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The influence of polyherbal formulation on the pharmacokinetic fate of amlodipine in healthy Nigerian volunteers

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Abstract
Concomitant administration of herbs with orthodox drugs has attracted more attention as it can modify the pharmacokinetics of drugs thus causing adverse drug reaction or ineffective drugs. Therefore, the influence of a polyherbal formulation and its constituent, *Markhamia tomentosa* (MT) on the pharmacokinetics fate of a commonly prescribed antihypertensive drug, amlodipine was investigated. The polyherbal and its constituent, were extracted with 70% methanol, concentrated and freeze dried. Acute toxicity, flavonoid and phenol contents were evaluated. Human volunteers were given amlodipine (10 mg/kg, p.o) with or without MT (200 mg/kg, p.o) or polyherbal (eq. of 200 mg/kg MT); blood was drawn at different time, plasma assayed for the concentrations of amlodipine. Co-administration of amlodipine with MT showed significant ($p \leq 0.05$) increase in k_a, $T_{max,}$ MRT and Vd with simultaneous decrease in k_{el}, C_{max}, and $AUC_{0\text{-}96}$. The administration of the polyherbal formulation showed little or no significance ($p \geq 0.05$); however, the k_a was slower, evident by decreased $t_{1/2}$ k_a and $AUC_{0\text{-}96.}$ These findings suggest cautious use of MT or the polyherbal formulation with amlodipine to prevent pharmacokinetic alteration of amlodipine and posing threat to health.

Keywords: Pharmacokinetics, *Markhamia tomentosa*, polyherbal, amlodipine, herb-drug interaction

Introduction
Herbs have long been associated with medicinal, therapeutic principles and culinary purposes [1]. Herbal products have attracted growing interest for the prevention and treatment of various diseases and the utilization of herbal medicine has become an integral part in treating human illness with a large population in developing countries relying on herbal medicines [2, 3]. Thus, there should be an effective need to evaluate the use of these herbs especially with a forecast that the global market for herbal products is expected to be rise to about 5 trillion US dollars by 2050 [4].

The concomitant use of herbs and orthodox drugs has created ample room for herb-drug interactions and adverse drug reactions **(**ADRs). These interactions are multifaceted, and the clinical effects may be alterations in either or both pharmacodynamic and pharmacokinetic fate of the drugs [5]. These effects may consequence in treatment failure or toxicity from delayed elimination [6, 7, 8], increase undesirable or desirable side effect and eventual life-threatening complications which may lead to death [9]. Harmful drug interactions are most likely to occur when the drug has a low therapeutic index, making it veer easily toward toxicity with the slightest increase in plasma concentration [10]. In Nigeria, more than one-third of the hypertensive and diabetic patients use herbal medicine [11, 12]. This has brought the need to thoroughly understand the fates of drugs when concomitantly administered with herbal medicines; more so that concomitant use of herbs and drugs are usually self-administered and information to their administration is not available to healthcare providers. Several herbs have been used in the treatment of various diseases with reports on their possible interactions. Garlic, *Ginkgo biloba* and the root plant, ginseng were reported to interact with warfarin enhancing its anticoagulant effects and raising the risk of bleeding problems [13]. Bitter melon and ginseng raises the risk of severe hypoglycemia by enhancing the hypoglycemic effects of insulin and metformin [14]. The root extract of *Calliandra portoricensis* was reported to interact with glibenclamide [15]. The leaf extract of *Vernonia amygdalina* has been reported to interact with nifedipine [6]. However, there are still many herb-drug co-administrations that have remained untapped.

With many people resorting to the use of herbs in the management of various diseases and at times in concomitant use with orthodox drugs has raised a concern on the risk of potentially

harmful herb-drug interactions, and a dearth of adequate research on the safety and efficacy of concomitant use of herbs with orthodox drugs is a cause for special concern and aroused interest. It is therefore worthwhile to study herb-drug interaction with an attempt to give scientific information on their possible side effects; pharmacokinetic fate of the orthodox drug, hence increasing patients' awareness on possible risks.

The administration of a polyherbal formulation which contains, *Markhamia tomentosa*, *Ipomea asarifolia*, *Phyllathus reticulatus* and *Adenopus breviflorus* by traditional healer in the treatment of diabetes in patient who have hypertension and are on orthodox drugs with no information on its possible interactions has become a cause for health and safety concern. It is thought that concomitant use of herbal preparation with synthetic antihypertensive drug, amlodipine may result in untold consequences. Therefore, it is worthwhile to investigate the possible effect of the polyherbal formulation on the pharmacokinetics of amlodipine following their concomitant administration. This study will provide scientific information on the concomitant administration of these agents and will lead to cautious use or deterrence in concomitant use of these herbs alongside prescription medicine.

