

Effects of the Leaf Extract of *Vernonia amygdalina* on the Pharmacokinetics of Dihydroartemisinin in Rat

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Abstract: Objective: The aim of this study was to evaluate the effect of the leaf of *Vernonia amygdalina* on the pharmacokinetics of dihydroartemisinin in rat. Artemisinin based drugs are the drugs currently used in malaria therapy. *V. amygdalina* is consumed as a vegetable and medicinal herb in Nigeria. Therefore, it is possible for a malaria patient on artemisinin based drug therapy to consume *V. amygdalina*. **Materials and Methods:** Albino rats were divided into five groups, each of which received 2 mg kg⁻¹ of dihydroartemisinin orally. In addition, Groups A and B were given 250 and 500 mg kg⁻¹ of the extract (single dose and simultaneously with dihydroartemisinin), respectively. Groups C and D received 250 and 500 mg kg⁻¹ of the extract, respectively once daily for seven days (sub-acute dose) before co-administration with dihydroartemisinin on the seventh day. Group E (control) received only dihydroartemisinin. **Results:** UV absorbance of serum of blood collected from the rats through cardiac puncture at 0, 0.25, 0.50, 0.75, 1.0, 2.0 and 5 h, after drug administration, was measured at 230 nm in a UV spectrophotometer. Both single and sub-acute treatments at the two dose levels reduced the bioavailability (F), absorption constant (K_a), peak concentration (C_{max}) and increased apparent volume of distribution (V_d) of the drug significantly. Single dose treatments of 250 and 500 mg kg⁻¹ decreased the Area Under the Curve (AUC) and elevated elimination constant (K_e). **Conclusion:** These results show that the leaf of *V. amygdalina* is capable of affecting the effectiveness of dihydroartemisinin. Therefore, patients on artemisinin-based therapy should consume the leaf of *V. amygdalina* with caution.

Key words: Pharmacokinetics, *V. amygdalina*, dihydroartemisinin, malaria, medicinal herb

INTRODUCTION

Food-drug interactions can lead to changes in some pharmacokinetic parameters of some drugs. These interactions may have clinical implications (Singh, 1999). Diet is one of the factors that can affect the pharmacokinetics of dihydroartemisinin (Ali *et al.*, 2002). *Vernonia amygdalina* is commonly used in traditional medicine. The leaf decoctions are used to treat diabetes, fever, malaria, diarrhea, dysentery, hepatitis and cough, as a laxative and fertility inducer. It is also used as medicine for scabies, headache and stomach-ache and joint pain associated with AIDS, gingivitis and toothache due to its proven antimicrobial activity (Akah and Okafor, 1992; Alabi *et al.*, 2005; De Boer *et al.*, 2005; Iwu, 1993; Fasuyi, 2006; Innocent and Deogracious, 2006). In Ethiopian local medicines the leaves are used against menstruation pain, wound dressing, urinary tract inflammation and other

sexually transmitted diseases (Farombi, 2003; Fasuyi, 2006). The bitter sesquiterpene lactones component of *V. amygdalina* extracts may help suppress, delay or kill cancerous cells (Jisaka *et al.*, 1993; Kupchan *et al.*, 1969; Sweeny *et al.*, 2005). It may also provide antioxidant activity (Igile *et al.*, 1994; Erasto *et al.*, 2007) and strengthen the immune system through many cytokines regulation (Sweeny *et al.*, 2005). *Vernonia amygdalina* (Bitter leaf) is used as condiments in human diet or as supplementary feed to livestock (Akah and Okafor, 1992). It has a hepatoprotective property (Fasuyi, 2006). *Vernonia amygdalina* leaf contains protein, fat, carbohydrate, crude fibre, energy and minerals such as calcium, phosphorus, iron and ascorbic acid. Its leaf extract has been found to reduce the rate of gastric emptying in rats. These effects of *V. amygdalina* may be important in influencing the bioavailability of drugs taken immediately before or after a meal containing

this vegetable (Anufuro and Lgboechi, 1986; Ayodele, 2000). Igboasoiki *et al.* (2008) reported that *V. amygdalina* affected the pharmacokinetics of Chloroquine in rat. Hence, there is need to investigate the effect of *Vernonia amygdalina* on the pharmacokinetics of Dihydroartemisinin. This study is designed to evaluate the effect of *Vernonia amygdalina* leaf extracts on certain pharmacokinetic parameters of Dihydroartemisinin in rats. The pharmacokinetic parameters include: Area under the plasma concentration curve (AUC), absorption rate constant (K_a), maximum concentration (C_{max}), elimination rate constant (K_e), time of peak plasma concentration (t_{max}), half life of the drug ($t_{1/2}$), volume of distribution (V_d), clearance (Cl) and bioavailability (F).

