Ruzu herbal bitters altered the pharmacokinetic profile of metformin tablets in healthy Nigerian volunteers following concurrent administration

Moshood O. Akinleye[1,3], Nkiruka Okonkwo[1], Ogochukwu U. Amaeze[2], Omonike O. Shonekan[1,3], Afeez I. Kareem[1], Emmanuel Eze[1], Grace E. Ukpo[1,3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy.
[2]Department of Clinical Pharmacy and Bio-pharmacy, Faculty of Pharmacy.
College of Medicine Campus, University of Lagos, Idi-araba, Lagos, Nigeria.
[3]African Center of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science, University of Lagos, Akoka, Lagos, Nigeria.

Corresponding author: Moshood Akinleye
E-mail: makinleye@unilag.edu.ng;
Telephone: +2348067533224

ABSTRACT

Background: Ruzu® Herbal bitters (RHB) is a commercial polyherbal formulation made from a blend of various parts and fruits of plant of *Uvaria chamae* P. *Beauv., Curculigo pilosa* Schumach. & Thonn and *Citrullus colocynthis* (L) Schrad. It is claimed to be used for the management of diabetes, cure weak erection in men, boost the immune system and effective against typhoid and malaria.

Objectives: This study was carried out to investigate the potential interactions between metformin tablets when co-administered with RHB.

Methods: A total of ten healthy volunteers were recruited and assigned into two groups. One group ingested 500 mg single dose metformin tablet orally using table water while the other group took 30 mL of the herbal formulation daily for 4 days, followed by 500 mg single oral dose of metformin tablet taken with same volume of the bitters on the fifth day. Blood samples were collected and analyzed using a validated High performance Liquid Chromatography.

Results: All the pharmacokinetic parameters evaluated were altered in the presence of RHB. The terminal half-life (t1/2, $p = 0.01$), apparent volume of distribution (Vd/F, $p = 0.008$), systemic clearance (Cl/F, $p = 0.034$) and mean residence time (MRT, $p = 0.03$) were reduced while terminal elimination rate constant (Kel, $p = 0.005$) was elevated with Ruzu herbal bitters. This influence is likely mediated by competitive inhibition or induction of the metformin transporters such as organic cation transporter-1(OCT-1), Plasma membrane monoamine transporter (PMAT) by the phytochemical constituents of RHB. The changes observed were statistically significant for the t1/2, Kel, Vd/F, Cl/F and MRT ($p < 0.05$).

Conclusion: The results obtained from the study showed that co-administration of metformin and RHB altered the pharmacokinetic parameters of Metformin.

Key words: -Herb-drug interactions, Metformin, Ruzu® Herbal Bitters, Pharmacokinetics.

Les amers à base de plantes Ruzu ont modifié le profil pharmacocinétique des comprimés de metformine chez des volontaires nigérians sains après une administration concomitante

Moshood O. Akinleye[1,3], Nkiruka Okonkwo[1], Ogochukwu U. Amaeze[2], Omonike O. Shonekan[1,3], Afeez I. Kareem[1], Emmanuel Eze[1], Grace E. Ukpo[1,3]

[1]Département de Chimie Pharmaceutique, Faculté de Pharmacie,
[2]Département de pharmacie clinique et de biopharmacie, Faculté de pharmacie,
Campus du Collège de médecine, Université de Lagos, Idi-araba, Lagos, Nigéria.
[3]Centre africain d'excellence pour la recherche sur les médicaments, le développement de la phytothérapie et la science réglementaire, Université de Lagos, Akoka, Lagos, Nigeria

Auteur correspondant: Moshood O. Akinleye
Email: makinleye@unilag.edu.ng
Téléphone: +2348067533224

RÉSUMÉ

Contexte: Ruzu® Herbal bitters (RHB) est une formulation commerciale à base de plantes faite à partir d'un mélange de diverses parties et fruits des plantes d'*Uvaria. chamae P. Beauv., Curculigo pileuse Schumach*. & Thonn et *Citrullus colocynthis* (L) Schrad. On affirme qu'il est utilisé pour gérer le diabète, guérir les faibles érections chez les hommes, renforcer le système immunitaire et être efficace contre la typhoïde et le paludisme.

Objectifs: Cette étude a été réalisée pour étudier les interactions potentielles entre les comprimés de metformine lorsqu'ils sont co-administrés avec le RHB.

Méthodes: Au total, dix volontaires sains ont été recrutés et répartis en deux groupes. Un groupe a ingéré 500 mg de comprimé de metformine en dose unique par voie orale en utilisant de l'eau de table, tandis que l'autre groupe a pris 30 ml de la formulation à base de plantes par jour pendant 4 jours, suivi d'une dose orale unique de 500 mg de comprimé de metformine prise avec le même volume d'amer le cinquième jour. Des échantillons de sang ont été prélevés et analysés à l'aide d'une chromatographie liquide à haute performance validée.

Résultats: Tous les paramètres pharmacocinétiques évalués ont été modifiés en présence de RHB. La demi-vie terminale (t1/2, p=0,01), le volume apparent de distribution (Vd/F, p = 0,008), la clairance systémique (Cl/F, p = 0,034) et le temps de séjour moyen (MRT, p = 0,03) ont été réduits, tandis que la constante de vitesse d'élimination terminale (Kel, p = 0,005) a été élevée avec Ruzu Herbal Bitters. Cette influence est probablement due à l'inhibition ou l'induction compétitive des transporteurs de la metformine tels que le transporteur de cations organiques-1 (OCT-1) et le transporteur de monoamine membranaire plasmique (PMAT) par les constituants phytochimiques du RHB. Les changements observés étaient statistiquement significatifs pour le t1/2, Kel, Vd/F, Cl/F et MRT ($p < 0,05$).

Conclusion: Les résultats de l'étude montrent que la co-administration de metformine et de RHB modifie les paramètres pharmacocinétiques de la metformine.

Mots clés: -Interactions plantes-médicaments, metformine, Ruzu® Herbal Bitters, Pharmacocinétique.