Materials and Methods

Plant collection, identification and preparation

The leaves of *Adenopus breviflorus*, *Ipomoea asarifolia*, *Markhamia tomentosa* and the stem bark of *Phyllanthus reticulatus* were collected from the Forestry Research Institute of Nigeria Ibadan in May, 2019. The plants were authenticated by Dr. Nodza George in the herbarium unit of the Department of Botany, University of Lagos where voucher specimens LUH 8737, LUH 8436, LUH 8909 and LUH 8479 respectively have been deposited. The plant materials were used to formulate the polyherbal mixture (2 g per sachet) as described by the traditional practitioner in Lagos, Nigeria. The leaves were dried at room temperature (25±3 ºC) in a free flowing air for 10 days while the stem bark was dried for 21 days; they were pulverized in a mechanical grinder. Each powdered plant, 1000 g and the polyherbal mixture, 2000 g containing appropriate plant ratio per sachet was exhaustively extracted in 70% methanol for 48 h using soxhlet extractor, concentrated in vacuum at 40 °C and then lyophilized in a freeze dryer to obtain 35.11% *(Adenopus breviflorus*), 31.08% (*Ipomoea asarifolia),* 29.96% (*Markhamia tomentosa*), 38.87% *(Phyllanthus reticulatus)* and 34.13% (polyherbal formulation) of the dried plants. Each dried extract was stored in an impervious amber bottle kept in a freezer at-20 ºC until used.

Chemicals and reagents

All chemicals were of analytical reagent grade from British Drug Houses, United Kingdom (BDH). Acetonitrile and methanol from sigma-Aldrich, MO, USA were HPLC grade. Atorvastatin (internal standard) was a gift from Fidson Pharmaceutical Company, Lagos. Amlodipine besylate standard powder was a gift from Bond Chemical Industries, Lagos, amlodipine besylate tablets were purchased from a pharmaceutical shop in Lagos, Nigeria.

Standard stock solutions

From the stock solution of amlodipine besylate, 500 ng/mL in methanol was prepared varying concentrations of working standard solutions (10, 25, 50, 70, 80 and 100) ng /mL used to construct the calibration curve.

Experimental animals

Albino rats of both sexes weighing between 150 and 200 g were housed in separate cages by sex in a well-ventilated standard laboratory animal care center of College of Medicine University of Lagos, under laboratory conditions of 12/12 h light/dark cycle and at a temperature of 24±2 ºC The experimental procedures and techniques used in the study were in accordance with Nation Research Council (US) Committee for the update of guide for care and use of animals in experimental studies [16]. The rats were fed with standard rodent diet and water *ad libitum* during the period of acclimatization and in the course of the experiment. Ethical approval was obtained from the Health Research and Ethics Committee of College of Medicine, University of Lagos (CMUL/HREC/07/19/976).

Phytochemical screening

The screening and identification of phytochemical constituent in the extracts were carried out using standard procedures as reported by Sharma *et al.* (2020) [17].

Determination of total phenol content

Total phenol content was estimated with Folin-Ciocalteu reagent (FCR) technique following the method of Oribayo *et al.* (2018) [18]. To 1 mL of each extract in a test tube was added 5 mL of distilled water, 0.5 mL of FCR and allowed to stand at room temperature for 5 min; then 1.5 mL of Na_2CO_3 (20%w/v) was added, and the volume was made up to 10 mL with distilled water. Each mixture was left to incubate at room temperature for 2 h and the absorbance of the developed colour was measured at 725 nm. The total phenol content of each extract was extrapolated from the gallic acid (10-100 µg/mL). Results were expressed as milligram gallic acid equivalent (GAE) per gram of dry extract.

Determination of total flavonoid content

The total flavonoid concentration was estimated using the method of Shraim *et al.* (2021) [19]. Procedurally, 1 mL of each extract and 4 mL of distilled water was placed in a 10 mL flask; 0.3 mL of 10% $AlCl_3$ was added, followed by 0.3 mL of 5% $NaNO_2$ after 5 min. Each mixture was incubated at room temperature for 6 min then 1 mL of 1 M NaOH was added to each reaction mixture and the volume made up to 10 mL mark with distilled water. The absorbance of each mixture was measured against the blank at 510 nm. Quercetin (100-1000 µg/mL) was used as standard. Results were expressed as milligram quercetin equivalent (QE) per gram of dry weight of extract.

