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FRUIT/VEGETABLE–DRUG INTERACTIONS: PHARMACOKINETIC ASSAY WITH A CYP3A4 SUBSTRATE

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ABSTRACT

Important health benefits of complex mixtures of phytochemicals in fruits and vegetables have been established. However, these phytochemicals have been shown to influence the pharmacological activity of drugs by modifying activities of key metabolizing enzyme systems, specifically the cytochrome P450 enzymes. Felodipine, an antihypertensive agent, has been identified as a substrate for CYP3A4.*Carica papaya* (pawpaw fruit) and *Telfairia occidentalis*(ugu/pumpkin vegetable) are common staple fruit and vegetable regularly consumed in Nigeria. However, the potential fruit/vegetable-drug interactions between felodipine and pawpaw/pumpkin are unknown. The objective of this study was to investigate the effects of pawpaw juice and pumpkin (ugu) vegetable extract on the pharmacokinetics of felodipine in rats. The pharmacokinetic profiles of orally administered felodipine (10 mg/kg) in the absence and presence of pawpaw juice (10 mL/kg) /pumpkin (ugu) extract (10 mL/kg) were investigated in rats. There was a significant ($p<0.05$) reduction in AUC and K_a while the clearance rate increased on concurrent administration with pawpaw and pumpkin extract. The AUC values for the groups that received pawpaw and pumpkin (ugu) extract were 84% and 48% lower than the group that received felodipine alone. The elimination rate in the group of rats that received both felodipine and pawpaw was 29% lower than the corresponding values obtained in the group that received felodipine alone. A 43% increase in clearance rate was observed in the group that received felodipine and ugu (pumpkin) extract as compared to the group that received felodipine alone while clearance rate was increased to about 548% in the group that received pawpaw juice. Reduction in

felodipine bioavailability was moderate in the group administered with pumpkin extract compared to the group that received pawpaw juice. These findings support activation or induction of CYP3A4-mediated pre-systemic felodipine metabolism as the most likely mechanism. This fruit juice–drug interaction rat model may be useful in prediction of potential food–drug interactions in humans.

KEYWORDS: fruit/vegetable-drug interactions, pharmacokinetics, bioavailability, enzyme induction.

INTRODUCTION

Consumption of balanced diets remains an important factor influencing human health and disease.[1] It is known that regular intake of fruits and vegetables could minimize the risk of some diseases, including cancer.[2] Studies have shown that complex mixtures of phytochemicals in fruits and vegetables can be beneficial for human health. However, it is becoming increasingly clear that phytochemicals can affect the pharmacological activity of drugs by influencing their absorption characteristics through interactions with drug transporters as well as drug- metabolizing enzyme systems.[1] Interactions could result to increased or decreased bioavailability of drugs. Many of the key pharmacokinetic interactions between drugs are mainly linked to cytochrome P450 (P450 or CYP) enzymes present in the liver and other extra-hepatic tissues.[3]

About thirty (30) CYP enzymes responsible for drug metabolism exist in humans.[4-6] It has been documented that 90% of drug metabolism can be attributed to six main enzymes: CYP 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4.[7] These CYPs have been shown to be induced or inhibited by the ingestion of some foodstuffs. For example, CYP1A2 is induced by cruciferous vegetables as well as barbecued or charbroiled food while CYP3A4 is inhibited by grape juice.[3,8-10] The most abundant CYP isoenzymes are CYP3A4 and CYP2D6. CYP3A4 is found not only in the liver but also in the intestinal wall.[3]

It is estimated that a large population of humans takes at least one pharmacologically active agent on a regular basis. Hence, with this level of medication use, coupled with differences in dietary lifestyle and food composition, there exists a high potential for drug–nutrient interactions.[1] It is important that healthcare providers are aware of important food–drug interactions in order to optimize the therapeutic outcome of drugs.

