

Herb-drug interaction: Effect of aqueous extract of *Bridelia ferruginea* leaves on the pharmacokinetics of metformin

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ABSTRACT

The concurrent use of herbal medicines and orthodox drugs, especially in the treatment and management of chronic ailments, may result in clinically significant herb-drug interactions. *Bridelia ferruginea* Benth (Euphorbiaceae) is a common medicinal plant with known anti-diabetic properties and has been reported to be taken alongside the orthodox medicine, metformin. The aim of this study was to investigate the effect of aqueous extract of *B. ferruginea* leaves on the pharmacokinetics of metformin in female Sprague-Dawley rats. Reconstituted freeze dried extract of *B. ferruginea* leaves (30 mg/kg), and metformin (7 mg/kg) were administered concurrently as a single dose to female Sprague-Dawley rats. Whole blood samples (1 ml) were aseptically withdrawn by tail bleeding at 1, 2, 4, 8, and 24 h after administration of the single dose for pharmacokinetics analyses. Concurrent administration of metformin and *B. ferruginea* significantly affected ($P < 0.05$) all the pharmacokinetics parameters of metformin except for the time to attain the maximum concentration (T_{max}), which increased but insignificantly. Whereas the area under the curve, maximum whole blood concentration (C_{max}) and half-life ($T_{1/2}$) of metformin decreased significantly in the presence of *B. ferruginea*, the elimination rate constant (K_{el}), clearance (Cl), absorption rate constant (K_a), and volume of distribution (V_d) of metformin increased significantly in the presence of *B. ferruginea*. Therefore, in clinical practice, patients should be advised on the implication of concurrent administration of metformin and *B. ferruginea*.

KEY WORDS: *Bridelia ferruginea*, concurrent administration, herb-drug interactions, pharmacokinetics

INTRODUCTION

Diabetes, a chronic disorder in metabolism of carbohydrates, proteins, and/or fat is now epidemic with the worldwide incidence of 5% in the general population. It is estimated that the number of adults with diabetes in the world will rise to 300 million in the year 2025 (Torben, 2002). Diabetic patients usually experience significant morbidity and mortality from microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (heart attack, stroke, and peripheral vascular disease) (Engelgau *et al.*, 2004).

The management of diabetes (especially Type 2) has evolved in recent times with many options available to patients. These include the use of sulfonylureas, the glucosidase inhibitors (biguanides), thiazolidinediones, insulin analogs, and D-phenylalanine derivatives (Christensen *et al.*, 2009;

Fowler, 2007). These drugs, although relatively effective, are not without untoward effects and are also costly. Thus, some patients explore the use of natural products (plants) for the management of diabetes. Ethnopharmacological studies have shown that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Kesari *et al.*, 2007).

Bridelia ferruginea Benth (Euphorbiaceae) is the common medicinal plant that has been widely reported for its pharmacological effects, especially its anti-diabetic properties (Iwu, 1983; Njamen *et al.*, 2012). The clinic at the Center for Plant Medicine Research (CPMR), Mampong-Akuapem, Ghana, prescribes *B. ferruginea* tea to Type 2 diabetic patients. Unconfirmed reports indicate that some patients, who visit the clinic for herbal treatment of diabetes, continue to take their orthodox medication (such as metformin) alongside. This practice can possibly result in herb-drug interaction, an area of

research affecting the modern practice of medicine but has received little attention.

Although the repercussion of herb-drug interaction could be beneficial or detrimental, there are no studies to ascertain the effect of such practice, especially on the pharmacokinetics of the orthodox anti-diabetic drug, metformin. The aim of this study, therefore, was to investigate the effect of aqueous extract of *B. ferruginea* leaves on the following pharmacokinetic parameters of metformin; concentration - Time course: The maximum whole blood concentration and time for the maximum concentration, bioavailability, elimination rate constant, half-life, clearance, volume of distribution and absorption rate constant. The outcome of this study would provide additional scientific information for the management of diabetes at the clinic of CPMR.

MATERIALS AND METHODS

Animals

A total of 20 female Sprague-Dawley rats weighing between 168 and 206 g were obtained from the Animal Unit of the CPMR, Mampong Akuapem, Ghana and standard laboratory chow diet was obtained from Ghana Agro Food Company, Tema, Ghana.

Preparation of Plant Extract

A hot infusion was made by boiling powdered leaves of *B. ferruginea* (6.49 g) in a 500 ml of water for 10-15 min and then decanted. The resultant extract was lyophilized into powder using a freeze dryer (EYELA, Tokyo Rikakikai Co. Ltd., Japan). The dried powdery extract was weighed (0.97 g) and stored in a desiccator at room temperature. The powder was reconstituted in distilled water before administration to the animals.