MATERIALS AND METHODS

Plant collection and identification: Fresh leaves of *Vernonia amygdalina* (Compositae) were bought in December 2009 in Akpan Andem market, Uyo, Akwa Ibom State. They were authenticated by Dr. (Mrs.) Margaret Bassey of the department of Botany and Ecological Studies, Faculty of Natural and Applied Sciences, University of Uyo, Nigeria.

Preparation of extract: The fresh leaf of *V. amygdalina* collected was washed thoroughly under tap to remove traces of sand and set aside to drain off. They were then cut into small pieces, weighed and macerated with 5 L of 96% ethanol for 72 h. The extract obtained was filtered, evaporated and concentrated using a rotary evaporator. The concentrated leaf extract obtained was dried to a constant weight of 48 g in a desiccator with silica gel as the absorbent. The extract was suspended in Tween 80 before being administered.

Administration of dihydroartemisinin and plant extract to animals: A total of one hundred and seventy five albino rats of either sex weighing between 182 and 212 kg were obtained and housed under standard conditions of light and temperature in University of Uyo animal house. They had access to water and feed *ad libitum*. They were divided into five equal groups (35 rats) and were treated as follows:

- **Group A:** It was given 250 mg kg⁻¹ of the extract and 2 mg kg⁻¹ of the drug simultaneously (single dose administration)
- **Group B:** It was given 500 mg kg⁻¹ of the extract and 2 mg kg⁻¹ of the drug simultaneously (single dose administration)

- **Group C:** It was given 250 mg kg⁻¹ of the extract once daily for seven days followed by the administration of 2 mg kg⁻¹ of the drug (sub-acute dose administration)
- **Group D:** It was given 500 mg kg⁻¹ of the extract once daily for seven days followed by the administration of 2 mg kg⁻¹ of the drug (sub-acute dose administration).
- **Group E (control group):** It was given 2 mg kg⁻¹ of the drug alone. All administration was done orally

Collection of blood samples: Blood was collected from each rat under chloroform anesthesia by cardiac puncture using 5 mL syringes at 0, 0.25, 0.50, 0.75, 1, 2 and 5 h after administration of Dihydroartemisinin (n = 5 for each time point). The blood samples were allowed to clot centrifuged for 25 min and the serum collected. 0.2 mL of the serum was taken and diluted to 3 mL using simulated intestinal fluid. The absorbance (A) of the serum solution was measured at 230 nm using serum of untreated rats as blank.

Statistical analysis: The significance of the result was calculated using student's t-test and was considered statistically significant at p<0.05.

RESULTS

The absorption constant (K_a) of Dihydroartemisinin (DHA) was reduced significantly by both single and sub acute dose of 250 and 500 mg kg⁻¹ of the plant extract. The reduction in K_a is dose dependent. Both the single and sub-acute dose of 500 mg kg⁻¹ reduced K_a (1075.7 and 1596.0) twice as much as the 250 mg kg⁻¹ dose (663.8 and 700.5, respectively) (Table 1).

Both single and sub-acute administration of the extract reduced C_{max} significantly in a dose dependent manner. 500 mg kg⁻¹ extract reduced C_{max} (886 and 10,643 $\mu\text{g mL}^{-1}$) better than the dose of 250 mg kg⁻¹ (8525 and 10,973 $\mu\text{g mL}^{-1}$), respectively for single and sub-acute administration. The reduction in C_{max} by single dose administration of both 250 and 500 mg kg⁻¹ doses (8525 and 5506 $\mu\text{g mL}^{-1}$) was greater than that by the sub acute administration of the extract (10,973 and 10,643 $\mu\text{g mL}^{-1}$, respectively).

The 250 mg kg⁻¹ dose of the extract did not significantly affect the t_{max} either when administered as a single dose or sub acutely. But 500 mg kg⁻¹ dose significantly increased t_{max} (0.006985 and 0.00977 h, when administered as a single dose and sub acutely,

respectively). The increase was greater in sub acute than single dose administered rats.

Single dose administration of the extract produced a reduction in Area Under the Curve (AUC) at the two dose levels (1392 and 1398, for 250 and 500 mg kg⁻¹, respectively). While sub acute administration of the extract increased AUC in both cases (1441 and 1423, for 250 and 500 mg kg⁻¹, respectively). These changes in AUC were not affected by dose of the extract.

