Journal of Medicinal Plants Research Vol. 5(13), pp. 2665-2669, 4 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Changes in the pharmacokinetics of chloroquine by the leaf extract of *Lasianthera africana* in rats

Eseyin Olorunfemi[1]*, Ebong Aniekan[1], Anefiok Udobre[1] and Imo Udoh[2]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.
[2]Department of Biopharmacy and Clinical Pharmacy,Faculty of Pharmacy, University of Uyo, Nigeria.

Accepted 19 April, 2011

Sixty albino Wistar rats were used in the *in vivo* study of the effects of ethanolic leaf extract of *Lasianthera africana* on the pharmacokinetic parameters of chloroquine. After an overnight fast, the rats were divided into two groups designated A and B. Group A was given chloroquine only (15 mg/kg) and group B received chloroquine (15 mg/ml) and *L. africana* extract (200 mg/ml) concurrently. All administrations were done orally. Blood was collected through cardiac puncture under chloroform anaesthesia at 15, 30, 60, 120, 240 and 480 min, respectively. The serum was analyzed for chloroquine and protein using UV-Vis spectrophotometer. Results obtained indicated significant ($p<0.05$) relative increase in $t_{½}$ (115%), t_{max} (100%), V_d (14%) and AUC $_{(0-\infty)}$ (59%) and decrease in K_a (54%), K_{el} (53%), C_{max} (38%), C_L (47) and $AUC_{(0-8)}$ (39%). Protein induction was relatively increased by 74%. Elemental analysis of the leaf showed high content of Na, K, Ca and Mg. Proximate analysis of the leaf revealed very high content of carbohydrate (73.36 mg/100 g) and moderate concentration (3.70 to 13.90 mg/100 g) of crude fat, crude fat, crude fibre, crude protein, ash and moisture. Phytochemical analysis proved that the leaf contains moderate amount of steroid, trace amount of flavonoids, alkaloids and saponins. The pharmacokinetics results revealed that *L. africana* significantly altered the pharmacokinetic parameters of chloroquine and is thus capable of altering therapeutic effect of chloroquine when administered concurrently.

Key words: Pharmacokinetics, chloroquine, *Lasianthera africana.*

INTRODUCTION

The only hope of successful treatment of parasitic infections is through chemotherapy. In the last decade, malaria parasites have shown resistance to chloroquine and the mechanism of its resistance remains unknown and controversial (Sanchez et al., 2005). Yet chloroquine is still being used as the front line antimalaria drug in some malaria endemic areas.

A number of factors are responsible for the reduction in chloroquine concentration in the plasmodia cells which leads to their resistance. Some foods affect the way the body handles some drugs thereby altering the therapeutic effects of such drugs (Mason, 2002). Numerous plant materials have the potentials of interfering with absorption and disposition of chloroquine within the body. Owoyale et al. (1995) reported peak delay and high blood level of chloroquine when fed with spinach to rabbit. Bitter leaf is also reported to decrease AUC and therapeutic effect of chloroquine in rabbit. Other plant materials reported to alter pharmacokinetic parameters of chloroquine include grape fruit juice which has the potential to increase C_{max} and bioavailability (Ali et al., 2001, 2002), *Azadirachta indica* which is capable of reducing serum concentration and prolonging $t_{½}$ (Shannon et al., 2006) and *Telfairia occidentalis* (Eseyin, 2007). *Vernonia amygdalina* and *Heinsia crianta* are also known to affect some pharmacokinetic parameters of chloroquine (Igboasoiyi et al., 2008; Eseyin et al., 2010). Nigerians exploit vegetables as cheap means of vitamins and minerals in their food (Akoroda, 1990; Okafor, 1994). *L. africana* is a shrub widely used in the southern Nigeria culinary. However, its medicinal uses include the

*Corresponding author. E-mail: femieseyin2@yahoo.co.uk Tel: +2348065161478.

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

Time (min)	Chloroquine alone	Chloroquine and *L. africana*
15	2.60 ± 0.02	1.00 ± 0.01*
30	7.00 ± 0.01	3.60 ± 0.01*
60	19.50 ± 0.01	4.50 ± 0.01*
120	24.00 ± 0.02	10.00 ±0.01*
240	22.00 ± 0.04	15.00 ± 0.03*
480	15.50 ± 0.02	13.00 ± 0.02*

Mean ± S.D n=5, *P < 0.05.

treatment of stomach disorder and internal heat and topical application to enhance healing of wound (Isong and Idiong, 1997) and serves as antimicrobial agent (Itah, 1996). Since it has been established that chloroquine interaction with plant materials results in altered pharmacokinetic parameters of the drug, this study was undertaken to determine the effect of ethanolic leaf extract of *L. africana* on the pharmacokinetics of chloroquine.

