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Changes in Some Pharmacokinetics Parameters of Chloroquine by *Gnetum Africana*

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Abstract

Effect of *Gnetum africana* on the pharmacokinetic parameters of chloroquine was investigated. Chloroquine phosphate (200 mg/Kg) was concurrently administered to overnight fasted albino rats. Blood samples were collected 15, 30, 60, 120, 240 and 480 minutes after administration through cardiac puncture. Serum concentration of chloroquine was evaluated spectrophotometrically. The results indicated that the extract significantly decreased Cmax (9.17%), Ka (3.06%), Ke (45.38%), C_l(48.46%) and AUC ($_{0\text{-}8}$) (16.90%). The extract also increased the values of $t_{1/2}$ (83.11%), t_{max} (100.00%). The LD50 of the extract was 5.25 g/Kg. Phytochemical screening revealed the presence of steroids, tannins, flavonoids and traces of phlobatanins and alkaloids. Proximate and elemental analyses showed that the plant contained Sodium (26.37 + 0.01ppm), Potassium (32.84 + 0.02 ppm), Copper (0.01 + 0.00 ppm), Calcium (6.53 + 0.01ppm), Magnesium (7.58+ 0.02 ppm), Iron (2.01 + 0.02 ppm), Zinc (0.93 + 0.03 ppm), Lead (0.02 + 0.01 ppm), Moisture (10.90 + 0.91 mg/100 g), Crude protein (15.31 + 1.12 mg/100g), Crude fibre (4.20 + 0.21 mg/100 g), Crude fat (10.60 + 0.65 mg/100 g), Ash (5.40 + 0.30 mg/100g), Carbohydrate (74.49 + 0.23 mg/100 g) and Energy (407.80 Kcal). The results of this work showed that concurrent administration of the extract of *Gnetum africana* affected some of the pharmacokinetic parameters of chloroquine. Malaria patients on chloroquine therapy should therefore be advised to beware of consuming *Gnetum africana.*

Introduction

The primary means of treating protozoan infections is chemotherapy. Unfortunately, the ability of the pathogens to mutate and become drug resistant poses a serious challenge to chemotherapy [1] . Although the mechanism of chloroquine resistance is largely unknown and remains controversial [2] many factors contribute to chloroquine concentration at the target site, which is a major cause of its resistance by the parasite.

Some foods are known to affect the way in which the body handles drugs and therefore have the potentials to either increase or decrease a drug's therapeutic or adverse effects [3]. Some plant materials have been reported to alter some pharmacokinetic parameters of the chloroquine by interfering with absorption, distribution or elimination of the drug. For example, Grapefruit juice is noted for its ability to increase Cmax and AUC [4]; Spinach is capable of maintaining high blood level of chloroquine [5]; Bitter leaf is noted for its ability to

decrease AUC and therapeutic effect of chloroquine [6]. *Azadirachta indica* is reported to significantly reduce serum concentration and prolong $t^{1/2}$ of chloroquine [7].

Vegetables are commonly eaten in Nigeria. Incidentally, some of these vegetables have the potentials of interfering with drug absorption and its bioavailability [8]. For example, *Telfairia occidentalis* and *Vernonia amygdalina* affect the pharmacokinetics of chloroquine [9,10]. The leaves and seeds of *Gnetum africana* are used for the traditional treatment of enlarged spleen, throat and reduction of pains at childbirth [11]. The seeds are used as antidote for some form of poison and snake bite, and as fungicide for dressing septic or fresh wound [12, 13]. It is popularly used in salad and soup preparation [11].

This study was carried out to evaluate the effects of concurrent oral administration of *Gnetum africana* on the pharmacokinetics of chloroquine.

Material and Methods

Plant Collection: *Gnetum africana* leaves were collected in 2007 from Uyo, Akwa Ibom State, Nigeria and was identified by Dr Kola Ajibesin of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction: The fresh leaves were oven dried in a Gallenkamp oven for 48 hours at 40°C. 5.0kg of the dry leaves were macerated in 15L of 96% ethanol for six days at room temperature. The extract obtained was filtered and concentrated in a Rotary evaporator. The residue was dried in a desiccator containing Silica gel.

Animals: Albino rats (Wistar strain) with a mean weight of 189±30.1g were obtained from the Animal house of the University of Uyo and were given free access to food and water *ad libitum.*

Evaluation of acute toxicity: The Up and Down method of Bruce (1985) was used to determine the LD50 of the extract [14].

Administration of Chloroquine and Extract: Sixty animals were starved overnight and later divided into two groups of 30 rats each. Group I received chloroquine (15 mg/kg) and extract (200 mg/kg). Group II served as control and was administered chloroquine only (15 mg/kg). Both Chloroquine and extract were administered orally.