Felodipine (a calcium antagonist) is an important substrate for a cytochrome P450 enzyme known as CYP3A4. Studies have revealed that grapefruit juice increases the oral bioavailability of felodipine by inhibiting pre-systemic metabolism mediated by cytochrome P450 (CYP) 3A4 and possibly also by inhibiting P-glycoprotein (P-gp) – mediated intestinal efflux.[11] Research has also indicated that one or more substances in grapefruit juice can produce irreversible inactivation of CYP3A4. Recent studies have shown these substances to be furanocoumarins.[12,13] Several furanocoumarins in grapefruit juice are effective *in vitro* CYP3A4 inhibitors and have been shown to be clinically active constituents.[14] Nevertheless, a question arises as to whether some commonly consumed Nigerian vegetables and fruits such as *Carica papaya* (pawpaw) and *Telfairia occidentalis* (ugu) could contain substances that could influence the bioavailability of co-administered drugs. In Nigeria (as well as many other parts of the world), felodipine is widely used as a potent antihypertensive drug, while *Carica papaya* (pawpaw) and *Telfairia occidentalis*(ugu) are common staple fruit and vegetable regularly consumed in Nigeria. Therefore a possible co-administration exists.

Minimal documentation exists for drug interactions involving vegetables and fruits. To the best of our knowledge, little or no data exists with regards to possible interactions of drugs with common fruits and vegetables commonly consumed in Nigeria. This work investigates the potential nutrient-drug interaction involving *Carica papaya* (pawpaw) and *Telfairia occidentalis* (ugu/pumpkin vegetable), with felodipine- a calcium channel blocking anti-hypertensive agent in rats.

MATERIALS AND METHODS

Chemicals

Hydrochloric acid (Sigma-Aldrich, Germany), Vanillin^{R} (Qualikems), methanol (JHD, China), distilled water, methylated spirit (JHD, China), ether (Kermel, China), felodipine 5mg tablets (Plendil® tablets-Astra Zeneca).

Fruit/Vegetable Sample collection

Telfairia occidentalis (pumpkin /ugu) vegetable and *Carica papaya* (pawpaw) fruit were purchased from Choba, Port Harcourt, Rivers State.

Fruit and vegetable processing and extraction

Carica papaya (pawpaw) fruit (4 kg) was washed, peeled and mashed to a pulp with a mortar and pestle, and then the pulp was filtered to extract the juice. Leaves of *Telfairia occidentalis*

(pumpkin /ugu) (3.5 kg) were washed, cut in small pieces, and squeezed to obtain a liquid extract.

Pharmacokinetic study

Fifteen (15) healthy male albino rats with weight range 125-185g used for this study were obtained from the animal house of Department of Pharmacology and Toxicology, University of Port Harcourt, Rivers State, Nigeria. The animals were placed in standard cages and housed in a controlled environment for at least two weeks for acclimatization. Animal ethics and proper handling methods were strictly adhered to. The cages were cleaned daily, ensuring proper and adequate bedding using saw dust. The animals were fed daily with standard diets and water *ad libitum*. The animals were starved a night before the experiment and fed afterwards.

The animals were divided into three (3) groups containing 5 animals each. The animals in the first group were given 10 mg/kg of felodipine plus water (which corresponds to the clinical dose in humans). The second group received 10 mg/kg felodipine plus 10 mL/kg of pawpaw juice, while the third group received 10 mg/kg felodipine plus 10 mL/kg of pumpkin (ugu) juice. This was done according to the method previously described by.[16] At predetermined intervals of 0, 2, 6, 12, 24 and 48 hours, blood samples withdrawn from the rat tail vein in heparinized tubes and plasma was obtained by centrifugation at 2000 rpm for 10 minutes and stored in a freezer until analyzed.