INTRODUCTION

Herbal medicinal products are increasingly used for health maintenance, prevention and treatment of diverse disease conditions globally. In Nigeria, the use of herbal medicine has been reported in the general population,[1,2] pregnant women,[3] and various patient populations.[4,5,6] Populations in developed nations have increasingly embraced herbal medicines as a complement to their standard health care in line with Sustainable Development Goal (SDG 3) which is for good health and well-being. Consumers of herbal medicine believe that herbal medicines are harmless because they are from natural sources, and so, they can be used alongside conventional therapies.[7] Concurrent herbal and conventional medicine use was estimated at 20-30% in the United States of America.[8] It is reported that up to 72.8% of people with diabetes used herbal medicine, dietary supplements and other Complementary and Alternative medicine (CAM) therapies[9] and this is common among older age within their cultural practice,[10] Age, marital status, residence, family history of Diabetes Mellitus (DM), presence of diabetic complications, and duration of DM were found to be strong predictors of CAM use among diabetic patients. Reports from Nigeria reveal that up to 46%, 65%, and 42.7% of diabetes mellitus, cancer, and HIV-infected patients respectively used herbal medicines alongside conventional drugs for the management of their disease conditions.[11,12,13]

Although, they are considered safe having passed toxicity tests, herbal remedies can interact with co-administered medications and exert beneficial effects like cancer prevention[14] or potentially toxic and harmful effects. Phytochemicals have been recognized as modulators of drug disposition.[15] They can alter the pharmacokinetics of co-administered medicines resulting in herb-drug interactions (HDIs), which can alter therapeutic outcomes.[16] Again, unlike conventional drugs which often composed of single, known active components, herbal medicines usually contain a complex mixture of chemical constituents that may or may not be pharmacologically active. This dramatically increases the potentials of interactions, and theoretically, the likelihood of HDIs is higher than drug-drug Interactions (DDIs).[17] Like DDIs, HDIs can occur either by pharmacokinetic (PK) or pharmacodynamic (PD) mechanisms; however, the most reported HDIs are of PK mechanism.[18] PK HDI results from altered time course of liberation, absorption, distribution, metabolism, or excretion of co-administered conventional drug or its metabolites. PD HDI arises from changes in the pharmacological activity of a co-administered medicine, and. mediated through additive, synergistic or antagonistic actions.

Metformin is a synthetic derivative of galegine, a natural product from the plant Galega officinalis, used in herbal medicine in medieval Europe.[19] Metformin, which is a biguanide drug, acts directly or indirectly on the liver to lower glucose production and acts on the gut to increase glucose utilization, increase glucagon-like peptide 1 and alter the microbiome. At the molecular level, metformin inhibits the mitochondrial respiratory chain in the liver, leading to activation of 5' adenosine monophosphate-activated protein kinase, enhancing insulin sensitivity (via effects on fat metabolism), and lowering cAMP, thus reducing the expression of gluconeogenic enzymes.[20] Metformin is the first-line drug for treating type 2 diabetes mellitus and the most prescribed glucose-lowering medicine globally, making it a potential candidate for herb interaction when co-administered with herbal remedies that are popularly acclaimed to cure diabetes. The clinical pharmacokinetics of metformin showed a low lipid solubility making rapid passive diffusion through cell membranes unlikely,[21] absorption largely taken place in small intestines with oral bioavailability of 50 - 60% and organic cation transporter 1; multidrug and toxin extrusion protein (MATE 1,2); Plasma membrane monoamine transporter (PMAT); Serotonin transporter (SERT) and High affinity choline transporter are the main transporters of metformin.[22] The transporters is inhibited by drugs such as cocaine, atropine, cimetidine, St John's wort etc.[23,24] The apparent volume of distribution after intravenous administration ranges from 63 - 276 L. Metformin is not metabolized but cleared from body by tubular secretion and excreted unchanged in urine.[21]

Ruzu® herbal bitters (RHB) is one of the most consumed polyherbal formulations in Nigeria and is listed by National Agency for Food and Drug Administration and Control (NAFDAC) with identification Number A7-1102L. NAFDAC is a Nigerian Federal agency under the Federal Ministry of Health that is responsible for regulating and controlling the manufacture, importation, exportation, advertisement, distribution, sale and use of food, drugs, cosmetics, medical devices, chemicals and package water. RHB is a greenish-brown aqueous solution with a characteristic bitter taste and ginger-like odour. It

comprises three major constituents namely Uvaria chamae P. Beauv. (Bush Banana), Curculigo pilosa Schumach. & Thonn. (Squirrel Groundnut) and Citrullus colocynthis (L) Schrad (Bitter Apple) in the ratio of 20:40:20. The plant name has been checked with "World Flora Online". Scientific and evidence-based studies have reported the antidiabetic effect,[25,26,27,28] antilipidemic, hepato-protective,[26] antioxidant, anti-inflammatory,[29,30] and biochemical effect[31] of RHB in experimental animals.

Several clinically significant herb-drug interactions have been recognized in the last decade,[16] prompting a surge of research to their cause and mechanism. This study thus aimed to investigate the effects of co-administration of Ruzu herbal bitters on the pharmacokinetic profile of metformin in healthy human volunteers, when taken concurrently, and propose the potential for drug interaction between the two medicines. Such studies have the potential to inform the safety of such herbal and conventional drugs co-use.

MATERIALS AND METHODS

Materials

Metformin 500 mg (Glucophage®), Ranitidine, Ruzu® Bitters (NAFDAC Reg. No: A7-1102L), a poly-herbal mixture product of Natural Health Products & Services Limited, Nigeria. Glucophage brand of metformin tablets 500 mg was purchased from a registered pharmacy in Lagos, Nigeria. Gallic acid, Rutin, Quercetin, Luteolin, formononetin and Biochanin A bioactive standards (Sigma-Aldrich) were obtained from African Center for Excellence for Drug Research, Herbal Medicine Development and Regulatory Science, University of Lagos, Nigeria. These standards were used for HPLC profiling of Ruzu Herbal Bitters. Ranitidine (Sigma-Aldrich) was used as internal standard in quantification of metformin in plasma samples. All reagents used for analysis were of analytical and HPLC grades. Instrument and equipment such as centrifuge (JP Selecta®), vortex mixer (JP Selecta®), Agilent® HPLC 1100 series, pH meters (HANNA Instrument™) and ultrasonic bath (JP Selecta™) were from Faculty of Pharmacy, Central Research Laboratory, College of Medicine campus of University of Lagos, Nigeria.