Blood Collection: Blood samples were collected with syringe through cardiac puncture at 15, 30, 60, 120, 240 and 480 minutes under chloroform anesthesia (5 rats per each time point).

Determination of Serum Chloroquine Concentration and Protein Level: The blood samples were left overnight in the refrigerator for the serum to separate. The supernatant serum was collected with a syringe and chloroquine and protein levels determined using UV-VIS spectrophotometer (Unicam 8625). The chloroquine concentration in the serum was determined by extrapolation from a chloroquine standard curve taken at 344 nm.

Elemental and Proximate Analyses: The proximate composition (moisture, crude protein, fibre, ash, fat and carbohydrate) and mineral contents (Na, K, Cu, Ca, Mg, Fe, Zn and Pb) of the plant were determined using the standard methods as outlined by AOAC, 1990 [15].

Phytochemical Screening: The plant was screened for alkaloids, tannins, flavonoids, steroids, terpenes, phlobatannins and anthraquinones using standard procedures [16, 17].

Statistical Analysis: The results were expressed as Mean + SD. Student's t- test was used to evaluate the significance level at $P < 0.05$.

Results

Concurrent administration of the extract significantly affected the pharmacokinetics parameters of chloroquine. Table 1 shows the concentration of chloroquine values at different time points. Most of the parameters including Cmax (9.17%), Vd (5.58%), $AUC_{(0-8)}$ (16.90%) and Ka (3.06%) were moderately decreased relative to the control while severe decrease was

Table 1: Serum concentration of chloroquine (µg/ml) administered alone and concurrently with *Gnetum africana* extract.

Time (minutes)	Chloroquine alone	Chloroquine and *Gnetum africana*
15	2.60 ± 0.02	0.80 ± 0.01*
30	7.00 ± 0.01	2.60 ± 0.01*
60	19.50 ± 0.01	4.50 ± 0.01*
120	24.00 ± 0.02	15.00 ± 0.04*
240	22.00 ± 0.04	20.50 ± 0.01*
480	15.50 ± 0.02	17.80 ± 0.02*

Mean + S.D.; n = 5; * P < 0.05.

Table 2: Pharmacokinetic parameters of chloroquine phosphate alone and with ethanolic leaf extract of *Gnetum africana*.

Parameter	Chloroquine alone	Chloroquine and *Gnetum africana*
K_a (hr^{-1})	0.9061 ± 0.011	0.8784 ± 0.015*
K_{el} (hr^{-1})	0.026 ± 0.007	0.0142 ± 0.002*
$T_{½}$ (hr)	26.65 + 1.551	48.80 + 1.550*
t_{max} (hr)	2.00 ± 0.000	4.00 ± 0.001*
C_{max} (µg/ml)	24.00 ± 0.400	21.80 ± 0.289*
C_L (L kg^{-1})	4.56 ± 0.050	2.35 ± 0.020*
V_d (L Kgr^{-1})	175.32 ± 2.010	165.53 ± 2.887*
$AUC_{(0-8)}$ (µg $hrml^{-1}$)	150.30 + 4.500	124.90 + 2.236*
$AUC_{(0-\infty)}$ (µg$hrml^{-1}$)	734.92 ± 5.000	1378.42 ± 4.220*

Mean + S.D.; n = 5; * P < 0.05.

observed in Ke (45.38%) and Cl (48.46%). The extract significantly increased $t_{1/2}$ (83.11), t_{max} (100.00%) and $AUC_{(0-8)}$ (87.56%) (Table 2). Phytochemical screening revealed the presence of steroids, tannins, flavonoids and traces of phlobatanins and alkaloids (see Table 3). The LD50 of the extract was 5.25 g/Kg. Table 4 gives the results of the elemental and Proximate composition of *Gnetum africana* leaves.

Table 3: Phytochemical components of *Gnetum africana*.

S/No.	Component	Result of Phytochemical test
1	Saponins	–
2	Tannins	++
3	Anthraquinones	–
4	Phlobatanins	+
5	Alkaloids	+
6	Cardiac glycosides	
	-Lieberman test : Steroid	+++
	Terpenes	–
	-Salkowski test	+++
	-Keller Kiliani test	++
7	Flavonoids	++

+++, Present in high concentration; ++, Present in moderate concentration; +, Present in trace concentration; -, Not present.