Felodipine Assay

The spectrophotometric assay of felodipine was performed according to the method described by.[15] A drug free plasma sample was used as blank. To 200µL aliquot of a plasma sample, 20µL methanol was added and volumes were made up to 1.5ml with 35.5 %v/v HCl. Vanillin solution (0.5% w/v) was added and the resultant solution centrifuged at 3000 rpm for 5 minutes. The supernatant was collected and the absorbance was measured at 500 nm using a UV spectrophotometer (UV-Visible SP6 Pye Unicam). The felodipine concentration in each sample analyzed was then calculated using a calibration curve constructed from the absorbance of the following standard felodipine concentrations of 20, 40, 80, 160, 320 and 640 µg/mL.

Data Analysis

Plasma felodipine concentrations were analyzed by a non-compartmental method. The concentration–time data for each study period 0–48 hours after completion of felodipine administration were fitted by a non linear least squares regression to the following equation, $C = C_0.e^{-(kt)}$, where C is the concentration at time t and C_0 is the concentration when t = 0.The terminal elimination rate constant (K_{el}) was determined by log-linear regression. The apparent elimination half-life of the log-linear phase ($T_{1/2}$) was calculated as $0.693/K_{el}$. The area under the plasma drug concentration-time curve (AUC) was calculated from 0 to 48 hours [AUC (0-48)] by the linear trapezoidal method. The AUC from time 0 to infinity [AUC (0-∞)] was calculated as AUC (0-48) plus AUC from 48 hours to infinity [AUC(48-∞)]. C_{max} and the time to reach C_{max} (t_{max}) were obtained directly from the experimental data. The volume of distribution (V_d) was calculated for each treatment by dividing the dose of felodipine by the initial plasma concentration of felodipine when t is equal to zero. The clearance rate (Cl_T) was obtained by multiplying V_d by the elimination rate constant (K_{el}).The method of residuals was used to determine the absorption rate (K_a), whereas the concentration maximum (C_{max}) and the corresponding time (T_{max}) were read off from the plasma concentration versus time curve. Results were expressed as mean ± SEM (standard error of the mean). Statistical analysis of data obtained was performed using chi-squared test and results were regarded as significant at $p < 0.05$.

RESULTS

The mean plasma concentration of felodipine when given alone and when concurrently administered with pawpaw and ugu (pumpkin) vegetable extract is shown in fig1. The results of the pharmacokinetic analysis were expressed as mean plasma concentration ± standard error of the mean (SEM). The corresponding log concentration graphs are shown in figs 2 and 3 respectively from where the elimination rate constants (K_{el}) were calculated using the relationship slope = $-0.434K_{el}$. The absorption rate constant was derived from the plot of mean plasma concentration versus time using the method of residuals.

The result of the pharmacokinetic analysis is represented in Table 1. Data revealed a significant ($p<0.05$) reduction in AUC and K_a and increased clearance rate when felodipine was administered concurrently with pawpaw and pumpkin vegetable extracts as compared to when it was administered alone (Table 1). Furthermore, results showed higher mean plasma

felodipine concentrations, when administered alone than when administered with pawpaw and pumpkin (ugu) extract respectively (Fig 1). Similar $T_{1/2}$ (Half life) and K_{el} (elimination rate constant) values were observed for the groups administered felodipine alone and with ugu (pumpkin) extract.

The AUC values for the groups that received pawpaw and ugu extract were 84% and 48% lower than the group that received felodipine alone. Peak plasma concentrations of felodipine in the three groups occurred after two (2) hours with plasma concentration of felodipine in the group that received pawpaw and ugu extract being 85% and 32% lower than the group that received felodipine alone respectively. The elimination rates in the group of rats that received both felodipine and pawpaw was 29% lower than the corresponding values obtained in the group that received felodipine alone. A 43% increase in clearance rate was observed for the group that received felodipine and ugu (pumpkin) extract as compared to the group that felodipine alone while clearance rate was increased to about 548% in the group that received pawpaw juice. At 48 hours, there was no detectable felodipine in the plasma of the experimental animals.

Table 1: Plasma pharmacokinetics parameters of felodipine with and without pawpaw/ pumpkin (ugu) vegetable extract.