Evaluation of acute toxicity following single dose administration of the extracts

The acute toxicity was evaluated in accordance with Food and Drug Administration (FDA) limit test for acute toxicity [20]. Eighty albino rats closely matched for weight were allowed feed and water *at libitum*; they were equally shared into 16 groups and fasted for 12 h prior to the study. Group I acted as the control and received equivalent volume of *acacia* gum solution which was used as vehicle for the extracts. For each extract, the rats were shared into 3 groups of 5 rats per group. Different doses of each extract, 2000, 4000 and 5000 mg/kg b.wt., was administered by oral gavage to the rats. The rats were observed for behavioural changes at 1 h then intermittently for 4 h, thereafter for over a period of 24 h [21]. Mortality was observed and 50% lethality (LD_{50}) was evaluated according to the method of Litchfield and Wilcoxon

(1949) [22]. The rats were further observed for two weeks to check for delayed toxicity.

Human volunteers

Twenty healthy adult volunteers (12 males, 8 females) aged, 24-45 y; weight, 54-78 kg; height, 1.50-1.76 m; body mass index, 19-24.7 kg/m participated in the study after careful explanation of the objectives and procedures. The health of the volunteer subjects was assessed by Dr. K. Otokiti on the basis of physical clinical examination including screening for Human Immunodeficiency Virus (HIV), hepatitis B and C viruses. Volunteer subjects were prevented from taking any beverage containing flavonoid and its derivatives throughout the study period.

Exclusion criteria for the study

Exclusion criteria included pregnant and nursing mothers, volunteers with blood pressure lower than 120/80 mmHg; allergic to amlodipine and with history of alcoholism, reactive for hepatitis B. Volunteers who had donated blood in 6 months and taken any medications in 4 months prior to the study that is capable of interfering with the pharmacokinetic of amlodipine. Obese or underweight individuals as predicted from their body mass index (BMI).

Ethical approval

All laboratory procedures were conducted according to standard operating procedures. The study was approved by the Health Research and Ethics Committee of the College of Medicine, University of Lagos, Nigeria (CMUL/HREC/05/23/1195). All volunteers signed a written informed consent form that was approved by the Health Research and Ethics Committee of the College of Medicine, University of Lagos, Nigeria and the study was conformed to the principle of the Declaration of Helsinki.

Administration of drugs (Amlodipine and extracts) and blood sample collection

The study followed a crossover design. In the first phase, each volunteer, ingested amlodipine besylate tablet 10 mg with 250 mL of water after 10 h overnight fast. Five milliliter of whole blood was withdrawn from the cubital fossa vein before (0) and at ¼, ½, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 h after dosing into lithium heparin vacutainers using vacutainer needles and immediately centrifuged at 5000 rpm for 10 min. The plasma samples were carefully separated using Pasteur pipette into plain bottles and stored frozen at-80 ºC until assayed. In the second phase, after a three-week washout period, volunteers were shared into two groups of 10 participants per group. One group received 200 mg of *Markhamia tomentosa*; the second group received polyherbal mixture containing 200 mg *M. tomentosa*. Each group concomitantly received 10 mg of amlodipine besylate tablet with 250 mL of water. Blood samples, 5 mL were withdrawn and treated as in the first phase and stored frozen at-80 ºC until assayed. In all the procedures, volunteers were not allowed food till 4 h after dosing.

Chromatographic system and conditions

The high performance liquid chromatographic system, (Agilent 1260 series, Hewlett-Packard, Germany) consisted of an auto sampler (Serial No. DEAAC15585, Product No. G1329B) having Rheodyne 7725i (20 µL capacity loop) injector and a quat pump (Serial No. DEAB706149, Product No. G1311B), to maintain a constant flow of mobile phase through the column. The analytical column was Agilent Eclipse XDB C18 (ODS) column (stainless steel column, 150 mm x 4.6 mm i.d., packed with 5 µm particle size) protected by a guard column, 30 mm x 4.6 mm i.d., placed between the injector and the analytical column; the degasser (Serial No. DEACN16928, Product No. G1316A) was used to remove gasses from the mobile phase. The detector was a UV-Visible spectrophotometer (Serial No. DEABB05196, Product No. G1314F) set at a wavelength of 240 nm. The chromatographic peaks were integrated and recorded using chemstation software (Agilent Chemstation, Germany).

Calibration curve of amlodipine in human plasma

All assays were performed under subdued light. Drug-free plasma in cryo-bottles was spiked with standard solution of amlodipine to yield concentrations 10 to 100 ng/mL. To each of the spiked plasma, was added 100 µL of atorvastatin (I.S) and deproteinized with acetonitrile. The peak area ratio of amlodipine to I.S. was plotted against their corresponding concentration; the calibration equation obtained from the plot was used to extrapolate for the concentration of amlodipine in the plasma sample.