METHODS

Study participants and setting

Ten healthy Nigerian male volunteers (age,43 ± 13 years; weight, 68 ± 9 kg; height, 1.7 ± 0.07 m) were recruited but only six completed the study. All study participants were adjudged healthy as indicated by their medical history, physical examination and clinical laboratory testing. The laboratory tests conducted were haematological, biochemical and HIV screening. All participants were non-smokers and were not on any prescription medicine during the research period. They were instructed to abstain from any herbal dietary supplements, coffee, herbal bitters, grape juice, and alcohol two weeks prior to and throughout the study period. Participants were excluded if they had any history of significant gastrointestinal condition that could potentially impair the absorption or disposition of the studied drug; previous history of allergy to metformin or any herbal remedies; need for any chronic medication; use of the investigating agent within 30 days of study entry, and the use of any medication within two weeks before commencement of the study. Participants were asked to abstain from taking any medication throughout the study.

Ethical approval

The study protocol was explained to the participants after which they signed a consent form expressing their understanding, agreement, and voluntary participation in the study. The study protocol conformed to the ethical guidelines of the 1975 declaration of Helsinki and was approved by the Health Research Ethics Committee (HREC) of the College of Medicine, the University of Lagos, Nigeria with reference number CMUL/HREC/06/18/355.

Study design and treatments

The study design was an open-label, two sequence, single-dose, randomized, cross-over pharmacokinetic study. The volunteers who had fasted for 12 hours prior to metformin ingestion were assigned into two treatment groups A and B. Each member of group A was administered 500 mg metformin tablet orally using 250 mL table water at 8.00 am on the day of blood collection while the Group B who had earlier been taken 30 mL of RHB daily for 4 days of Ruzu bitter supplementation, was administered 500 mg metformin with 30 mL of Ruzu bitters on the 5th day. Venous blood samples (5 mL) were collected through the indwelling catheter connected to forearm vein of each volunteer during the following time periods: 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 hours into heparinized bottles. A wash-out period of fourteen days was observed after which the study participants interchanged the treatment sequence.

Assessment of adverse events

All the study participants were monitored for adverse effects throughout the study period by the physician member of the team. Documentation of self-report by the volunteers were done during and after drug administration.

Plasma sample preparation

The blood samples were immediately centrifuged at 4000 rpm for 5 minutes at room temperature of 25^{o}C to separate the plasma from the blood cells. The plasma was collected using a sterile Pasteur pipette and transferred into a sterile plain sample bottle, labeled appropriately and stored at -20^{o}C until the day of analysis. To prepare metformin standard curve, a stock Metformin reference standard solution (100 µg/mL) was prepared in methanol and diluted 1/10th fold with the same solvent to obtain a working concentration of 10 µg/mL. From the working concentration, a gradient calibration concentration of 250 -1500 ng/mL in plasma was prepared by measuring 15 - 90 µL respectively into plain sample bottles and made up to 600 µL with plasma.

Determination metformin concentrations

Metformin concentration was determined by High Performance Liquid Chromatography (HPLC) as previously described.[32] To extract metformin from the plasma samples (calibration standard, quality control and participants), 600 µL of trichloroacetic acid was added to 600 µL of plasma samples in eppendorf tube, and 30 µL of the internal standard (ranitidine 100 µg/mL in methanol) was added. The tubes were vortexed for two minutes, centrifuged at 2000 rpm for 10 min, and all the clear supernatant was transferred into HPLC sample bottle containing 36 µL of 4N NaOH. The resulting solution was vortex-mixed and 20 µL was injected into the HPLC to quantify the metformin concentration in the samples. The mean peak area ratio obtained from metformin and internal standard were plotted against concentration of metformin prepared to obtain line of regression. The metformin plasma concentrations for the participants were extrapolated from the calibration equation.

HPLC Chromatographic Conditions

For plasma sample

Chromatographic separation was carried out using a Nucleosil® C18 125 x 4.0 mm, 5 µm column and Agilent™ 1200 series HPLC with Ultra violet variable detector set at 232 nm coupled with an on-line degasser. The mobile phase consisted of Acetonitrile: 10 mM KH_2PO_4/Sodium dodecyl sulphate (40:60 v/v) adjusted to pH 7.0 with an isocratic flow rate of 0.5 mLmin^{-1}. The mobile phase was filtered through a 0.45µm membrane filter, then de-aerated ultrasonically prior to use, and the samples were run at ambient temperature of 25^{o}C. The sample injection volume was 20 µL. The analysis was done using Agilent® Chem-Station software. The HPLC method was validated for linearity, precision, recovery rate, limit of detection (LOD) and quantification (LOQ) in line with International Conference on Harmonization (ICH)-Harmonized Tripartite Guidelines FDA, USA.

For RHB extract

Chromatographic analysis was done using Agilent Technologies® HPLC 1200 series, Binary pump, micro-vacuum Degasser, Standard and Preparative Autosampler, Thermo-stated Column Compartment, Diode Array and multiple Detector with Chem-Station software. The Column used was Agilent® Eclipse XDB-C18, 4.6 x 150 mm, 5 µm diameter particle size. The mobile phase was Methanol: Acetonitrile: H_2O) [40:15:45 in 1% glacial acetic acid]. The mobile phase was filtered through a 0.45 µm membrane filter, then de-aerated ultrasonically prior to use. The flow rate, injection volume and wavelength were 0.8 mL/minute, 10 µL and 257 nm respectively. RHB formulation was freeze dried and 1 g was weighed into 50 mL volumetric flask and dissolved in methanol. The sample was sonicated, filtered through 0.45 µm syringe filter and kept in the refrigerator at 2^{o}C prior to analysis. Stock solutions of gallic acid, rutin, quercetin, luteolin, formononetin and Biochanin A bioactive standards were also prepared in methanol. Gallic acid (25 µg/mL), Rutin (100 µg/mL), Quercetin (100 µg/mL), Luteolin (100 µg/mL), Biochanin A (80 µg/mL) and Formononetin (20 µg/mL) were injected separately and in combination into the HPLC machine to obtain chromatograms for the standards. RHB methanol extract was injected into the HPLC to obtain the respective chromatographic fingerprints. Poly-phenolic compounds identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards by one-point assay. This is a modified HPLC method of Y. Zu *et al*.[33]

Pharmacokinetic analysis

The plasma concentration-time profiles were analyzed

using a non-compartmental model analysis extracted from WinNonlin® Professional Edition version 2.1 (Pharsight Corporation). The pharmacokinetic parameters obtained were terminal elimination half-life ($t_{1/2}$ (h), Elimination rate constant K_{el} (h^{-1}), maximum plasma concentration, Cmax (µg/mL), time to maximum plasma concentration, Tmax (h), apparent volume of distribution, Vd/F (L/Kg), systemic clearance, Cl/F (L/h), Mean residence time, MRT (h), absorption rate constant, K_{abs} (h^{-1}), half-life of absorption, $t_{1/2abs}$ (h), and area under the plasma time curve to infinity, $[AUC]_{0-8}$ (µg.h/mL).