Experimental Design

The rats were randomly divided into four groups of five animals each, fed on the standard laboratory chow and water *ad libitum*, and acclimatized for a week before the commencement of the treatment.

Group I: Received metformin, as a single dose (7 mg/kg; based on the recommended dosage in humans).

Group II: Concurrently, received reconstituted freeze dried *B. ferruginea* at the therapeutic dose (30 mg/kg) (CSRPM, unpublished data) and a single oral dose of metformin.

Group III: Received reconstituted freeze dried *B. ferruginea* at the therapeutic dose (30 mg/kg).

Group IV: Received distilled water.

Blood Sampling

Whole blood samples (1 ml) of animals in each group were aseptically withdrawn by tail bleeding at 1, 2, 4, 8, and 24 h after administration of the single dose of metformin. Blood was collected into tubes containing trisodium citrate as an anticoagulant and stored at -20°C for pharmacokinetic studies.

Extraction of Metformin from Whole Blood

The extraction of metformin from whole blood was carried out as described by Chhetri *et al.* (2014) with modifications. Whole blood (380 μl) was transferred into a 1.5 ml eppendorf tube. 20 μl of perchloric acid (60-62% m/m) was added and vortex mixed for 1 min. 100 μl of the mobile phase was then added and vortex mixed for 1 min. The mixture was centrifuged at 9400 g for 3 min and the supernatant layer was transferred into another tube and filtered through a 0.45 μm filter. 10 μl of the filtrate was injected onto the high-performance liquid chromatography (HPLC) column. The mobile phase was composed of water (pH 3.2 adjusted with orthophosphoric acid) and acetonitrile (35:65 v/v); at a flow rate of 0.8 ml/min with run time of 10 min.

HPLC Analysis of Metformin

Calibration curve

Standard stock solutions of 100 $\mu\text{g}/\text{ml}$ of metformin were prepared using diluent (HPLC grade water adjusted to pH 3.2 with orthophosphoric acid). A calibration curve of metformin concentration versus peak area in the range of 0-10 $\mu\text{g}/\text{ml}$ was prepared. Reference standard (0.625 $\mu\text{g}/\text{ml}$ of metformin) was used to check the system suitability. Peak area was plotted against concentrations and linearity accepted for $r^2 \geq 95\%$.

Instrumentation and conditions for chromatography

Instrumentation

An HPLC analysis of metformin was performed with the Agilent Chemstation HPLC system, consisting of 1260 Quart HPLC pump, ASL Prep autosampler, and multiple wavelength detectors.

Chromatographic conditions

A Supelco C18 reversed phase column (250 mm \times 4.6 mm, 5 μm) was used for the chromatographic analyses of metformin. The mobile phase was water (pH 3.2

adjusted with orthophosphoric acid) and acetonitrile (35:65 v/v) adjusted to a flow rate of 0.6 ml/min with the ultraviolet detector set at 233 nm.

Pharmacokinetic Analysis

Concentration-time profiles were analyzed using non-compartmental methods (Shargel and Yu, 1993). The concentration-time profile was generated from the original data obtained. The maximum whole blood concentration (C_{max}) and time to C_{max} (T_{max}) were obtained directly from original data. The elimination rate constant, K_{el} , was first estimated from log-linear regression of the straight terminal part of the curve; in all instances this was made up of the last three or four concentration-time points.

The K_{el} was calculated from slope of the equation;

$$\text{Log } C_p = -kt / 2.3 + \text{log} C_{po}$$

Where C_p is concentration at any time (t), k is the elimination rate constant; C_{po} is the intercept on the Y-axis.

Therefore,

$$\text{Slope} = -k / 2.3$$

The elimination half-life ($t_{1/2}$) was calculated using the equation

$$t_{1/2} = \ln 2 / K_{el}$$

The area under the concentration-time curve (AUC), a measure of bioavailability, was calculated by the trapezoidal rule as shown below;

$$AUC = [C_n - 1 + C_n][t_n - t_{n-1}] / 2$$

Where, t_n is the time of observation of drug concentration C_n , and t_{n-1} is the time of the prior observation of drug concentration corresponding to C_{n-1} .

The clearance rate (Cl) was calculated as Dose/AUC.

The volume of distribution (V_d) was calculated as $Cl \times K_{el}$

From a concentration-time semi-log graph, the absorption rate constant (K_a) was estimated from the intercept of the equation below;

$$\text{Log } C_p = \text{Log} [(FK_a D_o) / V_d (K_a - K_{el})] - k_t / 2.3$$

Where, C_p is the concentration at time t , K_{el} is the elimination rate constant, V_d is the volume of distribution, D_o is the dose and F is the fraction of the drug absorbed given as peak concentration divided by Dose (Shargel and Yu, 1993; R ath et al., 2004).