Chromatographic assay of amlodipine

The plasma concentration of amlodipine was evaluated by a validated HPLC method previously developed in our laboratory (Owolabi *et al.* 2023) [23]. To each volunteer blood sample with or without the polyherbal extract was added 100 µL of I.S. followed by 1 mL of acetonitrile to deproteinize the protein and vortex mixed for 3 min. The mixture was centrifuged at 5,000 rpm for 10 min; the clear supernatant was filtered through a 0.22 µm syringe filter and 20 µL was injected into the HPLC for chromatographic separation. The mobile phase composition was acetonitrile: phosphate buffer (25 mM KH_2PO_4) adjusted to pH 3.1 with orthophosphoric acid in the ratio of 45:55 v/v pumped at a flow rate of 1.2 mL/min and monitored by ultraviolet detection wavelength set at 240 nm.

Pharmacokinetic parameters

The pharmacokinetic parameters, time to peak plasma concentration (T_{max}), peak plasma concentration (C_{max}), volume of distribution (Vd), elimination half-life ($t_{1/2el}$), absorption half-life ($t_{1/2ab}$), clearance (CL) were estimated using PKSolver software in Microsoft Excel [24]. The systemic exposure (area under the curve; AUC) was estimated by the trapezoidal rule method for observed values and this was extrapolated to infinity ($AUC_{0\rightarrow\infty}$).

Statistical analysis

Data were expressed as mean±SEM (standard error of mean). The statistical difference was assessed using one-way ANOVA followed by Dunnett's multiple comparison T-test. Values were considered to differ significantly if $P < 0.05$.

Results

Phytochemical investigation of the constituent plant materials

Phytochemical investigation of the aqueous methanol extract of the constituent plant in the polyherbal preparation (Table 1) revealed the presence of alkaloids, tannins, flavonoids, saponins, reducing sugars, steroids and protein and the absence of anthraquinone.

Table 1: Phytochemical investigation of the 70% methanol extract of the plant constituent

	Phytochemical test							
Plants material	**Alkaloids**	**Tannins**	**Flavonoids**	**Saponins**	**Reducing Sugars**	**Anthraquinones**	**Proteins**	**Steroids**
A breviflorus	+	+	+	+	+	-	+	+
I. asarifolia	+	+	+	+	+	-	+	+
P. reticulatus	+	+	+	+	+	-	+	+
M. tomentosa	+	+	+	+	+	-	+	+

(+) indicates presence; (-) indicates absence

Total flavonoids and phenol contents in the polyherbal extract and its constituent plant materials

The estimation of the total flavonoids and phenols content in the plant materials are presented in Table 2. The plant extracts showed rich polyphenolic contents (flavonoids and phenol). The polyherbal extract showed significantly higher content of flavonoid and phenol ($p<0.001$) compared to other extracts. The extract of *M. tomentosa* contained higher flavonoid and phenol followed by *A. breviflorus, P. reticulatus* and *I. asarifolia* ($p<0.01$). The extract of *I. asarifolia* had more phenolic content than *A. breviflorus* and *P. reticulatus.*

Table 2: Total flavonoid and total phenol content of the plant extracts

Plant materials	Total Flavonoid mg QE/g DPM	Total Phenolic mg GAE/g DPM
A. breviflorus	136.89±13.35**	110.99±12.47*
I. asarifolia	106.45±12.53*	241.56±42.16**
P. reticulatus	113.39±53.12**	131.66±10.49**
M. tomentosa	192.41±16.32***	256.63±1.10***
Polyherbal preparation	240.65±16.40***	426.2±11.53***

Values are expressed as mean±SD. Mean values followed by superscripts are significantly different (*$p<0.05$, **$p<0.01$, ***$p<0.001$), n=5. QE = quercetin equivalent, GAE = gallic acid equivalent, DPM is dry plant material.

Acute Toxicity Study

Animals remained stable for the dose studied up to 5 g/kg b. wt. The animals did not show any undesirable effect after 14-day observation. The animals that show transient irritation recovered within 4 h.

Anthropometric and biochemical parameters: Table 3 presents the anthropometric and clinical data of the volunteer subjects. All the volunteer subjects successfully completed the study. The anthropometric parameters were well matched. They had within limit biochemical parameters. The average serum creatinine was 0.83±0.13 mg/dL which is within the normal range of 0.6-1.3 mg/dL (53.0-114.9 µmol/L). They did not have any viral infection following the screening and all oral doses were well tolerated by the subjects without any clinical adverse effects.

