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The Effect of Ethanolic Extract of *Vernonia Amygdalina* Leaves on Some Pharmacokinetics Parameters of Chloroquine in Rats

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Abstract: Studies were done to find out if there are significant pharmacokinetics interactions between chloroquine and ethanolic extract of *Vernonia amygdalina* when the extract was administered 1 h before chloroquine or simultaneously with chloroquine to different groups of albino rats (Wister strain). Three groups of rats were used, each group comprising 25 rats with one group acting as control. The chloroquine level in the serum was measured using UV-spectrophotometer. The results indicate significant interactions ($p< 0.05$) in the groups to which the extract was administered simultaneously with chloroquine. There was a decrease in the level of drug in circulation as evidenced by the lower values of AUC (297.52±8.45 vs. 333.22±24.99) and Cmax (74.6±1.02) vs. 76.6±3.07) comparing experimental group with control. There was increased rate of elimination of the drug between the experimental group and control (Ke 0.088±0.035 vs. 0.027±0.017). There was no significant pharmacokinetics interactions ($p>0.05$) when the extract was administered 1 h before chloroquine. The result indicate that concomitant administration of chloroquine and *Vernonia amygdalina* resulted in significant interactions ($p<0.05$) and should be avoided to maintain a good plasma level of the drug and prevent possible development of resistance by Plasmodium due to sub-clinical concentration of the drug in the blood.

Key words: Pharmacokinetics interactions, chloroquine, *Vernonia amygdalina*

INTRODUCTION

A drug must be present in appropriate concentrations at its sites of action to produce its desired effects (Hardman, 2001). For an orally administered drug like chloroquine, it must be absorbed from the gastrointestinal tract to an extent and at a rate that will ensure adequate blood levels to elicit pharmacological response of desired magnitude and duration (Ayodele *et al*., 1995). The efficiency with which a drug is absorbed is a function of many variables (Ganellin and Roberts, 1999). One of such variables is food-drug interaction, which has been described for several drugs including chloroquine (Ali *et al*., 2002).

Vernonia amygdalina (Compositae) is a shrub, which grows predominantly in the tropical Africa. The macerated leaves of the plant is used in making soup while the aqueous extract serves as a tonic for the treatment of several illnesses including malaria (Akah and Okafor, 1992; Igile *et al*., 1994; Abosi and Benjamin, 2003; Bergeron *et al*., 2005; Bricerno *et al*., 2006). Chloroquine, on the other hand, has remained a useful drug in the treatment of malarial infection (WHO, 1994). It is still widely used for chemotherapy in Nigeria (Tekoba *et al*., 2004). The drug is also safe in pregnancy and breast feeding (Tekoba *et al*., 2004). Vegetable diets are known to interact with and influence drug blood levels (Sunday *et al*., 2003). This study therefore, seeks to establish if there is any significant interaction between *Vernonia amygdalina* and chloroquine when administered simultaneously since there is a great probability of using the 2 concurrently given the daily and prevalent consumption of *Vernonia amygdalina* in many parts of the malaria belt. The result obtained could be used as a possible explanation for the treatment failures associated with chloroquine therapy (Ariemughare *et al*., 2003).

MATERIALS AND METHODS

Plant collection, identification and authentication: Fresh leaves of *Vernonia amygdalina* (Compositae) were collected from a garden in the University of Uyo Staff Quarters, Uyo Akwa Ibom State of Nigeria in November 2005. The leaves were identified and authenticated by the taxonomist of the Department of Pharmacognosy and

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Traditional Medicine, University of Uyo, Nigeria, where a voucher specimen has been preserved for future reference.

Processing of the leaves: The fresh leaves of *V. amygdalina* collected were cut into small pieces, weighed and then extracted with 3L of 96% ethanol over 72 h. The extract was filtered and the filtrate dried in vacuo with a rotary evaporator, weighed and stored in a desiccator prior to use.

Animals: A total of 75 healthy albino rats (Wistar strain) of average weight 120±20 g were used in the study. They were maintained under standard environmental conditions and had free access to feed and water at the animal house, University of Uyo.