Statistical analysis

The results were expressed as mean and standard deviation (SD). The data of the control (metformin alone) and test (metformin ingested with RHB) were statistically compared using student's t-test with excel Microsoft package. The data was considered significant if p value is ≤0.05.

RESULTS

HPLC chemical profiling of RHB

HPLC analysis revealed the presence of polyphenolic compounds such as gallic acid, Rutin, Quercetin, Luteolin and formononetin in the methanol extract of RHB as shown in figures 1a & b. This HPLC fingerprint serves as a means of identifying, authenticating, quantification and quality control of RHB. The concentration of gallic acid, rutin, quercetin, luteolin and formononetin found in the methanol extract of RHB were 0.41, 2.39, 4.42, 0.29 and 0.0079 mg/mL respectively.

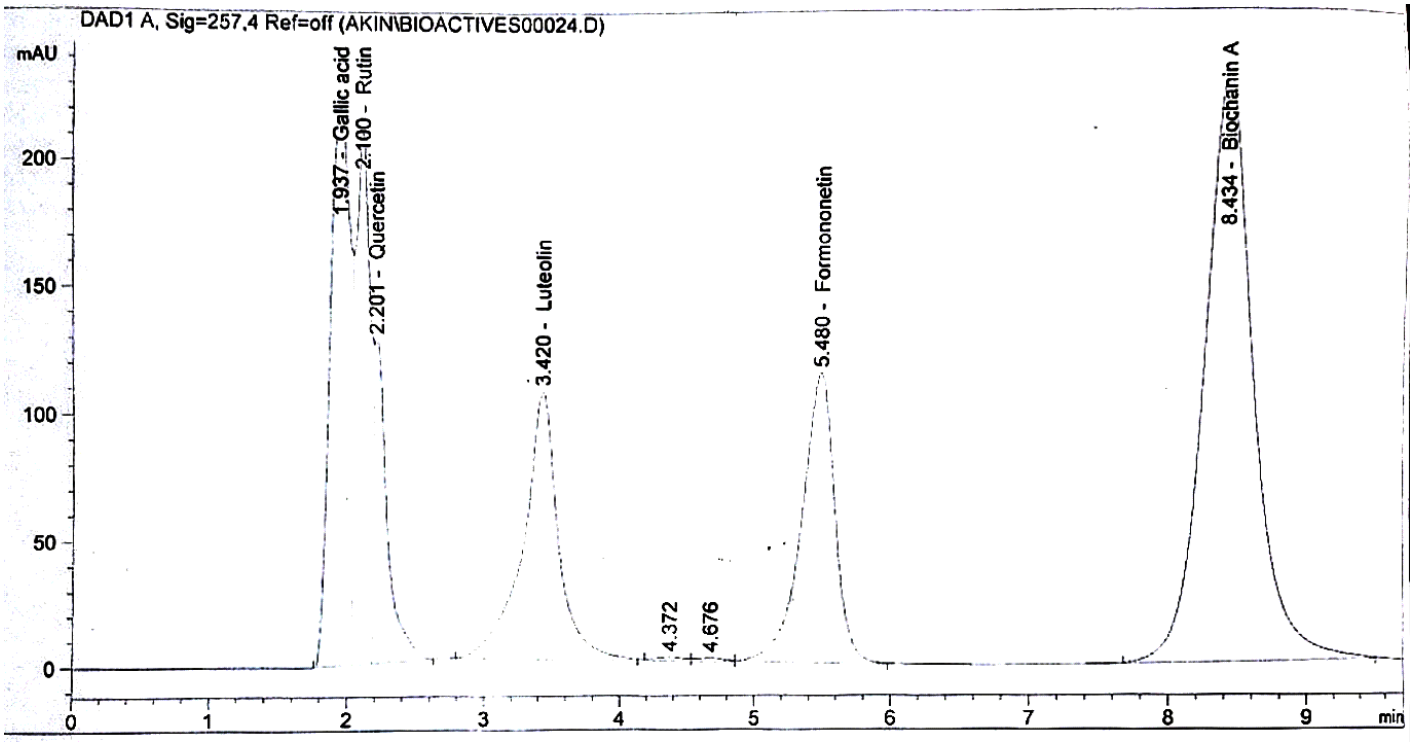

Figure 1a: High performance liquid chromatography chromatogram of 5 pure standard phytoconstituents (Gallic acid, Rutin, Quercetin, Luteolin, Formononetin and Biochanin A).