Table 3: The anthropometric and biochemical parameters of the volunteer subjects

Volunteer subjects	Parameters						
	Age (y)	**Height (m)**	**Weight (kg)**	**BMI (kg/m^2)**	**Hb (g/dL)**	**Serum creatinine (mg/dL)**	**Creatinine clearance (mL/min)**
Females (8)	32.00±2.15 (24-43)	1.55±0.12 (1.50-1.61)	62.81±2.15 (54-78)	23.03±0.13 (19-24)	12.43±0.07 (13.20-13.90)	0.82±0.06 (0.70-0.83)	109±2.73 (91-116)
Males (12)	36.30±2.73 (28-45)	1.61±1.02 (1.56-1.76)	66.23±2.93 (59-76)	23.64±1.04 (19.00-24.7)	14.93±0.16 (14.20-15.40)	0.85±0.21 (0.70-0.91)	114±3.41 (99-128)
Total (20)	34.80±3.43 (24-45)	1.60±0.83 (1.50-1.76)	65.81±6.1 (54-78)	23.42±0.72 (19.00-24.7)	14.21±0.69 (13.20-13.90)	0.83±0.13 (0.70-0.91)	112±4.01 (91-128)

Values are mean±SD, N are as indicated in the parenthesis for females and males

Pharmacokinetic analysis

Pharmacokinetics fate of amlodipine after concomitant administration with *Markhamia tomentosa* or polyherbal formulation

The mobile phase provided a good resolution for amlodipine and the internal standard, artovastatin. Representative chromatograms of the standard mixture of amlodipine and artovastatin (I.S.) or plasma spiked with the same concentration of amlodipine and artovastatin (I.S.) are shown in Figure 1a-b respectively. The mean plasma concentration-time curve of the oral administration of 10 mg of amlodipine besylate with or without concomitant administration of *M. tomentosa* or the polyherbal formulation to the volunteers is illustrated in Figure 2 and the mean pharmacokinetic parameters obtained from the investigation are presented in Table 4. The oral doses were well tolerated by the subjects without any clinical adverse effects. The absorption of amlodipine was most rapid in the volunteers who consumed *M. tomentosa* extract (0.27±0.15 h^{-1}) than in the volunteers who consumed polyherbal formulation (0.18±0.02 h^{-1}) as well as the control group (amlodipine alone, 0.22±0.06 h^{-1}). There was significant difference in the T_{max} between the control group (10.08±2.21 h) compared to the volunteers who concomitantly consumed *M. tormentosa* (15.00±5.85 h; $p\geq0.001$) and the polyherbal formulation (11.00±3.11 h; $p\geq0.05$). There was a 48.81% increase in T_{max} of amlodipine in volunteers who received concomitant administration of *M. tormentosa* extract. However, concomitant administration of polyherbal extract gave a slightly increased T_{max} by 9.13%. There was no significant difference in the peak plasma concentration (C_{max}) between volunteers in the control group (10.57±2.03 ng/mL) and the volunteers who concomitantly consumed polyherbal extracts (10.03±.64 ng/mL). However, C_{max} of amlodipine in volunteers who concomitantly consumed *M. tomentosa* extract was reduced by 42.01%. There was significant difference ($p\geq0.001$) in the elimination

half-life ($t_{½el}$) between the control (21.58±3.02 h) and the volunteers who concomitantly consumed *M. tormentosa* (86.00±18.16 h). No significant difference was seen between control and polyherbal formulation groups. The significant ($p \leq 0.001$) increase in $AUC_{0-\infty}$ in the *M. tomentosa* extract group (575.21±57.53 ng/mL/h) compared to the control group (455.20±12.71 ng/mL/h) was also observed in the polyherbal formulation group (417.84±13.49 ng/mL/h). The volume of distribution (Vd) was higher in the *M. tomentosa* extract group compared to the control and polyherbal groups. Total clearance (CL/F) was not significantly different.