Discussion

Phytochemicals interact with drug transporters and cause impairement of pharmacological activity [18-20]. Some elements, notably Zn, may induce intestinal proteins which bind drugs and prevent their transfer from the intestine to the body [21]. Reduction in the $AUC_{(0-8)}$ by the extract of *Gnetum africana* might have been a consequence of the interaction between the drug and some components of the extract. It was reported by O'Brien and Haddad, 2002 [8] that flavonoids inhibited Cyt P450, thereby prolonging the $t_{1/2}$ of chloroquine. The flavonoid content of this extract could therefore contribute to the elongation of $t_{1/2}$ of chloroquine. In addition, alkalinization of urine by the extract could also contribute to the increase in t1/2 since about 50% of chloroquine is excreted in urine, of which about 70% is unchanged.

Table 4: Elemental and Proximate composition of *Gnetum africana* leaves.

Composition	Concentration (ppm)
Sodium	26.37 ± 0.01 (ppm)
Potassium	32.84 ± 0.02 (ppm)
Copper	0.01 ± 0.00 (ppm)
Calcium	6.53 + 0.01 (ppm)
Magnesium	7.58 ± 0.02 (ppm)
Iron	2.01 ± 0.02 (ppm)
Zinc	0.93 ± 0.03 (ppm)
Lead	0.02 ± 0.01 (ppm)
Moisture	10.90 + 0.91 (mg/100g)
Crude protein	15.31 ± 1.12 (mg/100g)
Crude fibre	10.60 ± 0.65 (mg/100g)
Crude fat	5.40 ± 0.30 (mg/100g)
Ash	4.20 ± 0.21 (mg/100g)
Carbohydrate	74.49 + 0.23 (mg/100g)
Energy (Kcal)	407.80

Mean + S.D.; n = 5.

Excretion of chloroquine in urine is decreased by alkalinization of the urine and green leafy vegetables are among the foods that can alkalinize the urine [8, 22, 23]. The effect of *Gnetum africana* on half-life of chloroquine was similar to that of *Azadirachta indica* [7]. Increased tubular reabsorption caused by the alkalinization of the urine could also be responsible for the decrease in the Ke of chloroquine by the plant extract [24, 25]. The presence of carbohydrates in ileum can slow down gastric emptying by altering gastrointestinal contraction patterns [3]. Decrease in Cmax of chloroquine could be attributable to the presence of the extract which is rich in carbohydrates (74.49%) in the gastrointestinal tract.

Gnetum africana have in common with Bitter

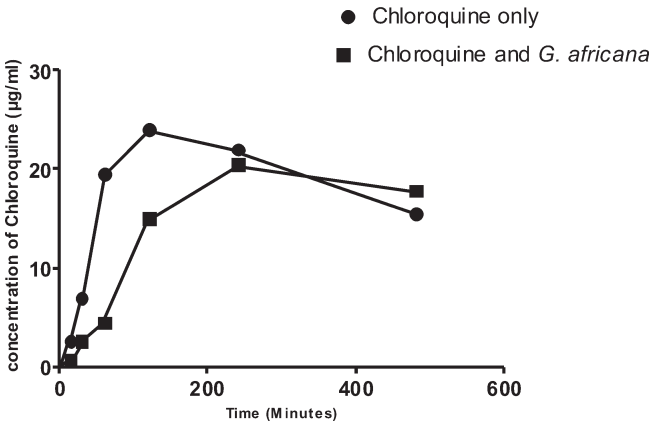

Figure 1: Concentration versus time curve.

leaf the ability to decrease C_{max} [6]. Chloroquine has a t_{max} range of of between 1.5 hours and 3.0 hours [26] and there is always a change in the height and duration of plasma drug peak whenever a drug is taken orally with food [27]. Food rich in fat and dietary fibre delay the entry of an orally administered drug into the duodenum, delaying absorption and increasing tmax [3]. This may account for the increase in tmax and Ka of chloroquine by *Gnetum Africana.* The high LD50 value of 5.25 g/Kg shows that the extract is safe for consumption. Okafor et al., 1996 revealed that *Gnetum africana* leaves contained 37.39, 4.72, 14.20 and 10.18% moisture, ash, crude fat and crude protein, respectively [28]. Results of proximate analysis of the plant agree with the above in the content of ash and crude protein. Similarly, results of elemental analysis of the plants agree in only in the content of Sodium with the report of Okafor et al., 1996 [28].

In conclusion, the results of this work revealed that concurrent administration of the extract of *Gnetum africana* affected some of the pharmacokinetic parameters of chloroquine. Malaria patients on chloroquine therapy should therefore be advised to beware of consuming *Gnetum africana* with chloroquine decreased Cmax and AUC of chloroquine by the plant extract may lead to reduced therapeutic effect of the drug and resistance.

However, further work needs to be done using human subjects to ascertain the correlation between the result of this study which was obtained in rat with that of human beings.

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