RESULTS

Samples of HPLC Chromatograms of Metformin Extracted from Whole Blood

A sample of the chromatogram of metformin extracted from whole blood is shown in Figure 1. The constituents of the blank blood did not interfere with the metformin peak (Figure 1a). The blank (drug-free whole blood) absorbed minimally around 2.0 min and 3.5 min but did not interfere with metformin peak. The retention time of metformin under the conditions used was about 3.63 ± 0.02 min (Figure 1b).

Chromatograms showing peaks after extraction of: Drug-free whole blood as blank (a) and metformin contained whole blood (b). Retention time (R_t) in min is shown adjacent to the peak: ($R_t = 3.628$). For HPLC condition and procedure refer to the section on "Method."

Calibration Curve

Figure 2 shows the calibration curve from which the concentration of metformin, extracted from whole blood, was extrapolated. From the calibration curve, regression coefficient (R^2) was 0.996 (99.6%). This signifies a strong positive correlation between the concentration and peak area in the HPLC analyses of metformin.

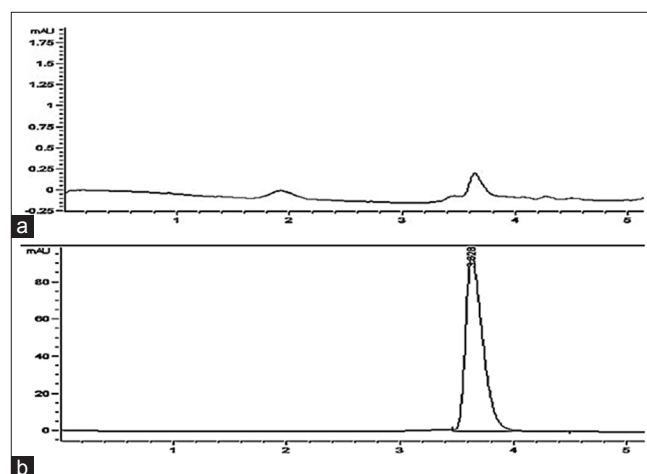


Figure 1: High-performance liquid chromatography chromatograms of metformin and blank blood after extraction

Concentration-time Profile of Metformin in the Presence and Absence of *B. ferruginea*

Figure 3 shows the concentration-time curve of metformin in the presence and absence of *B. ferruginea*. The blood metformin level of the control group (metformin-treated group) reached its maximum level ($83.36 \pm 15.53 \mu\text{g/ml}$) after about 2 h and decreased rapidly by 82% to $13.94 \pm 0.04 \mu\text{g/ml}$ within 2 h and then gradually to undetectable level by 24 h (Figure 3). However, the whole blood metformin level of the test group (metformin and *B. ferruginea* treated group) peaked after about 2 h to $45.08 \pm 10.60 \mu\text{g/ml}$; a lower concentration relative to the control. The drug level then decreased gradually to an undetectable level after 10 h.

Effect of aqueous extract of *B. ferruginea* leaves at 30 mg/kg concentration on the whole blood levels of metformin given as single oral dose (7 mg/kg). Concentrations are given as mean \pm standard error of the mean, $n = 5$.

Pharmacokinetic Parameters of Metformin in the Presence and Absence of *B. ferruginea*

The pharmacokinetic parameters of metformin in the presence and absence of *B. ferruginea* are shown in Table 1. From Table 1, concurrent administration of metformin and *B. ferruginea* significantly affected ($P < 0.05$) all the pharmacokinetics parameters of metformin except for the time to attain the maximum concentration (T_{max}), which was not significantly affected. Whereas the AUC, maximum whole blood concentration (C_{max}) and elimination half-life ($T_{1/2}$) of metformin decreased significantly in the presence of *B. ferruginea*, the elimination rate constant (K_{el}), clearance (Cl), absorption rate constant (K_a), and volume of distribution (V_d) of metformin increased significantly in the presence of *B. ferruginea*.

DISCUSSION

The concurrent use of orthodox medicines and herbs to facilitate or hasten recovery especially in the treatment and management of chronic ailments such as diabetes, hypertension, HIV/AIDS and cancer is on the rise (Obodozie, 2012). One of the consequences of concurrent use of herbal medicines and orthodox medicines is the possibility of interactions. This study investigated the effect of aqueous extract of *B. ferruginea* leaves on the pharmacokinetics of metformin.