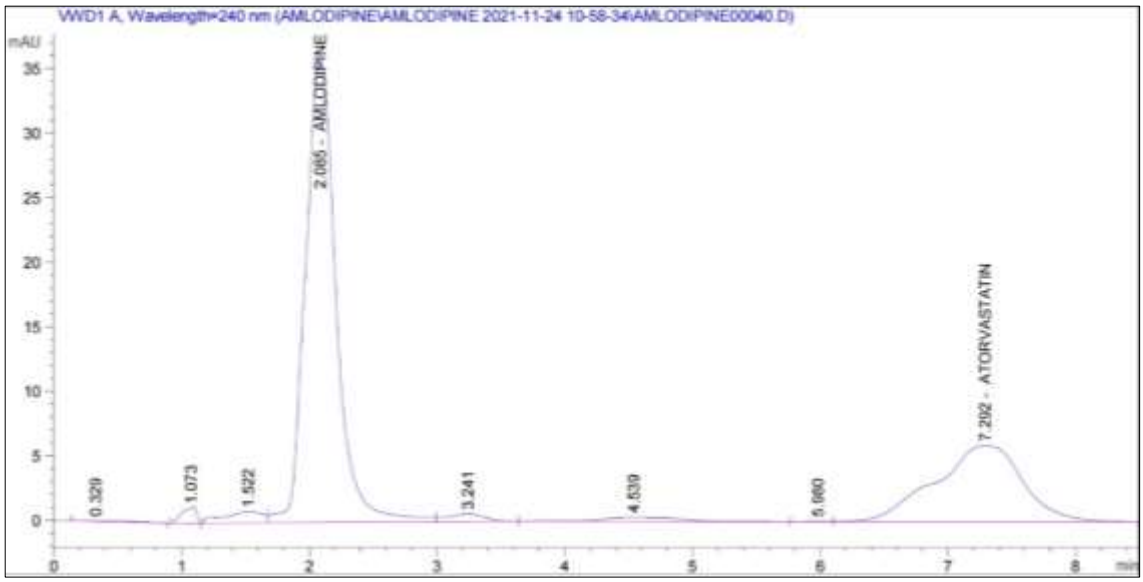

Fig 1a: Representative chromatogram of amlodipine and atorvastatin in solvent.

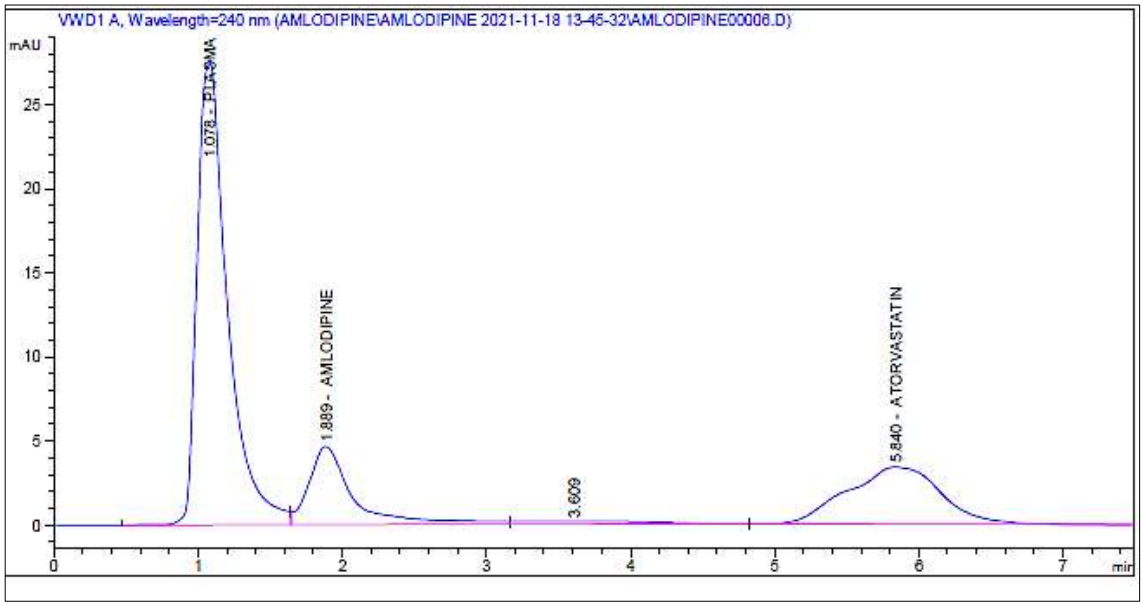

Fig 1b: Representative chromatogram of plasma spiked with amlodipine and atorvastatin.

