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Herb-drug interaction of Nisha Amalaki and Curcuminoids with metformin in normal and diabetic condition: A disease system approach

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ABSTRACT

Nisha Amalaki (NA), formulation with *Curcuma longa* Linn (Turmeric, Haridra, Nisha in Sanskrit; Family: Zingiberaceae) and *Phyllanthus emblica* Linn (Indian gooseberry, Amlaki in Sanskrit; Family: Phyllanthaceae) which is described for various diseases including diabetes in ayurvedic texts and Nighantus. The aim of the present study was to assess the pharmacokinetic (PK) and pharmacodynamic (PD) interactions of chemically standardized NA and Curcuminoids (CE) with metformin (MET) in normal and diabetic animals. Oral administration of NA (200 mg/kg) and CE (30 mg/kg) was carried out for seven days followed by co-administration of MET till fifteen days. MET plasma PK parameters including C_{max}, $AUC_{0-\infty}$, $t_{1/2}$, CL and V_d were measured on the eighth day. PD parameters including plasma glucose AUC followed by oral glucose tolerance test, high-density lipoproteins (HDL), total cholesterol (TC) and triglycerides (TG) were measured on the fifteenth day. In normal animals, co-administration of NA + MET and CE + MET resulted in significant increase ($p < 0.05$) in C_{max}, $AUC_{0-\infty}$, $t_{1/2}$, and reduction of CL and V_d. We report that co-administration of NA + MET and CE + MET significantly ($p < 0.01$, $p < 0.001$) reduced plasma glucose level, HDL level while a notable reduction in TG and TC level was observed. Interestingly, in diabetic condition, co-administration of NA + MET and CE + MET indicated a significant decrease ($p < 0.05$) in C_{max}, $AUC_{0-\infty}$, $t_{1/2}$ and enhanced CL and V_d. Hence, to conclude, co-administration of NA + MET and CE + MET resulted in beneficial PK and PD interactions leading to antihyperglycemic and antihyperlipidemic effects in both conditions. However, PK interaction was drastically different in diabetic and normal conditions.

1. Introduction

Incidence of drug-drug interactions (DDIs) and herb-drug interactions (HDIs) can lead to beneficial or severe and sometimes lethal clinical outcomes [1–3]. In contrast to DDIs, the study of HDIs remains a challenge due to inherent physicochemical complexity of herbs and multi-target modulatory potential [4,5]. These interactions need to be detected, analyzed and assessed during the early stage of botanical drug development. Several studies on pharmacokinetic HDIs on traditional Chinese herbal medicines and Western herbal medicines have been documented [6]. These studies mainly focus on the use of normal animal models or healthy human volunteers. However, this data is often extended to relevant disease conditions. It is known that compared to normal condition, PK parameters can be significantly altered during disease condition [7]. This is mainly because of altered expression of metabolizing enzymes such as cytochrome p450 isoenzymes (CYPs) and transporter proteins involved in absorption and excretion of co-administered drugs and also, due to neuroendocrine imbalance [8,9]. Therefore, there is a need for more comprehensive ways to study such interactions. The systems-based approach has been considered to study these complex HDIs [10]. We propose that a disease system approach can provide unique insights into the dynamic changes in pathophysiological, structural and functional aspects in the body during such interactions.

Abbreviations: NA, Nisha Amalaki; CE, Curcuminoids; MET, metformin; DDI, drug-drug interactions; HDI, herb-drug interactions; CYPs, cytochrome p450 isoenzymes; IS, internal standard; MWR, Male Wistar rats; STZ, streptozotocin; MNC, mononuclear cells; OCT, organic cation transporter

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Ayurveda, a traditional Indian system of medicine, prescribes several formulations for health and therapeutic benefits. Among them, Nisha Amalaki (NA) is a very popular formulation containing *Curcuma longa* Linn (Turmeric, Haridra, Nisha in Sanskrit; Family: Zingiberaceae) and *Phyllanthus emblica* Linn (Indian gooseberry, Amlaki in Sanskrit; Family: Phyllanthaceae). NA described in Ayurvedic literatures and Nighantus, is recommended for various diseased conditions including diabetes [11]. Traditionally, *C. longa* is a popular Indian spice that has been used for the treatment of a variety of ailments such as rheumatism, cancer; wound healing, anorexia, cough, sinusitis and diabetic ulcers [12]. Curcuminoids (CE) (diferuloylmethane) such as curcumin or derivatives of curcumin like desmethoxycurcumin and bisdemethoxycurcumin are considered as bioactive compounds present in *Curcuma longa* Linn. CE has shown to possess significant anti-inflammatory, antioxidant, anti-carcinogenic, anti-coagulant, anti-infective and anti-diabetic effects [13–16].