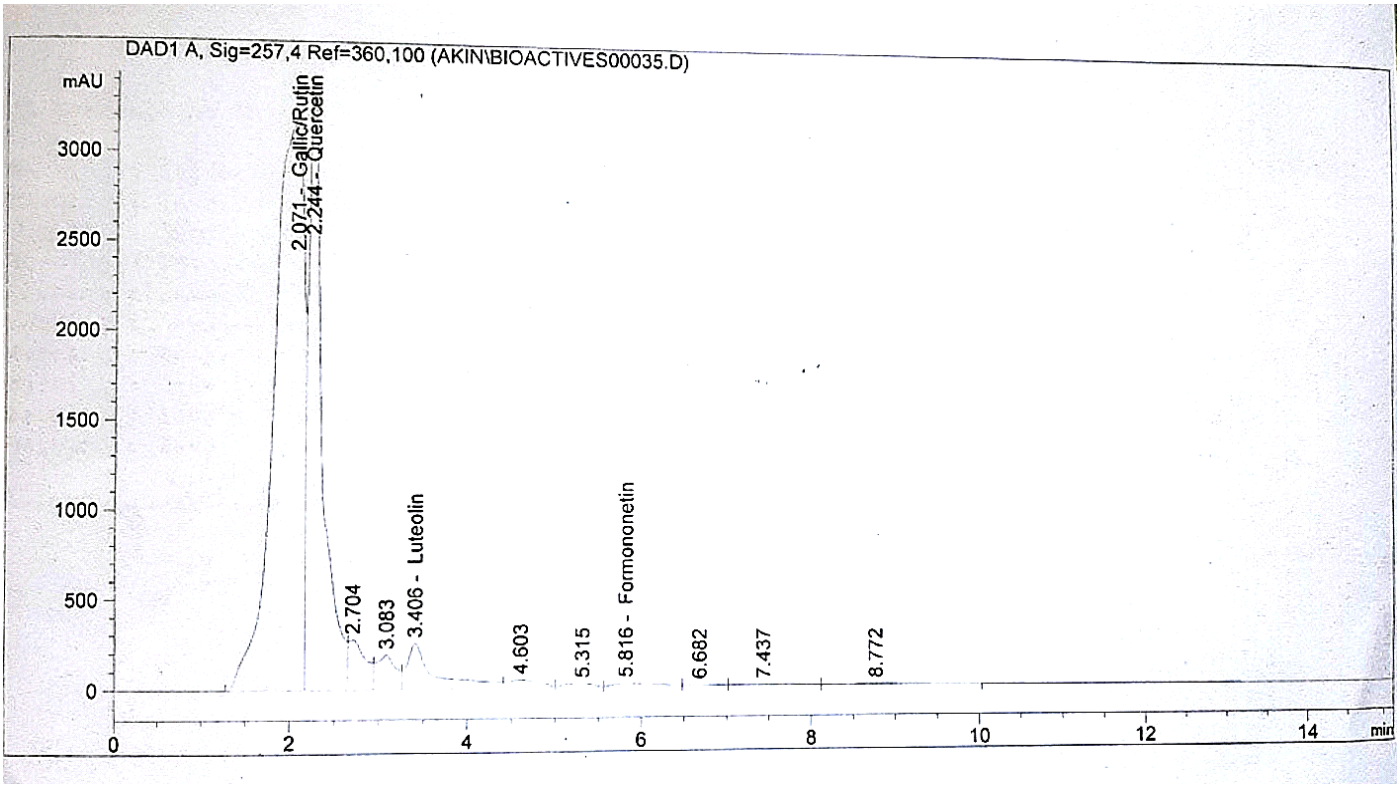

Figure 1b: HPLC chromatogram of methanol extract of RHB indicating the presence of gallic acid, rutin, quercetin, luteolin and formononetin

Demographic data of the volunteers

The base-line demographic characteristics are as shown in Table 1. Generally, the average body mass index of the participants recruited was within normal body weight of healthy individuals. No serious adverse effect was observed or self-reported by the participants.

Table 1: Baseline characteristics of volunteers

Characteristics	Value
Age (years), mean ±SD	43.5 ± 13.2
Weight (Kg), mean ±SD	68.1 ± 9.1
Height (m), mean ± SD	1.7 ± 0.1
Body Mass Index (kg/m^2), mean ± SD	23.6 ± 1.5

SD = Standard deviation

Method of validation for plasma assay

The method was specific, with baseline resolution of ranitidine (internal standard) and metformin without lack of interfering peaks from endogenous components in plasma. The method for the procedure validation was found to be selective, precise and accurate as the calibration curve gave linearity with coefficient of determination (r^2) of 0.9799. The limit of detection (LOD) and limit of quantification (LOQ) were 45 and 150 ng/mL respectively. The intra- and inter-day precision was evaluated based on the coefficient of variation (% CV) and found to be within 7-15%. The average recoveries obtained for concentrations 250, 750 and 1500 ng/mL quality control samples were within 78-90 %.

Effects of Ruzu bitters on the PK of metformin tablets

The terminal half-life and mean residence time of metformin were significantly reduced (p= 0.01, 0.03) by Ruzu bitters from 9.26 to 5.44 h and 13.80 to 9.05 h respectively while the elimination rate constant was increased from 0.10 to 0.14 h^{-1} at a high significant level (p=0.005). All other pharmacokinetic parameters such as Tmax, V.d/F., Cl/F, Kab, $t_{i/2ab}$ and AUCs assessed though altered in the presence of Ruzu bitter but were found to be statistically insignificant. Figure 2 shows the comparative plasma concentration time plot of metformin in the presence and absence of Ruzu bitter. The rate of metformin absorption was faster with Ruzu bitter than when metformin was taken with water. Table 2 represents the comparative summary of the pharmacokinetic parameters obtained with and without Ruzu bitters.