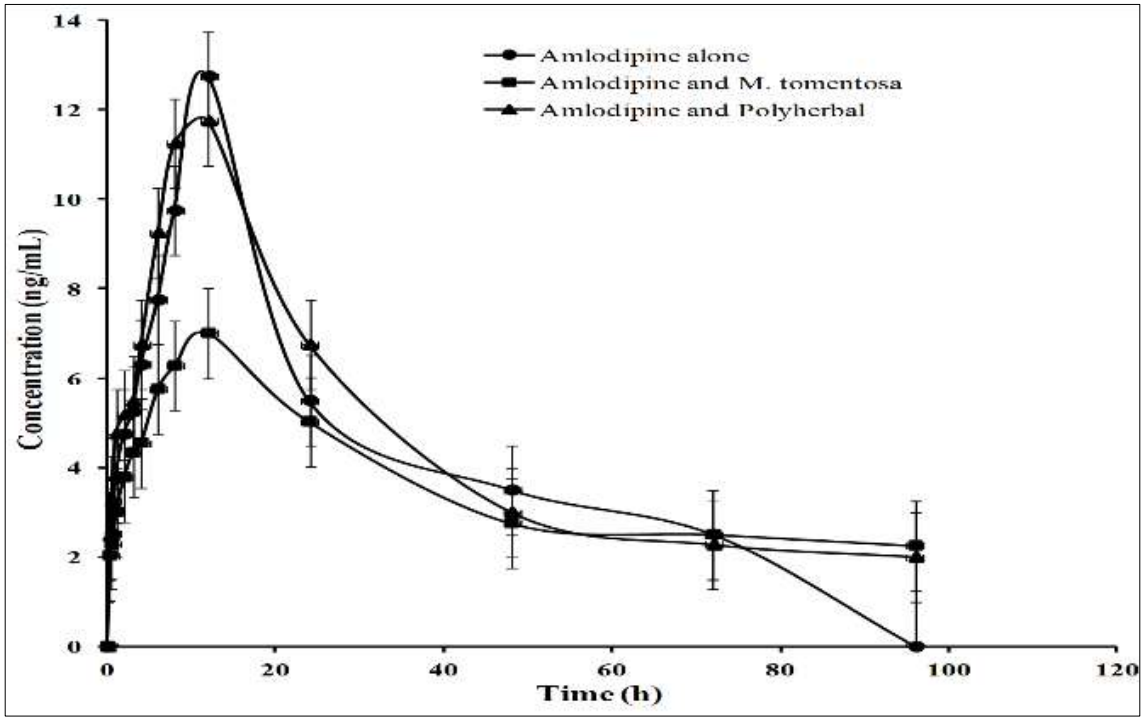

Fig 2: Mean plasma concentration-time curve of amlodipine and in the presence of *M. tormentosa* and polyherbal formulation

Table 4: Pharmacokinetic parameters of amlodipine besylate after concomitant administration of *M. tomentosa* and Polyherbal formulation

Pharmacokinetic Parameters	Amlodipine alone	Amlodipine with *M. tomentosa*	Amlodipine with polyherbal formulation
$t_{1/2ka}$ (h)	3.15±0.67	2.75±2.98***	3.85±0.93*
$t_{1/2kel}$ (h)	21.58±3.02	86.00±18.16***	23.92±1.41
Vd (L)	659.97±16.65	2100.96±393.86***	610.50±10.21
CL/F (mLh^{-1})	21.93±6.54	18.61±1.57***	22.88±4.08
T_{max} (h)	10.08±2.21	15.00±5.85***	11.98±3.11
C_{max} ($ngmL^{-1}$)	10.57±2.03	4.40±1.19***	10.03±1.64
AUC $_{0-96}$ ($ngmL^{-1}h^{-1}$)	430.88±9.26	306.68±64.91***	377.76±7.53*
AUC $_{0-\infty}$ ($ngmL^{-1}h^{-1}$)	455.20±12.71	575.21±81.75***	417.84±13.49

Values are expressed as mean±SEM using Pksolver 2.0 application Two-way ANOVA, Dunnett's multiple comparisons test using graphpad prism. * (p<0.05) ** (p<0.01) *** (p<0.001)

Discussion

Herbs contain various phytochemicals, which may interact with prescription drugs when taken concomitantly and could be harmful to health. These herbs could alter pharmacokinetic fate and or pharmacodynamic properties of the drugs leading to therapeutic failure or toxicities. Also, polypharmacy, defined as concurrent use of several drugs could increase risk of adverse drug interaction or benefit either drug to produce a strong synergistic effect which may have favourable impact in pharmacotherapy or produce antagonistic effect that would completely alter the activity of the drug [25, 26]. Therefore, the pharmacokinetic fates of amlodipine in combination with single herb and polyherbal formulation were investigated.

The polyherbal formulation contains the leaves of *A. breviflorus*, *I. asarifolia*, *M. tomemtosa* and stem bark of *P. reticulatus* and revealed the presence of various secondary metabolites (Table 1). Flavonoids are known antioxidants which scavenge free radicals that are generated in disease states including diabetes mellitus [27, 28]. Therefore, the high content of flavonoid and phenols in *M. tomentosa* and the polyherbal formulation (Table 2) may be responsible for the blood glucose lowering activity as observed by the traditional practitioners.