Bioavailability (F value) was reduced by the extract. Both 250 and 500 mg kg⁻¹ dose administration reduced F (0.0240 and 0.2045) more than sub acute administration (0.02297 and 0.0427). While the single dose administration did not show any dose dependent effect, the sub acute administration of the extract gave a dose dependent effect. The reduction in F by 250 mg kg⁻¹ extract (0.2867) was greater than that by 500 mg kg⁻¹ extract (0.0427) (Table 1).

Volume of distribution (V_d) was increased by 250 mg kg⁻¹ of the extract to the same extent when

administered as a single dose (0.0290) or sub acutely (0.2867). But at the 500 mg kg⁻¹ dose level, the increase in V_d was greater in the sub acute (0.0505 kg⁻¹) than in the single (0.2867 kg⁻¹) dose administration (Table 1).

Single dose administration of the extract significantly raised the value of K_e (Elimination constant) at the two dose levels of 250 and 500 mg kg⁻¹ (8.350 and 6.747 h⁻¹, respectively). The 250 mg kg⁻¹ extract elevated K_e higher than 500 mg kg⁻¹. Only 250 mg kg⁻¹ dose of the extract raised K_e value significantly when administered sub acutely. The 500 mg kg⁻¹ extract did not. The extracts reduced t_{1/2} (half-life) in a manner opposite to that of K_e (Table 1).

The extract at 250 mg kg⁻¹ did not have any significantly effect on Cl. But both single and sub acute administration of 500 mg kg⁻¹ extract reduced Cl to the same extent (0.1805 and 0.1843, respectively) (Table 1).

Figure 1 graphically depicts the concentration-time curve of each group.

Table 1: Pharmacokinetic parameters of dihydroartemisinin after a single and sub-acute dose administration of *Vernonia amygdalina*

Parameters of dihydroartemisinin (2 mg kg ⁻¹)	Control	Single dose (mg kg ⁻¹)		Sub-acute dose (mg kg ⁻¹)	
		250	500	250	500
K _e (h ⁻¹)	1865.4±23.03	1075.7±153.50*	663.8±1.56**	1596.0±53.19*	700.5±10.69**
K _a (h ⁻¹)	4.025±0.001	8.350±0.290	6.747±0.243**	7.097±0.383**	3.460±0.189
AUC (µg h mL ⁻¹)	1401±1.00	1392±0.125**	1398±1.188	1441±3.185*	1423±2.500**
T _{1/2} (h)	0.1715±0.006	0.083±0.000	0.1025±0.001**	0.098±0.001**	0.201±0.001
V _d (l kg ⁻¹)	0.1715±0.006	0.0290±0.300	0.0269±0.000	0.2867±0.002*	0.0505±0.005*
Bioavailability (F)	0.0535±0.004	0.0240±0.009**	0.2045±0.008**	0.02297±0.007*	0.0427±0.002*
Clearance, Cl (mL h ⁻¹)	0.2045±0.002	0.2415±0.002	0.1805±0.001**	0.2023±3.330	0.1843±0.008**
T _{max} (h)	0.003282±1.80	0.00465± 0.015	0.006985±0.35**	0.003413±9.82	0.00977±0.002*
C _{max} (µg mL ⁻¹)	28381±2920	8525± 361*	5506± 886**	10973±3504*	10643±3504**