Chloroquine for Experimental animals: A freshly prepared stock solution of chloroquine in water for administration to experimental animals was made using chloroquine phosphate tablet containing 150 mg chloroquine base such that 1 mL of stock solution contained 1 mg of the chloroquine base.

Extract of *V. amygdalina*: A freshly prepared stock solution of the extract in water was made such that 1 mL contained 25 mg of the crude extract.

Chloroquine for standard curve: A stock solution of chloroquine in O. IM HCl was prepared using chloroquine phosphate tablet containing 150 mg chloroquine base such that 1 mL of the stock solution contained 1 mg of chloroquine base.

A serial dilution of the stock solution was carried out to produce 0.001, 0.002, 0.003, 0.004, 0.005, 0.006 mg mL^{-1} chloroquine solution, respectively. The absorbances of the standard solutions were measured at 344 nm and the results obtained were used to produce a standard curve.

Administration of Test Materials (Chloroquine *V. amygdalina*): The rats were fasted overnight prior to the testing. They were divided into 3 groups; A, B and C comprising 25 rats each. Group A was sub-divided into 5 groups of 5 rats each. Each sub-group representing a time point-15, 30, 60, 120 and 300 min, respectively. The stock solution of chloroquine in water was administered orally to the different sub-groups of group A. The dose of chloroquine solution given was calculated based on 10 mg kg^{-1} body weight schedule. Blood samples were collected from the heart of each group of rats at the end of the designated time point, under chloroform anesthesia following chloroquine administration.

Table 1: Serum concentration of chloroquine

Time points (min.)	Group A (CQ only) μg mL^{-1}	Group B (VA before CQ) μg mL^{-1}	Group C (VA +CQ) μg mL^{-1}
15	22.32±6.82	26.98±17.09	24.96±21.79
30	62.10±11.71	62.37±27.55	71.33±3.27
60	75.63±3.92	75.44±2.71	70.16±2.82
120	69.31±9.27	65.19±8.05	68.14±6.55
300	67.56±1.57	59.86±1.73	51.71±5.83*

Mean±SD n =5; * $p<0.05$; CQ only: Groups to which only chloroquine was administered (control); VA before CQ: Groups to which chloroquine was administered 1 h after administration of leaf extract of *Vernonia amygdalina*; VA+CQ: Groups to which both the extract and chloroquine were administered simultaneously

Group B was similarly sub-divided into 5 sub-groups of 5 animals each, labeled with same time points as above but they were orally administered with *Vernonia amygdalina* extract 1 hbefore the administration of the required dose of chloroquine as calculated above. The dose of the extract given was calculated based on 250 mg kg^{-1} body weight. The animals representing each time point were sacrificed at the end of the time period under chloroform anaesthesia and blood samples collected from the heart. Group C was also sub-divided into 5 groups of 5 animals each and were equally labeled with same time points as above. The extract and chloroquine were concomitantly administered to this group. Blood samples were collected from the heart of the animals under chloroform anaesthesia. The blood samples collected were properly labeled and stored in a refrigerator pending analyses (Table 1).

Analyses of blood samples: The blood samples were centrifuged for 20 min and the serum collected into sterile sample tubes. 0.2 mL of the serum was taken and diluted to 3 mL using 0.1M HCl. The absorbance of the diluted serum solutions were measured at 344 mnm using blank diluted serum solution as reference sample.

RESULTS AND DISCUSSION

Area Under the Curve (AUC): The area under the concentration-time curve was calculated using the trapezoidal rule as described by Shargel and Yu (1984).

Absorption rate constant, Ka: The absorption rate is given by first order rate equation:

$$\text{Log } C_t = -K_t/2.303 + \log C_o$$

The graph of concentration against time using semi-log paper gave the slope as Ka.

Elimination rate constant, Ke: The elimination rate constant was evaluated from the elimination phase of the serum concentration-time graph on semi-log paper.

Maximum Serum Concentration (Cmax) and the time required for Cmax to be attained, t max: The Cmax and tmax were obtained from the serum concentration-time graph on semi-log paper.

Elimination half-life, t½el: This is the time required for half the total drug in circulation to be eliminated. It was calculated from the formula:

$$t½el = 0.693/Ke$$

Table 2 is a summary of the values generated from these pharmacokinetics parameters.