MATERIALS AND METHODS

Plant collection

The leaves of *L. africana* were collected from the rural community in Uruan Local Government Area of Akwa Ibom State, Nigeria in January 2007. Identification of the plant was done by Dr K. Ajibesin, Department of Pharmacognosy, University of Uyo, Nigeria.

Extraction

The fresh leaves were washed with distilled water, chopped and oven-dried for 48 h at 40°C. 5.5 kg of the dry leaves was macerated in 17 L of 96% ethanol for six days at ambient temperature. The extract was filtered, concentrated and dried in a desiccator.

Animals

60 albino Wistar rats of both sexes weighing averagely 182± 24.82 g were purchased from the experimental animal unit of University of Jos, Nigeria. They had access to standard food and water *ad libitum*. Ethical standard of University of Uyo was adhered to during the experiment.

Administration of chloroquine and extract

15 mg/kg of chloroquine only was administered to group A and a mixture of 15 mg/kg of chloroquine and 200 mg/ml of *L. africana* extract given to group B orally. Both groups A and B were starved overnight and randomly sub-divided into six sub-groups of 5 animals each.

Blood collection

The rats were dissected under chloroform anaesthesia and blood samples were collected with syringe through cardiac puncture at 15, 30, 60, 120, 240 and 480 min (5 rats per each time point).

Analysis of blood samples

The blood samples were left overnight in the refrigerator for the serum to separate. The supernatant serum was collected with a syringe and absorbance measured using UV-VIS spectrophotometer (Unicam 8625) at 344 nm. Drug and extract free serum was used as blank for zero calibration of the instrument. The chloroquine concentration in the serum was determined by extrapolation from a chloroquine standard curve taken at 344 nm. Protein level was estimated using Bradford assay method for the determination of unknown protein with possible nucleic acid contamination (Carprette, 2000). This was done by measuring the UV absorbance of serum at 260 and 280 nm, using drug and extract free serum as blank

Proximate analysis

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).

Phytochemical screening

The plant was screened for alkaloids, tannins, flavonoids, steroids, terpenes, phlobatannins and anthraquinones using standard procedures (Harbone, 1973; Sofowora, 1980).

Statistical analysis

Student's t –test was used to analyze significance at $p< 0.05$. While pharmacokinetics parameters were estimated using standard methods (Shargel and Yu, 1984).

RESULTS AND DISCUSSION

Co-administration of chloroquine and *L. africana* caused a relative decrease of 62, 49, 79, 50, 32 and 15%) at 15, 30, 60, 120, 240 and 480 min, respectively in the serum concentration of chloroquine (Table 1). Relative increase of 115, 100, 597 and 14% was recorded in $t_{½}$, t_{max}, $AUC_{(0-\infty)}$ and V_d, respectively; and relative decrease of 51, 53,

Table 2. Pharmacokinetic parameters of chloroquine phosphate alone and with ethanolic leaf extract of *L. Africana*.

Parameter	Chloroquine alone	Chloroquine and *L. africana*
K_a (h^{-1})	0.9061 ± 0.011	0.4181 ± 0.091*
K_{el} (h^{-1})	0.026 ± 0.007	0.0121 ± 0.001*
$T_{½}$ (h)	26.65 ± 1.551	57.27 ± 2.500*
t_{max} (h)	2.00 ± 0.000	4.00 ± 0.00*
C_{max} (µg/ml)	24.00 ± 0.400	15.00 ± 0.287*
C_L (L kg^{-1})	4.56 ± 0.050	2.42 ± 0.029*
V_d (L Kg^{-1})	175.32 ± 2.010	199.99 ± 2.440*
$AUC_{(0-8)}$ (µg hml^{-1})	150.30 ± 4.500	90.98 ± 3.126*
$AUC_{(0-\infty)}$ (µghml^{-1})	734.92 ± 5.000	1165.36 ± 4.872*

Mean ± S.D n=5, * P < 0.05.

Table 3. Crude protein (mg/ml) in rats.

Time (min)	Chloroquine alone	Chloroquine and *L. africana*
15	0.028 ± 0.038	0.030 ± 0.007
30	0.027 ± 0.036	0.031± 0.008
60	0.027 ± 0.068	0.046 ± 0.068*
120	0.029 ± 0.007	0.063 ± 0.002*
240	0.030 ± 0.014	0.069 ± 0.056*
480	0.033 ± 0.008	0.064 ± 0.014*

Mean ± S.D n=5,*P < 0.05.