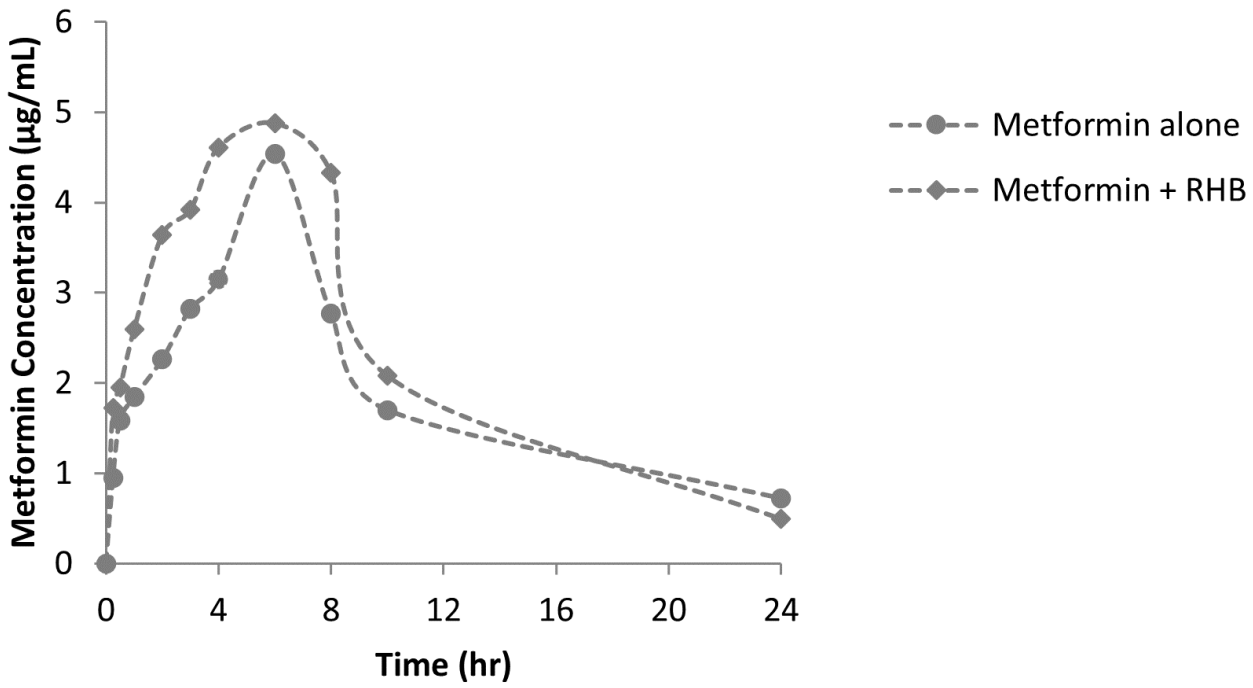

Figure 2: Metformin plasma concentration versus time profiles for metformin alone and in combination with RHB

Table 2: Comparative pharmacokinetic parameters of metformin with and without Ruzu Bitters

Parameter	Metformin alone mean ± SD	Metformin with RHB mean ± SD	p-value
$t_{1/2}$ (h)	9.26 ± 1.58	5.44 ± 0.24	0.01*
K_{el} (h^{-1})	0.10 ± 0.05	0.14 ± 0.02	0.005*
C_{max} (μgmL^{-1})	4.67 ± 2.16	5.05 ± 1.12	0.84
t_{max} (h)	4.50 ± 1.54	4.67 ± 1.34	0.74
Vd/F (LKg^{-1})	127.36 ± 16.98	72.97 ± 9.66	0.008*
CL/F (LKg^{-1})	9.28 ± 0.28	8.74 ± 0.09	0.034*
MRT (h)	13.80 ± 8.54	9.05 ± 1.05	0.03*
K_{abs} (h^{-1})	0.75 ± 0.10	0.76 ± 0.09	0.84
$t_{1/2abs}$ (h^{-1})	1.20 ± 0.03	1.22 ± 0.11	0.96
$AUC_{0\text{-}8}$ ($\mu ghmL^{-1}$)	59.09 ± 6.10	55.35 ± 4.99	0.85

*Statistically significant , $p < 0.05$; SD = Standard deviation; $t_{1/2}$, elimination half -life; K_{el}, elimination rate constant; C_{max}, maximum plasma concentration; tmax; time to reach maximum plasma concentration; Vd/F, apparent volume of distribution; CL/F , apparent clearance; MRT, mean residence time; k_{abs}, absorption rate constant; $t_{1/2}$, absorption half-life; $AUC_{0\text{-}8}$, area under plasma concentration time curve.

DISCUSSION

Most herbal products or formulations contain more than one phytochemical, of which have been implicated singly or collectively to interact positively or negatively with conventional drugs resulting to clinically significant interactions. RHB is a poly-herbal remedies marketed to have profound therapeutic effects on some of the common non-communicable and communicable diseases such as diabetes, hypertension, malaria, typhoid and sexually transmitted diseases. Some of the active secondary metabolites found in plants have been reported in-vitro and in-vivo to alter the metabolic activities of metabolizing enzymes particularly with cytochrome P450-enzymes[34] including P-glycoprotein. In some instances, the herb-drug interactions could be as a result of physical incompatibility of the herbal phytoconstituents with the active drug molecules leading to complexation, or the adhesion of excipients of herbal mixtures to drug or vice versa. The outcome of these interactions most often leads to reduced bioavailability of active pharmaceutical principle in the conventional medicine. Significant reduction in bioavailability ultimately will result to drug therapeutic failure or render the drug impotent.

In this study, the impact of concomitant administration of RHB and metformin was investigated to identify any potential drug interaction between these two medicines that may likely be taken together. So much that the prescribers of the conventional medicine are not aware and the patient suffers severe adverse effects hence the need to investigate and assess the potential interactions of some of the complex phytoconstituents in RHB formulations on the pharmacokinetics of metformin. HPLC chemical profile of RHB revealed the presence of 5 polyphenolic bioactive compounds of which gallic acid (phenolic), quercetin and rutin (flavanols) Luteolin (flavone) and formononetin (isoflavone) classified as flavonoids and have been implicated in causing herb-drug interactions most especially in cytochrome P450 drug metabolism.[35]

The outcome of the co-administration of metformin and RHB altered the pharmacokinetic parameters of metformin significantly, and this could impact on the efficacy of metformin. The elimination rate constant ($p = 0.005$) of metformin was significantly increased while the terminal half-life ($p = 0.01$), apparent volume of distribution ($p = 0.008$), clearance ($p = 0.034$), mean resident time (MRT) ($p = 0.03$) were significantly reduced by concomitant administration of RHB. Metformin is not a substrate of cytochrome P450 enzymes; it is majorly

excreted unchanged in the urine. Reports have shown that the absorption, distribution and excretion of metformin depend on the transporters like organic cation transporter (OCT-1), MATE and PMAT,[36] hence the observed high clearance of metformin could be due to its negligible plasma protein binding; the presence of transporters most especially OCT-1, PMAT and the low lipid solubility which consequently reduces passive re-absorption.[37] This high clearance is expected if metformin was ingested alone but co-administration of RHB may have inhibited metformin transporter thereby causing increase in plasma concentrations as seen in this study but the impact was insignificant (p = 0.84). This impact may lead to elevated risk of metformin associated lactic acidosis.[38] Other parameters including absorption rate constant, maximum plasma concentration, time to maximum plasma concentration, volume of distribution, and area under plasma concentration time curve, even though altered by RHB, were not statistically different between the metformin plus RHB and metformin alone groups.