From the results obtained from the Areas under the curve (AUC) it could be seen that the relative bioavailability for experimental groups B and C compared with control (group A) were 0.92 and 0.89, respectively. The presence of *V. amygdalina* resulted in reduced bioavailability of chloroquine. The decrease, however, was statistically significant for group C ($p<0.05$) when chloroquine was administered concurrently with *V. amygdalina*. Administration of the extract 1 h before the drug did not result in any significant reduction in AUC ($p>0.05$).

The Ka, absorption rate constant, is a measure of the rate at which the given drug gets into systemic circulation from its site of absorption, which is mainly the small intestine (Hardman *et al*., 2001). The values obtained from the various groups revealed that the presence of the extract of the leaf of *V. amygdalina* resulted in a significant increase in the absorption rate constant when the extract was administered simultaneously with the chloroquine ($p<0.05$). This increase was not statistically significan when *V. amygdalina* was administered 1 h before chloroquine.

There was a rise in the value of the elimination rate constant, Ke in groups B and C (0.04 and 0.09 μg/h/mL, respectively) compared with control, that is Group A, which was 0.03 μg/h/mL. Elimination of drugs proceed by excretion or biotransformation or a combination of both (Shargel and Yu, 1984). The increased elimination rate, as observed, could be due to induction of metabolizing enzymes mainly CYP2C8 and CYP3A4, which constitute low affinity, high capacity systems and to a lesser extent CYP2D6 which account for most of the chloroquine N-desethylation (Progeanet *et al*., 2003).

There was a reduction in maximum serum concentration, Cmax with the administration of *V. amygdalina*. This reduction was greater when both the extract and chloroquine were administered simultaneously than when they were administered 1 h apart. The reduction in Cmax was statistically significant ($p<0.05$) for group C, to which both the extract and chloroquine were administered concurrently.

There was also a significant decrease ($p<0.05$) in the time required for half of the total drug in circulation to be eliminated (t½el) when both the extract and chloroquine were administered simultaneously (32.9±19.00 h for control as against 8.90±2.90 h for Group C in which *V. amygdalina* and chloroquine were given simultaneously).

In summary, the administration of leaf extract of *V. amygdalina* and chloroquine-either 1 h apart or concurrently resulted in increased rate of absorption and elimination but reduced elimination half-life, leading to reduction in total amount of drug in circulation as seen in the reduced values of AUC and Cmax. However, these effects were significant when *V. amygdalina* was administered simultaneously with chloroquine.

The study demonstrated the pharmacokinetics interactions between the extract of the leaves of *V. amgydalina* and chloroquine. The interactions were statistically significant ($p<0.05$) when *V. amygdalina* was administered concurrently with chloroquine. Chloroquine remains a good drug in the treatment of uncomplicated malaria with minimum effective concentration (MEC) values of 15 ng mL^{-1} (0.000015 μg mL^{-1}) and 30 ng mL^{-1} (0.00003 μg mL^{-1}) against *Plasmodium vivax* and *Plasmodium falciparum*, respectively (White, 1985; Krishna and White, 1996). Thus concomitant administration of chloroquine while taking food or herbal preparations made from V amygdalina should be avoided since this could lead to significant reduction in the amount of chloroquine available in circulation as demonstrated by the study. However, administration of chloroquine 1 h after food or herbal preparation made from the leaves of *V. amygdalina* would not result in any significant reduction ($p>0.05$) of the amount of chloroquine in circulation.

Table 2: Comparison of pharmacokinetics parameters

Pharmacokinetics	Group A CQ only	Group B VA before CQ	Group C VA + CQ
AUC (μg/h/mL)	333.22±24.99	307.50±21.24	297.52±8.45
Ka (μg/h/mL)	4.25±1.68	5.45±2.23*	12.78±2.82*
Ke(μg/h/mL)	0.03±0.01	0.04±0.02	0.09±0.04
C_{max}(μg/h/mL)	78.60±3.07	77.40±2.24	74.60±1.02
t_{max} (h)	1.26 ± 0.28	1.01±0.07	0.86±0.33
$t_{½el}$ (h)	32.9±19.00	21.70±11.2*	8.90±2.90*

Mean±SD n =5 * $p<0.05$

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