	AUC (mg. h/ mL^{-1})	$T_{1/2}$ (h)	K_a (h^{-1})	K_{el} (h^{-1})	Clearance rate (mL/h)
GROUP F	355.97 ± 52.45	11.17± 0.10	1.51± 0.02	0.06 ± 0.00	0.07 ± 0.00
GROUP FU	185.44 ± 28.13*	11.17 ± 0.10	1.30 ± 0.01*	0.06 ± 0.00	0.10 ± 0.00*
GROUP FP	57.13 ± 10.56*	15.75 ± 0.20*	0.55 ± 0.00*	0.04 ± 0.00*	0.48 ± 0.01*

GROUP F = group administered felodipine only, GROUP FP = group administered felodipine + pawpaw, GROUP FU = group administered felodipine + ugu vegetable extract.
*Significant $p < 0.05$

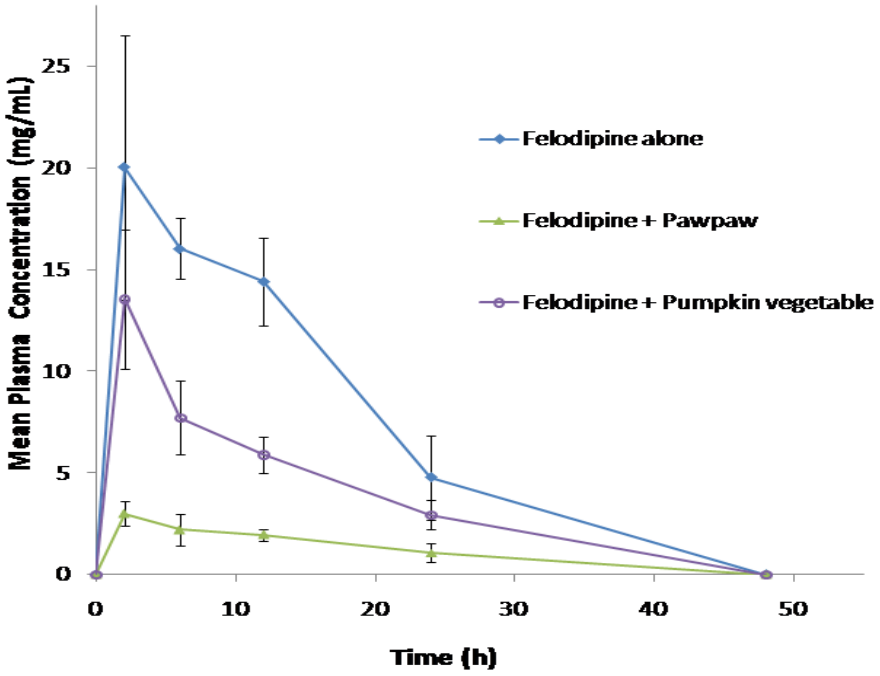

Fig 1: Mean plasma concentration of felodipine versus time with and without pawpaw/pumpkin (ugu) extract.