Plant materials contain compounds that could show beneficial or detrimental effects to man, thus toxicity study is important to ascertain its safety and efficacy for human use. The evaluations of the acute toxicity of the polyherbal formulation as well as its constituent plants in rats following the oral administration of the extracts did not record any deaths within 24 h of their administration (Table 3). The rats showed no changes in their physical appearance, respiratory rate and central nervous system effect even at the highest dose of 5 g/kg. However, rats that showed transient irritation following the administration of 5 g/kg extracts of *A. breviflorus* and *I. asarifolia* recovered within 4 h and were similar to the study of Mukhtar *et al.* (2022) [29] who reported that the extract has low acute toxicity profile when administered orally**.** The result obtained from the administration of *Markhamia tomemtosa* concurs with the findings of Sowemimo *et al.* (2013) [30] as reported by Ibrahim *et al.* (2016) [31]. There was neither side effect nor untoward reaction seen with a dose of up to 5 g/kg of *M. tomentosa* extract and the polyherbal formulation which may be attributed to possible synergistic effect of the extracts that attenuated the transient irritation. Following the Hodge and Sterner toxicity rating scale [32] the extracts could be classified as relatively safe to humans by the oral route.

Anthropometric measurements are non-invasive quantitative measurements used to evaluate the general health status of an individual. We employed the anthropometric measurement in this study to assist the inclusion or exclusion criteria in the pharmacokinetic study. All the twenty volunteers who enrolled in the study were healthy as revealed by their biochemical parameters. The volunteers were not anaemic and

their kidney function was optimum evident from the haemoglobin and the serum creatinine results respectively (Table 3).

The co-administration of amlodipine with *M. tomentosa* compared to the control group showed significant interaction evident by increased k_a, $T_{max,}$ Vd and $AUC_{0-\infty}$ with simultaneous decrease in C_{max}, k_{el} and AUC_{0-96}. These findings indicate rapid absorption, delayed elimination, prolonged exposure and potential tissue accumulation. It could have been thought that *Markahmia tomentosa* extract increasing the absorption rate of amlodipine would lead to faster onset of drug action; however the long T_{max} which controls the onset of drug action would have hindered this, probably through induced drug metabolism leading to lower blood level. Increased systemic exposure, volume of distribution, and mean residence time may result in drug accumulation in certain tissues potentiating the risk of side effect or toxicity. The decrease in k_{el} further explains possible increased exposure and confirms high plasma concentration (C_{max}) since the rate of drug elimination is directly proportional to plasma concentration of drug [33]. The result of polyherbal extract compared to the control group showed little or no statistical difference, this may be due to the presence of the constituents in the polyherbal formulation. However, the rate of absorption was slower, evident by decreased k_a and $t_{1/2ka}$ and $AUC_{0-96,}$ indicating delayed onset of drug action and potentially reduced efficacy. These finding suggest that polyherbal formulation decreasing the absorption rate of amlodipine may make blood pressure lowering effect exhibit a longer onset of action time thus leading to medication failure. The polyherbal extract contains-*Adenopus breviflorus*, *Ipoemea asarifolia*, *M. tomemtosa* and *Phyllanthus reticulatus*, this may suggest that some constituents in the polyherbal formulation may have hindered the rate of absorption compared to the *M. tomentosa* extract group. The polyherbal formulation group when compared to *M. tomentosa* group showed a statistically significant difference for AUC_{0-96} and $AUC_{0-\infty}$ ($P<0.001$).

Amlodipine is extensively metabolized in the liver via the cytochrome P_{450} system and various studies have shown that CYP3A4 is the major isoenzyme involved in the metabolism of this drug [34, 35]. Flavonoids such as quercetin, kaempferols, rutin, apigenin have been reported to alter the pharmacokinetics profiles of concurrently administered drugs in some animal models [36]. They inhibit the metabolism of these drugs by interfering with the activity of cytochrome P_{450} 3A4 metabolizing enzyme in the human liver [37]. It is also reported that flavonoids found in fruits inhibit cytochrome P_{450} 3A4 metabolizing enzymes responsible for conjugation of calcium antagonists [38]. Similarly, Tabassum *et al.* (2022) [39] reported the effect of *G. bibloba* extract on the pharmacokinetic profile of nifedipine and glibenclamide, attributing the effect to the presence of many kinds of flavonoids in the plant including quercetin, a potent CYP3A4 inhibitor. Thus the result of this study may well be attributed to the action of abundant flavonoids present in the study plants as revealed by phytochemical screening which might interact with the cytochrome P_{450} isoenzymes thus interfering with the metabolism of amlodipine, a calcium antagonist.

Conclusions

The plant extracts are rich in secondary metabolites with notable amount of flavonoids and phenols in *M. tomentosa* and the polyherbal formulation. The pharmacokinetic interaction between the extracts and amlodipine underscored caution. Advocacy for standardized pharmacovigilance echoes for monitoring possible adverse reactions. This interaction can be avoided by taking the amlodipine at least 3 h prior to the administration of the extracts.

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Conflict of Interest

No conflicts of interest are associated with the authors whatsoever.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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