Table 4. Elemental composition of *L. africana* leaves.

Element	Concentration (ppm)
Sodium	13.16 ± 0.03
Potassium	41.47 ± 0.04
Copper	0.01 ± 0.01
Calcium	26.91 ± 0.03
Magnesium	18.53± 0.02
Iron	0.79 ± 0.01
Zinc	0.42 ± 0.01
Lead	0.01 ± 0.01

Mean ± S.D n=5,*P < 0.05.

47, 38 and 39% in K_a, k_{el}, C_L, C_{max} and $AUC_{(0-8)}$, respectively (Table 2). Microsomal protein induction of 74% was also observed (Table 3). Elemental, proximate and phytochemical composition of the leaves of *L. africana* are shown in (Tables 4, 5 and 6), respectively. The delay in absorption and subsequent drop in the K_a of chloroquine is not unconnected with the high fibre content of the leaf (Udosen et al., 1999). High dietary fibre was noted by Mason (2002) for being capable of delaying the rate of absorption of orally administered drug. Zinc binds to drug and prevents its absorption from gastrointestinal tract into the body (Schrauzer, 1984). Bioavailability ($AUC_{(0-8)}$) is a function of absorption rate of the drug (Svenssion, 2007). The serum drug peak is proportional to the amount of drug in the adaptive phase (O'Brien and Haddad, 2002), thus the relative reduction in C_{max} and increased t_{max} of the drug.

Increased microtonal protein induction (Table 3) resulted in high binding ratio and a decrease in the rate of metabolism (Wallace and Amsden, 2002). These may account for the low rate of elimination and clearance of chloroquine (Walker et al., 1983; Bauer et al., 1991).

Table 5. Proximate composition of *L. africana* leaves.

Composition	Amount present (mg/100 g)
Moisture	13.90 ± 1.10
Crude protein	10.94 ± 0.91
Crude fibre	10.60 ± 0.41
Crude fat	3.70 ± 0.22
Ash	11.40 ± 0.83
Carbohydrate	73.36 ± 0.30
Energy (Kcal)	370.500

Mean ± SD n=5,* P < 0.05.

Table 6. Phytochemical screening of the *L. africana* leaves.

Test	Result
Saponins	+
Tannins	-
Anthroquinones	-
Phlobatanins	-
Alkaloids	+
Cardiac glycosides: steroid	++
Terpenes	-
Flavonoids	+

+++ = concentrated amount present, ++ = moderate amount present, + = trace amount present, - = not present.

Since alkalinization of urine is noted with vegetable meals (O' Brien and Haddad, 2002) and the $t_{½}$ of chloroquine might have been increased in an alkaline medium (Olaniyi, 2000), the long $t_{½}$ of chloroquine is enhanced by its concurrent administration with the extract.

The significant drop in the AUC $_{(0-8)}$ of chloroquine by 39% may be because of interaction of the drug with the components of the leaf extract. However, the 597% increase of AUC $_{(0-\infty)}$ may be related to the induction of microsomal protein which might have increased the $t_{½}$ of the drug. A small initial serum drug concentration results in large volume of distribution because of high concentration of drug in the peripheral tissues and organs (Holmberg et al., 1983). The 14% increase in apparent volume of distribution of chloroquine may be an indication that the drug is concentrated in some compartments probably because of increased microsomal protein. By the results of this work, *L. africana* can be listed among plants that are known to affect some pharmacokinetics parameter of chloroquine such as *A. indica, T. occidentalis, V. amygdalina and H. crinata* (Shanon et al., 2006; Eseyin et al., 2007; Igboasoiyi et al., 2008; Eseyin et al., 2010).

The result of the elemental analysis (Table 4) showed that the leaf of *L. africana* is rich in K (41.47), Ca (26.91), mg (18.53) and Na (13.16) while Fe, Zn, Cu and Pb are present in trace amount of 0.79, 0.42, 0.01 and 0.01 ppm, respectively. The proximate composition of the leaf revealed crude fat (3.70), crude fibre (10.60), crude protein (10.94), ash (11.40), moisture (13.90) carbohydrate (73.36) and energy (370.50 Kcal). The leaf is safe for consumption as the antinutrient composition was very low. Phytochemical screening proved moderate presence of steroid, trace amount of saponins, alkaloids and flavonoids and absence of tannins, anthraquinones, terpenes and phlobatannins. It is not known which of these components is responsible for the effect of the plant on the pharmacokinetics of chloroquine. The study proved that concurrent administration of chloroquine and the leaf extract of *Lasianthera africana* significantly altered the pharmacokinetic parameters of chloroquine and microsomal protein in rat.