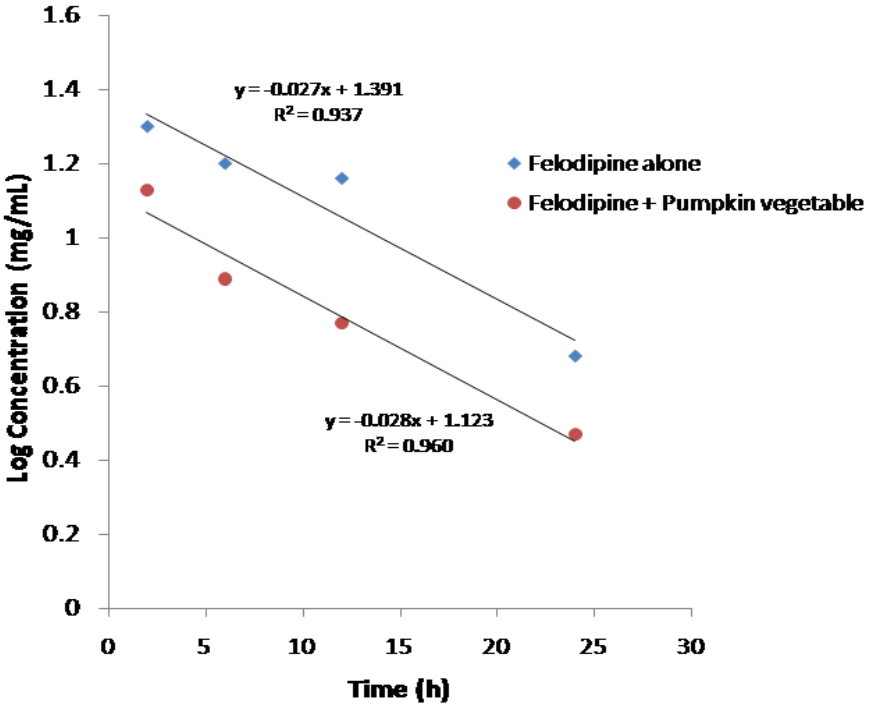

Fig 2: Graph of log concentration of felodipine versus time in the absence and presence of pumpkin vegetable extract

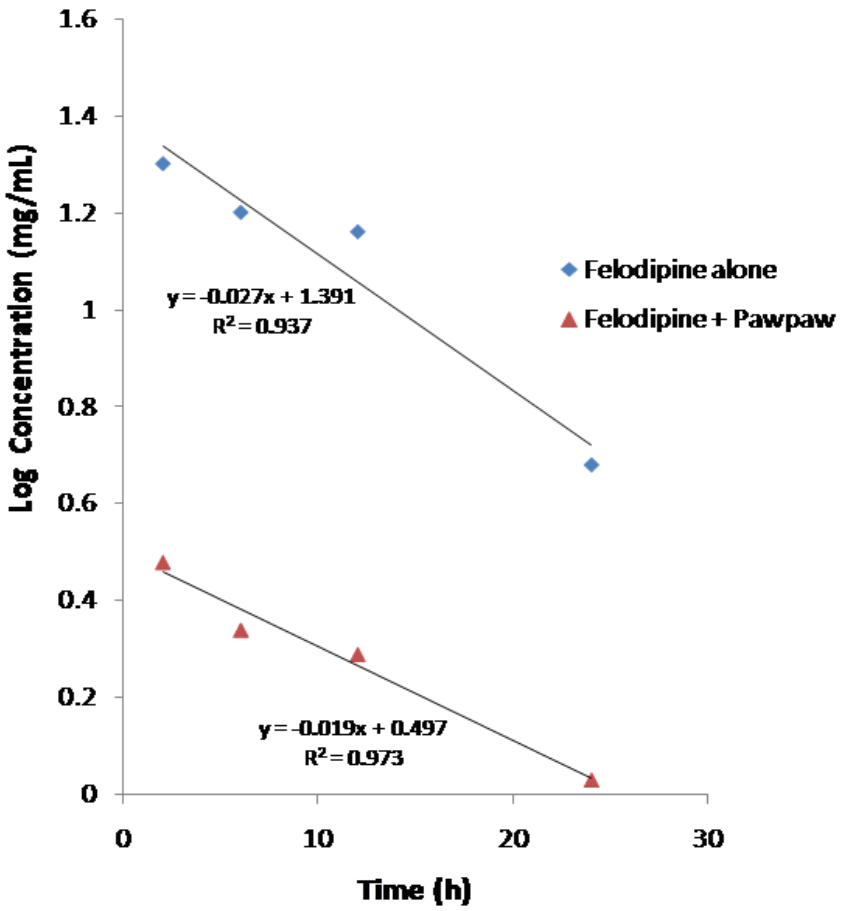

Fig 3 : Graph of log concentration of felodipine versus time in the absence and presence of pawpaw juice.