The consequential effect of altered pharmacokinetics of a drug by any polyherbal products could be detrimental to the user more so if it leads to increased plasma concentration. It has been recently reported that RHB at high doses and chronic or prolong administration may result in renal, hepatic and cardiac toxicity (Odangowei *et al.*, 2022).[31]

CONCLUSION

This study established that co-administration of metformin and RHB altered the pharmacokinetic parameters of metformin. Although RHB is perceived beneficial for the management of diabetes, caution should be applied when co-administering it with metformin. Patients with diabetes, especially those who are not achieving the desired therapeutic outcomes, should be asked about herbal medicine use and counseled appropriately.

The authors declare that there is no financial/personal interest and no potential conflicting interest.

REFERENCES

1. Oreagba IA, Oshikoya KA, Amachree M (2011). Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Complementary and Alternative Medicine* 11: 117. doi: 10.1186/1472-6882-11-117. PMID: 22117933; PMCID: PMC3252251.
2. Awodele O, Amagon KI, Usman SO, Obasi PC (2014). Safety of herbal medicines use: case study of ikorodu residents in Lagos, Nigeria. *Current Drug Safety* 9(2): 138-44.
3. El Hajj M, Holst L (2020). Herbal Medicine Use During Pregnancy: A Review of the Literature with a Special Focus on Sub-Saharan Africa. *Frontiers in Pharmacology* 11, 866. doi: 10.3389/fphar.2020.00866.
4. Olisa NS, Oyelola FT (2009). Evaluation of use of herbal medicines among ambulatory hypertensive patients attending a secondary health care facility in Nigeria. *International Journal of Pharmacy Practice* 17(2): 101-5. PMID: 20214258.
5. Onyeka TC, Ezike HA, Nwoke OM, Onyia EA, Onuorah EC, Anya SU, Nnacheta TA (2012). Herbal Medicine: a survey of use in Nigerian presurgical patients booked for ambulatory anaesthesia. *BMC Complementary and Alternative Medicine* 12, 30. http://www.biomedcentral.com/1472-6882/12/130.
6. Amaeze OU, Aderemi-Williams RI, Ayo-Vaughan MA, Ogundemuren DA, Ogunmola DS, Anyika EN (2018). Herbal medicine use among Type 2 diabetes mellitus patients in Nigeria: understanding the magnitude and predictors of use. *International Journal of Clinical Pharmacology* 40(3): 580-588. doi: 10.1007/s11096-018-0648-2.
7. Amaeze OU, Olugbake OA, Lawal M (2020). Knowledge of Herbal Medicines and Herb-drug Interaction Among Medical and Pharmacy Students of the University of Lagos, Nigeria. *Nigerian Journal of Pharmaceutical Research* 16 (1): 61-70
8. Bent S (2008). Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center. *Journal of General Internal Medicine* 23(6): 854-9. doi: 10.1007/s11606-008-0632-y.
9. Chang HY, Wallis M, Tiralongo E (2007). Use of complementary and alternative medicine among people living with diabetes: literature review. *Journal of Advanced Nursing* 58(4): 307-319. doi: 10.1111/j.1365-2648.2007.04291.x. [PubMed] [CrossRef] [Google Scholar]
10. Radwan H, Hasan H, Hamadeh R, Hashim M, AbdulWahid Z, Gerashi MH, Hilali MA, Naja F (2020). Complementary and alternative medicine use among patients with type 2 diabetes living in the United Arab Emirates. *BMC Complementary Medicine and Therapies* 20: 216.

https://doi.org/10.1186/s12906-020-03011-5

11. Ezeome ER, Anarado AN (2007). Use of complementary and alternative medicine by cancer patients at the University of Nigeria Teaching Hospital, Enugu, Nigeria. *BMC Complementary and Alternative Medicine* 7: 28. doi: 10.1186/1472-6882-7-28.
12. Ogbera AO, Dada O, Adeyeye F, Jewo PI (2010). Complementary and alternative medicine use in diabetes mellitus. *West African Journal of Medicine* 29(3): 158-62. doi: 10.4314/wajm. v29i3.68213. PMID: 20665458.
13. Ilomuanya MO, Okubanjo OO, Azubuike C, Adeyemi O, Ajiboye D, Maduka C (2017). Evaluation of the frequency of use of herbal drugs with concomitant administration of highly active antiretroviral therapy and its effect on medication adherence in two healthcare facilities in South-Western Nigeria. *Journal of AIDS and HIV Research* 9 (1): 8-16. https://doi.org/10.5897/JAHR2016.0399
14. Gerber W, Steyn JD, Kotzé AF, Hamman JH (2018). Beneficial Pharmacokinetic Drug Interactions: A Tool to Improve the Bioavailability of Poorly Permeable Drugs. *Pharmaceutics* 10(3): 106. doi: 10.3390/pharmaceutics10030106.
15. Gurley BJ (2012). Pharmacokinetic herb-drug interactions (part 1): origins, mechanisms, and the impact of botanical dietary supplements. *Planta medica* 78(13): 1478-1489. https://doi.org/10.1055/s-0031-1298273.
16. Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD (2017). Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetology and Metabolic Syndrome* 9: 59. doi: 10.1186/s13098-017-0254-9.
17. Izzo AA (2012). Interactions between herbs and conventional drugs: overview of the clinical data. *Medical Principles and Practice* 21(5): 404-28. doi: 10.1159/000334488. Epub 2012 Jan 11. PMID: 22236736.
18. Shi S, Klotz U (2012). Drug interactions with herbal medicines. *Clinical Pharmacokinetics* 51(2): 77-104. doi: 10.2165/11597910-000000000-00000. PMID: 22257149.
19. Bailey CJ (2017). Metformin: historical overview. Diabetologia 60: 1566-1576. https://doi.org/10.1007/s00125-017-4318-z
20. Rena G, Hardie DG, Pearson ER (2017). The mechanisms of action of metformin. *Diabetologia* 60: 1577-1585 https://doi.org/10.1007/s00125-017-4342-z
21. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM (2011). Clinical Pharmacokinetics of Metformin. *Clinical Pharmacokinetics* 50(2): 81-98.
22. Kawoosa F, Shah ZA, Masoodi SR, Amin A, Rasool R, Fazili KM, Dar AH, Lone A, ul Bashir S (2022). Role of human organic cation transporter-1 (OCT-1/SLC22A1) in modulating the response to metformin in patients with type 2 diabetes. *BMC Endocrine Disorders* 22: 140. https://doi.org/10.1186/s12902-022-01033-3
23. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, Ianculescu AG, Yue L, Lo JC, Burchard EG, Brett CM, Giacomini KM (2007). Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *The Journal of Clinical investigation* 117(5): 1422-31. doi: 10.1172/JCI30558. PMID: 17476361; PMCID: PMC1857259.
24. Zolk O, Solbach TF, König J, Fromm MF (2009). Structural determinants of inhibitor interaction with the human organic cation transporter OCT2 (SLC22A2). *Naunyn-Schmiedeberg's Archives of Pharmacology* 379(4): 337-48. doi: 10.1007/s00210-008-0369-5. Epub 2008 Nov 11. PMID: 19002438.
25. Kale OE, Akinpelu OB, Bakare AA, Yusuf FO, Gomba R, Araka D C, Ogundare T O, Okolie A C, Adebawo O, Odutola O (2018). Five traditional Nigerian Polyherbal remedies protect against high fructose fed, Streptozotocin-induced type 2 diabetes in male Wistar rats. *BMC complementary and alternative medicine* 18(1): 160. https://doi.org/10.1186/s12906-018-2225-6
26. Obasi DC, Ogugua VN (2021a). Effect of Ruzu Herbal Bitters on the Liver Function and Lipid Profile Parameters of Alloxan-Induced Diabetic Rats. *Journal of Clinical and Experimental Hepatology* DOI:10.1016/j.jceh.2021.09.012
27. Obasi DC, Ogugua VN (2021b). GC-MS analysis, pH and antioxidant effect of Ruzu herbal bitters on alloxan-induced diabetic rats. *Biochemistry and Biophysics Reports* 27: 101057. doi: 10.1016/j.bbrep.2021.101057.
28. Briggs ON, Elechi-amadi KN, Aleruchi-Didia TN, Anyalebechi E O, Agwor S (2022). Effects of the Anti-diabetic Polyherbal (Ruzu Bitters) on Glucose, Hepatic and Renal Parameters in Alloxan-induced Diabetic Rats. *Journal of Advances in Medical and*