The study showed that concurrent administration of metformin and *B. ferruginea* significantly affected ($P < 0.05$) all the pharmacokinetic parameters of

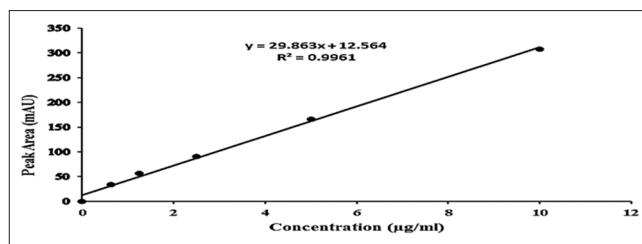


Figure 2: Calibration curve of metformin concentration against peak area

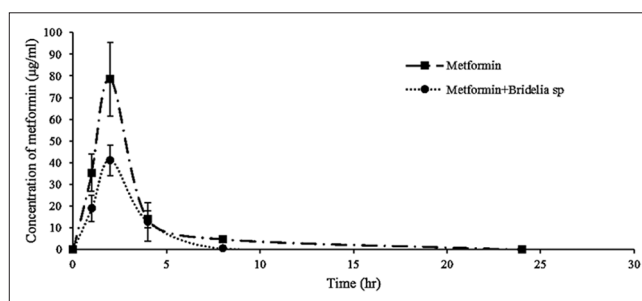


Figure 3: Effect of *Bridelia ferruginea* on whole blood concentration of metformin

Table 1: Pharmacokinetic parameters of metformin administered as a single oral dose in the absence and presence of *B. ferruginea*

Pharmacokinetic parameter	Metformin alone	Metformin and <i>B. ferruginea</i>	Comment
AUC ($\mu\text{g/ml}^{-1} \text{h}^{-1}$)	223.57 ± 28.96	123.87 ± 37.95	Decrease
C_{max} ($\mu\text{g/ml}$)	83.36 ± 15.53	45.08 ± 10.60	Decrease
T_{max} (h)	1.80 ± 0.20	2.40 ± 0.40	Increase
$T_{1/2}$ (h)	6.75 ± 0.36	0.54 ± 0.06	Decrease
K_{el} (h^{-1})	0.10 ± 0.01	1.13 ± 0.05	Increase
Clearance (L/h)	6.60 ± 0.65	16.10 ± 3.97	Increase
V_d (L/kg)	0.69 ± 0.09	18.24 ± 3.67	Increase
K_a (h^{-1})	0.15 ± 0.01	1.33 ± 0.17	Increase

Values are given as mean \pm SEM, $n=5$. *B. ferruginea*: *Bridelia ferruginea*. SEM: Standard error of mean, AUC: Area under the curve

metformin except for the time to attain the maximum concentration (T_{max}) which increased but insignificantly. From the study, concurrent administration of metformin and *B. ferruginea* significantly decreased the maximum whole blood concentration (C_{max}) and AUC (a measure of bioavailability) of metformin although the absorption rate constant (K_a) of metformin increased significantly.

The observed decrease in the C_{max} and bioavailability despite the increase in absorption rate constant could be attributed to the significant increase in the volume of distribution (V_d) of metformin in the presence of *B. ferruginea*. Metformin is reported to be widely distributed into body tissues such as intestine, liver and kidney via various organic cation transporters (Gong et al., 2012). From the study, in the presence of *B. ferruginea*, metformin appears to be

more distributed (about 25 times) into the body tissues as evident in the significant increase in V_d after concurrent administration of *B. ferruginea* and metformin. This observation could contribute to the observed decrease in C_{max} and bioavailability of metformin in the presence of *B. ferruginea*.

Furthermore, two major factors contribute significantly to the bioavailability of a drug. These are the degree of first-pass metabolism of the drug in the liver and intestine, and the extent of protein binding of the drug (Thomson, 2004). Unlike other biguanides, no metabolites or conjugates of metformin have been identified suggesting that metformin does not undergo liver metabolism (Scheen, 1996). Instead, it undergoes renal excretion. In addition, metformin does not bind to plasma proteins (Sambol *et al.*, 1996). This suggests that the observed decrease in the C_{max} and bioavailability could also be due to a higher rate of excretion of metformin. This assertion is evident in the observed decrease ($P < 0.05$) in the half-life ($T_{1/2}$) and significant increase in the elimination rate constant (K_{el}) and clearance (Cl) of metformin in the presence of *B. ferruginea*.

Therefore, in clinical practice, patients should be advised on the implication of concurrent administration of metformin and *B. ferruginea*. The study, however, cannot conclude with certainty the clinical effect of concurrent administration of metformin and *B. ferruginea* since metformin alone and *B. ferruginea* alone have been reported to be effective anti-diabetic medicines. Thus, further work including efficacy studies in animals, may be carried out to investigate the effect of concurrent administration of metformin and *B. ferruginea* in diabetic animal models.

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