DISCUSSION

The results of this study demonstrated that pawpaw juice and pumpkin (ugu) vegetable extract significantly reduced the bioavailability of felodipine in experimental animals. Pawpaw juice produced a pronounced decrease in bioavailability (indicated by reduced AUC and K_a) suggestive of a significant potentiation of CYP3A4 activity/enzyme induction. In contrast, pumpkin (ugu) juice/extract caused a much less decrease in bioavailability under the same conditions, indicating modest enzyme activation (Table 1). An increased clearance rate was validated by a corresponding increase in half life and increase in elimination rate constant in the group that received pawpaw juice. This is suggestive of some metabolic process favoring felodipine metabolism.

Felodipine has been shown to be a non-substrate for the efflux pump, P-glycoprotein, which eliminates possible confounding effects by these juices on this transporter.[17] The single primary inactive metabolite, dehydrofelodipine, and the major secondary metabolite,M3, are

formed by CYP3A4. This enzyme is located in the small bowel and liver and is responsible for the high presystemic metabolism and low absolute oral bioavailability of felodipine.[18] In the present study, felodipine AUC and K_a values were reduced, while clearance was increased for the groups co-administered with paw paw and pumpkin (ugu) vegetable extract. From the graphs of Log concentration of felodipine versus time (figures 2 and 3), pawpaw juice produced a pronounced left shift, illustrating marked activation of CYP3A4 activity. In contrast, ugu/pumpkin extract caused much less left shift under the same conditions, indicating modest mechanism-based enzyme activation. These findings support activation or induction of CYP3A4-mediated pre-systemic felodipine metabolism as the most likely mechanism.

Studies by[19,20] revealed that tangeretin, a flavonoid found in high levels in tangerine juice, stimulates CYP3A4 activity. It was shown to be a potent region-selective stimulator of midazolam 1′-hydroxylation by human liver microsomes CYP3A4.[1] In another research carried out by[12] on felodipine and grapefruit showed that grapefruit juice inhibited CYP3A4, leading to an increased plasma concentration. The first report of this interaction revealed that grapefruit juice tripled the mean plasma AUC compared to water in borderline hypertensive patients and was shown to elevate C_{max} but did not alter $T_{1/2}$ since felodipine was administered intravenously.[21] A similar research also revealed that grapefruit juice reduced presystemic metabolism of felodipine through selective post-translational down regulation of CYP3A4 expression in the intestinal wall.[13]Several furanocoumarins in grapefruit juice are believed to be responsible for the in vitro inhibition of CYP3A4, especially bergamottin and 6',7' dihydroxybergamottin.[13]Studies have revealed that ugu (pumpkin vegetable)contains saponins, glycosides, tannins, flavonoids, alkaloids, vitamins, minerals, proteins, phenolic compounds, phytosterols, resins and oils.[22,23] On the other hand pawpaw contains alkaloids, flavonoids, cardenolides, tannins, saponins, steroids, cardiac glycosides, anthraquinones and phenolics.[24] To the best of our knowledge, neither pawpaw nor pumpkin leaves have been shown to contain bergamottin and 6',7' dihydroxybergamottin.

The concurrent administration of felodipine with pawpaw juice and felodipine with ugu leaves extract in our study revealed a significant decrease in plasma concentration of felodipine compared to the group administered with felodipine alone. Pawpaw fruit and ugu

(pumpkin vegetable) extract could contain various bioactive compounds which may be responsible for these decreased concentrations through activation of CYP3A4 enzyme.

CONCLUSION

The results of this study demonstrated that concurrent administration of felodipine with pawpaw and pumpkin (ugu) extract significantly ($p<0.05$) reduced the bioavailability of felodipine. AUC and K_a were significantly reduced while the clearance rate was increased. In view of the data obtained from this study, we infer that this reduction in bioavailability/pharmacokinetic parameters could be due to CYP3A4 enzyme induction or activation. However, this needs to be established in studies in humans.

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