flip-flop kinetics,[46] its C_L result in this study is consistent with the documented range in literature.[46] On the other hand, the C_L of AQ is comparable to the report of Stepniewska *et al*.[45] C_L is an index of how well a drug is removed irreversibly from the circulation. After an extracellular administration, the average drug exposure is determined both by clearance and bioavailability. As a result, clearance determines the dose-rate (dose per unit time) required to maintain plasma concentration, and it only applies to drugs with first-order (exponential) kinetics. Enzyme inhibition can cause low or high clearance of drug[47, 48] and phytochemicals have the potential to both elevate and suppress cytochrome P450 activity.[32] A drug with high metabolic clearance is always subject to an extensive first-pass effect, resulting in low bioavailability. Antimalarial drugs are a class of agents known to utilize metabolic and elimination pathways prone to genetic variation.[49]

CYP2B6 is involved in the phase I metabolism of artemisinin drugs[35] and CYP2C8 is the main hepatic isoform responsible for the metabolism of AQ. Documented evidence in

literature reveals that phenolic compounds in fruits and vegetables are responsible for inhibition of CYP450 and drug transporters.[50] This implies that inhibition of CYP2B6 or CYP2C8 will lead to delayed clearance and thus cause accumulation of DHA or AQ. Therefore the delayed C_L of DHA and AQ when co-administered with the extract in this study may be caused by inhibition of metabolizing enzymes as a result of their interaction with phenolic compounds in the aqueous extract of *T. occidentalis* leaf, thereby reducing the rate of metabolism within the gastrointestinal tract and probably within the hepatic passage.

5.7 Volume of Distribution (V_d)

The result of the apparent volume of distribution showed a general reduction in the volume of distribution of DHA and AQ except in the group given DHA + AQ + Ext 250mg/kg in which the V_d increased sharply. This result shows that the V_d of DHA and AQ is in agreement with many documented results of V_d in the literature. [51,22,28,35] The V_d is the second most important pharmacokinetic parameter after clearance. However, V_d is not a physical space, but a dilution space, or in other words an apparent volume into which a drug appears to be distributed with a concentration equal to that of plasma. It is actually a proportionality constant relating the plasma concentration to the amount of drug in the body. Nevertheless, the volume of distribution is useful in estimating the dose required to achieve a given plasma concentration and it varies with individual height and weight. As the proportion of each body compartment varies with age, so does the V_d for most drugs. Variation of V_d mainly affects the peak plasma concentration and the bioavailability of the drug as is seen in this result (Table 2 and 3). Genetic variability in drug-metabolizing enzymes and drug transporters is known to influence the pharmacokinetics of many drugs. Highly protein bound drugs like AQ (92 %) and DHA (92 %)[52] usually exhibit small volume of distribution. This implies that the drugs are concentrated in the plasma and the drugs have low lipophilicity.[53]

5.8 Bioavailability (F)

The bioavailability (F) of DHA and AQ in this study was estimated from back extrapolation of the semilogarithm plot of the concentration time graph using Graph Pad Prism. The bioavailability of both DHA and AQ in this study showed a progressive increase in concomitant administration with the aqueous extract. The F of DHA was raised in the groups given DHA + AQ + Ext 500mg/kg, DHA + AQ + Ext 250mg/kg, DHA + Ext 500mg/kg and DHA + Ext 250mg/kg, respectively compared to the treatment group that was gavaged with DHA only. Similarly, the F of the groups that received AQ only, AQ + Ext 500mg/kg, AQ +

DHA + Ext 250mg/kg, AQ + DHA + Ext 500mg/kg and AQ + Ext 250mg/kg showed a progressive increase.

The finding in this study is consistent with most documented values of F for AQ and DHA. Bioavailability is the fraction of the dose of drug given orally that reaches the systemic circulation. Because the entire blood supply of the upper gastrointestinal tract passes through the liver before reaching the systemic circulation, the drug may be metabolized by the liver and gut wall during the first passage of drug during absorption. The relative high F values in this result again suggest inhibition of metabolizing enzymes by the bioactive components in the aqueous extract.

Food affects absorption and first-pass metabolism in opposite ways. Food usually decreases the F of drugs that are poorly absorbed, but increases the F of drugs that are subject to high first-pass metabolism. Some foods, e.g. grapefruit juice, have constituents that compete with drugs for presystemic elimination, thereby causing increased F of some drugs. This result shows that both AQ and DHA are rapidly absorbed as confirmed by the ka values (Table 2 and 3) in this result. The F values also are pointers to the fact that good interactions have occurred between the aqueous extract of *T. occidentalis* leaf and the drugs (AQ and DHA) *in vivo*.

However, this result also shows that the bioavailability of DHA and AQ in concomitant administration with aqueous extract of *T. occidentalis* is not dose-dependent. But there is a positive correlation between F and c_{max} as well as AUC in the respective treatment groups.

5.9 Statistical analysis

The two-way ANOVA revealed a very significant ($p < 0.05$) difference in the pharmacokinetic parameters among the test groups with respect to the groups administered with only DHA and AQ. The Bonferroni posttests also showed a very significant ($p < 0.05$) difference in the bioavailability of DHA in the DHA vs DHA + Ext 250mg/kg group and AUC of AQ in AQ + Ext 250mg/kg group.

CONCLUSION

The effect of aqueous extract of *T. occidentalis* leaf increased the total exposure and efficacy of AQ and DHA. Although this effect was observed not to be dose-dependent in all the pharmacokinetic parameters, but it is strongly suspected to result from the interaction of the

bioactive compounds present in the leaf of *T. occidentalis* with the metabolizing enzymes or transporting system of the drugs.

Conflict of Interest

We have no conflict of interest in the subject matter or materials discussed in this manuscript. Funding was by individual contribution by the authors.

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Ethical approval

Scientific and ethical clearance for this work was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Uyo in accordance with the recommendations of the proper care and use of laboratory animals.

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