REFERENCES

Akoroda MO (1990). Ethnobotany of *Talfaira ocidentalis* (*Cucurbitaceae*) among Igbos of Nigeria. Econ. Bot., 44: 29-33.

Ali BH, Al QA, Mousa HM (2002). Effect of grapefruit juice on plasma chlorquine kinetics in mice. Clin. Exp. Pharm. Physiol., 29(8): 704-706.

AOAC (1990). Official methods of Analysis. 15th ed. Association of Official Analytical chemists, Arlington.

Bauer P, Marie B, Weber M (1991). Full recovery after a chloroquine sucide attempt. Clin. Toxicol., 29: 23-30.

Eseyin OA, Edoho EJ, Igboasoiyi AC, Nwadiukwu E, Ekpo A (2007). Effects of the leaf extract of *Telfairia occidentalis* on the pharmacokinetic of chloroquine. Int. J. Biol. Chem., 1(4): 256-260.

Eseyin O, Ebong A, Attih E, Imo U (2010). Effects of the leaf of *Heinsia crinata* on the pharmacokinetics of chloroquine in rats. Int. J. Biol. Pharm. Sci., 1(2): 88-93.

Harbone JB (1973). Phytochemical methods. London, Chapman and Hill.

Holmberg FM, Bergovist Y, Englund U (1983). Chloroquine Intoxication. Br. J. Clin. Pharmacol., 15: 502-503.

Igboasoiyi AC, Eseyin OA, Udoma NF (2008). The effect of ethanolic extract of *vernonia amygdalina* leaves on some pharmacokinetic parameters of chloroquine in rats. Res. J. Pharmacol., 2(2): 24-27

Isong EU, Idiong UI (1997). Comparative studies on the nutritional and toxic composition of three varieties of *Lasianthera Africana*. Plant Foods Hum. Nutr., 51: 79-84

Itah AY (1996). Screening of plants for fungral properties. Trans. Nig. Soc. Biol. Conserv., 4(1): 26-40.

Mason P (2002). Drug-food interactions (1) food and Medicines. Continuing professional development. Pharm. J., 269: 571-573.

Okafor JC, Omoradion F, Amaja FI (1994). Non-timber forest products (Nigeria): Consultancy paper prepared by the Evaluation and Co-ordination Unit (FORMECU) and Federal Department of Forestry (FDP) Abuja, Nigeria, P. 8.

Olaniyi AA (2000). Essential medicinal chemistry (2nd edn.) Shaneson C. I. Ltd. Ibadan, Nigeria.

Owoyale JA, Aliu YO, Ayodele JT (1995). Influence of dietary spinach (*Amarathion* Linn.) on chloroquine blood level in the rabbit. Orient. J. Med., 7: 12-13.

O'Brien D, Haddad AR (2002). Consulting patients on drug-food interactions. U. S. Pharmacist, 22(6).

Sanchez CP, McLean JE, Rohrbach P, Fidock DA, Stein WD, Lanzer M (2005). Evidence for a pfcrt-associated chloroquine efflux system in the human malaria parasite *Plasmodium falciparum.* Biochemistry, 44(29): 9862-9870.

Schrauzer GN (1984). "The discovery of essential trace elements: An outline of the history of biological trace elements research". In: biochemistry of essential trace elements, edited by Earl Frieden. Plenum, New York, pp. 17-31.

Shannon LTS, Dands CC, Drissa AT, Mahamadon K, Ogobara D,

Christopher VP (2006). A high throughput method of quantifying alleles and haplotype of the malaria vaccine candidate *Plasmodium falciparum* merozoite surface protein 119 kDa. Malaria J., 5: 1475-2871.

Shargel L, Yu BCA (1984). Applied Biopharmaceutics and Pharmacokinetics. Application century Croft New York, pp. 31-85.

Sofowora ER (1980). Guidelines for research promotion and development in traditional medicine. Nig. J. Pharm., 11: 117-118.

Svensson CK (2007). Expanded answers for study problems index page. In: Rowland and Tozer's clinical pharmacokinetics: Concept and Applications (3rd edn.) 1995. On line contact. *Graig-Svensson@uiowa.edu.*

Udosen EO, Udok UE, Unuigbe OS (1999). The comparison of the nutrient composition of *Lasianthera africana* and *Heinsia crinata*. J. Food Biochem., 23: 571-576.

Walker O (1983). Characterization of chloroquine plasma protein binding in man. Br. J. Clin. Pharmacol., 15: 375-377.

Wallace AW, Amsden GW (2002). Is it really Ok to take this with food? Old interactions with new twist. J. Pharmacol., 42: 437-443.