Pharmaceutical Sciences 24(4), 44-51. https://doi.org/10.9734/jamps/2022/v24i430296

29. Ogunlana OO, Ogunlana OE, Ugochukwu SK, Adeyemi AO (2018). Assessment of the Ameliorative Effect of Ruzu Herbal Bitters on the Biochemical and Antioxidant Abnormalities Induced by High Fat Diet in Wistar Rats. *International Journal of Pharmacology* 14: 329-341. DOI: 10.3923/ijp.2018.329.341. https://scialert.net/abstract/?doi=ijp.2018.329.341
30. Ogunlana OO, Ogunlana OE, Adekunbi TS, Adetuyi BO, Adegboye BE, Iheagwam FN (2020). "Anti-inflammatory Mechanism of Ruzu Bitters on Diet-Induced Nonalcoholic Fatty Liver Disease in Male Wistar Rats", *Evidence Based Complementary & Alternative Medicine* https://doi.org/10.1155/2020/5246725
31. Odangowei IO, Frank-Oputu A, Shonubi OO, Ruth OA (2022). Biochemical Study on the Effects of Ruzu Herbal Bitters Formulation on Wistar Albino Rats. *Biomedical Journal of Scientific & Technical Research* 41(1): DOI:10.26717/BJSTR.2022.41.006558
32. Valizadeh H, Nayyeri-Maleki P, Ghanbarzadeh S, Sheikhloo A, Servat H, Nemati M, Zekeri-Milani P (2014). Pharmacokinetics and bioequivalence of two brands of metformin 500 mg tablets in Iranian healthy volunteers. *Journal of Pharmaceutical Investigation* 44: 61-68. 10.1007/s40005-013-0102-3 https://api.semanticscholar.org/CorpusID:256309835
33. Yuangang Zu, Chunying Li, Yujie Fu, Chunjian Zhao (2006). Simultaneous determination of catechin, rutin, quercetin,kaempferol and isorhamnetin in the extract of sea buckthorn (Hippophae rhamnoidesL.) leaves by RP-HPLC with DAD. *Journal of Pharmaceutical and Biomedical Analysis* 41: 714-719
34. Appiah-Opong R, Commanduer JN, Axson C, Vermeulen NP (2008). Interactions between cytochromes P450, glutathione S-transferases and Ghanaian medicinal plants. *Food and Chemical Toxicology* 46: 3598-3603. doi: 10.1016/j.fct.2008.09.002. [PubMed] [CrossRef] [Google Scholar].
35. Yannan Li, Jing Ning, Yan Wang, Chao Wang, Chengpeng Sun, Xiaokui Huo, Zhenlong Yu, Lei Feng, Baojing Zhang, Xiangge Tian, Xiaochi Ma (2018). Drug interaction study of flavonoids toward CYP3A4 and their quantitative structure activity relationship (QSAR) analysis for predicting potential effects. *Toxicology Letters* 294: 27 - 36. https://doi.org/10.1016/j.toxlet.2018.05.008.
36. Stage TB, Brøsen K, Christensen MM. 2015. A Comprehensive Review of Drug-Drug Interactions with Metformin. *Clinical Pharmacokinetics* 54(8): 811-24. doi: 10.1007/s40262-015-0270-6. PMID: 25943187.
37. Sheleme T (2021). Clinical Pharmacokinetics of Metformin. Metformin - Pharmacology and Drug Interactions. *IntechOpen* DOI: 10.5772/intechopen.99343.
38. Pakkir Maideen, NM, Jumale A, Balasubramaniam R (2017). Drug Interactions of Metformin Involving Drug Transporter Proteins. *Advanced Pharmaceutical Bulletin* 7(4):501-505. doi: 10.15171/apb.2017.062. Epub 2017 Dec 31. PMID: 29399540; PMCID: PMC5788205.