Mean±SD, n: 5, *p<0.05, **p<0.01

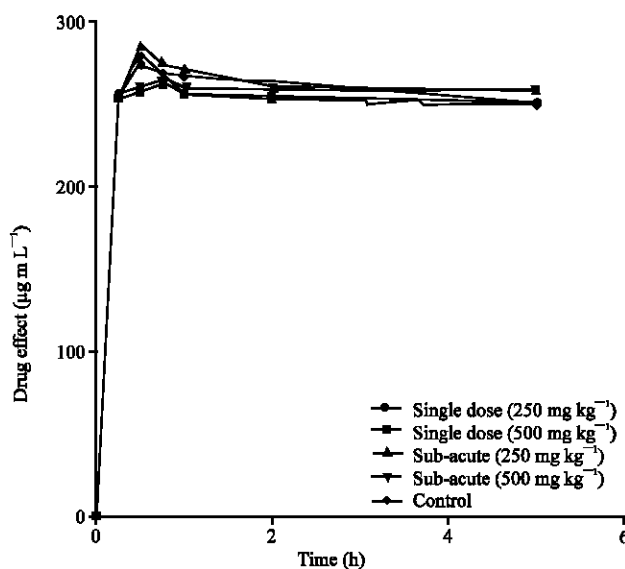


Fig. 1: Effect of concentration of dihydroartemisinin w.r.t. time

DISCUSSION

Dihydroartemisinin is rapidly and completely absorbed from the Gastrointestinal Tract (GIT) following oral administration and extensively distributed in the body. In most cases Dihydroartemisinin is detected in plasma after 30 min. The biliary metabolite is the biologically inactive Dihydroartemisinin glucuronide. The other metabolites are products of reductive cleavage and rearrangement of endoperoxide bridge, a process known to generate reactive radical intermediates and abolish antimalarial activity. Peak plasma concentration is 1-2 h and the drug is eliminated from the circulation within 8-10 h (Karbawang *et al.*, 1997; Maggs *et al.*, 1997; Hong *et al.*, 2008).

Interaction between plant extracts and drugs may increase or decrease the pharmacological or toxicological effects of either component (Fugh-Berman, 2000). The hypericum extract of St. John's wort (*Hypericum perforatum*) has been shown to cause multiple drug interactions through induction of the cytochrome P450 enzyme CYP3A4 and CYP2C9 resulting in increased metabolism of drugs metabolized by the system, leading to their decreased concentration and a reduced clinical effect. The principal extract thought to be responsible is hyperforin. This plant extract has also been shown to cause drug interactions through the induction of the P-glycoprotein (P-gp) efflux transporter resulting in decreased absorption and increased clearance of the drugs. This leads to a lower clinical concentration and efficacy. Examples of these drugs are Benzodiazepines, immune suppressants such as ciclosporin and other drugs like digoxin, theophylline (Gurley *et al.*, 2008; Linde *et al.*, 2008). Concurrent use of plant extracts with drugs may mimic, magnify or oppose the effect of drugs. This is seen in increased bleeding when warfarin is combined with garlic, decreased concentration of phenytoin when combined with anthranoid containing plants such as senna (Fugh-Berman, 2000).

Solid food, especially those rich in fat and dietary fibre delay the entry of orally administered drug into the duodenum, reducing the rate of absorption and hence, the onset of therapeutic action (Mason, 2002). Reduction in K_a by the extract of *V. amygdalina* may therefore, be as a result of physicochemical reaction such as chelation and adsorption of DHA by the extract of *V. amygdalina*. The same reasons could also be responsible for the decrease in C_{max} and increase in t_{max} of DHA by the extracts.

Phytochemicals are known to interact with drug transporters thereby causing impairment or exaggeration of pharmacological activity (Guengerich, 1997; Lin and Lu, 1998; Ioannides, 2003). Some elements, notably Zinc,

may induce intestinal proteins which bind drugs and prevent their transfer from the intestine into the body (Schrauzer, 1984). Reduction in AUC of DHA by single dose administration of the extract might have resulted from the interaction of the drug with components of the extract. It is not known why the sub acute administration of the extract increased AUC of DHA. Elevation of K_e of DHA by the plant extract may be a consequence of either increased metabolism or excretion of the drug. Elevation of V_d value of DHA by the extract is an indication that DHA is concentrated in some body compartments probably as a result of increased microsomal protein in the body. Low serum drug concentration is indicated by high value of V_d .

The bioavailability of chloroquine has been shown to increase when chloroquine was administered together with food: it appears food facilitates chloroquine absorption as the result from the bioavailability studies carried out by Tulpule and Krishnawamy (1982). They suggested that the AUC was significantly higher in postprandial than in the fasting state. Grapefruit juice has been shown to increase the plasma concentration of Chloroquine (Ali *et al.*, 2002). Kava kava beverage extract prepared from the rhizome of kava plant (*Piper methysticum* Forst) has shown that several kava lactones, the assumed active principles of kava beverages are potent inhibitors of several enzymes of the CYP450 system (CYP1A2, 2C9, 2C11, 2D6, 3A4 and 4A9/11), thus indicating that this beverage has high potential for causing pharmacokinetic drug interactions with other drug metabolized by the CYP450 enzymes (Gurley *et al.*, 2008). *Heinsia crinita*, *Telfairia occidentalis*, *Lasiathera africana* have been reported to affect some pharmacokinetic parameters of Chloroquine (Eseyin *et al.*, 2007; Eseyin *et al.*, 2010; Olorunfemi *et al.*, 2011). The result of this work is therefore, in agreement with this.

The bitterness of *V. amygdalina* is caused by sesquiterpenes lactone e.g., vernodalin, vernolepin and vernomygdin and steroid glucosides-vernon sides. The effects of the plant on some pharmacokinetic parameters of DHA could be attributable to any of these components. However, further work needs to be done to ascertain this.

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