Co-administration of NA with anti-diabetic drugs like Metformin (MET) has reported increasing the need for evaluation of their possible interactions in diabetic conditions. Previously, Puranik et al. have reported PK interaction of NA crude powder and its tablet formulations with the anti-diabetic drug MET, in healthy volunteers after single oral dose [17]. This study has shown that oral administration of NA crude powder significantly decreased MET pharmacokinetic parameters such as C_{max} and AUC_t could occur at absorption stage. However, this study on healthy volunteers has used only a single dose of MET and NA. In contrast to this, in rality diabetic patients would take multiple doses of MET and NA concurrently. Hence, this study was undertaken to evaluate the effect of co-administration of NA and its active constituents CE with MET on PK-PD interactions in normal and diabetic conditions.

2. Materials and methods

2.1. Chemicals and reagents

MET (purity ≥ 99.5%) and ranitidine (purity ≥ 99.5%) was a kind gift sample from Twilight Litaka Pharma, Pune, India. Ranitidine was used as an internal standard (IS). CE (purity ≥ 97%) was received from Pharmanza Herbal Pvt. Ltd., Mumbai, India. Streptozotocin (STZ) was procured from Sigma Aldrich, Bangalore, India. NA tablets were purchased from local pharmacy (Batch No. A-05, KD/AYU/004/05, Vishal Ayurvedic Pharmacy, India). HPLC solvents such as acetonitrile and methanol were obtained from Merck, Mumbai, India. All other solvents used were of analytical grade.

2.2. Test material phytochemical characterization

NA powder was standardized based on CE such as curcumin desmethoxycurcumin and bisdemethoxycurcumin using in-house standardised high-performance liquid chromatography coupled with photodiode array (HPLC-PDA) method [18]. Briefly, Prominence HPLC system (Shimadzu, Japan) was equipped with a binary pump, autosampler, a column oven and a photodiode array detector. Chromatographic separations were carried out using C-18 analytical column (150 × 4.6 mm, 5 µm particle size; Syncronis, Thermo Fisher Scientific, USA) with mobile phase consisting of a mixture of acetonitrile and 0.1% formic acid in the ratio of 50:50 (v/v) with the flow rate of 0.8 mL/min. The column temperature was maintained at 40 °C and detection wavelength was set at 425 nm.

2.3. HPLC-PDA method for estimation MET in plasma samples

Previously reported HPLC method was modified and used for the analysis of MET in plasma samples collected from PK study [19]. HPLC system (Dionex) with a gradient pump having photodiode array detector was employed. Chromatographic separation was carried out on a reverse phase Hypersil Gold Phenyl column (250 mm × 4.6 mm, 5 µm: Thermo Fisher Scientific, Mumbai, India) using isocratic mobile phase acetonitrile and phosphate buffer (0.020 M; pH 7.0 with 35: 65 v/v) at a flow rate of 1.5 mL/min. The column and autosampler were maintained at 40 °C and 4 °C, respectively. The total analytical run time was 10 min. Data acquisition was performed with Chromeleon software.

2.4. Preparation of calibration standards and quality control (QC) samples

The working standard solution of MET (0.025 mg/mL) was prepared in methanol. A stock solution of the ranitidine (IS), was prepared (1 mg/mL) in methanol and stored at 4 °C. The working solution of IS was prepared freshly by diluting aliquots of the IS stock solution to 30 µg/mL with methanol.

Rat plasma calibration standards of MET (0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 µg/mL) were prepared in pooled rat plasma. The QC sample for MET (0.8, 6.4 and 51.2 µg/mL) was prepared in bulk by the addition of appropriate working standard solutions to drug-free rat plasma. Aliquots of the QC samples (100 µL) were stored in polypropylene tubes at −70 °C.

2.5. Sample preparation/Extraction procedure

Plasma samples including blank, calibration and QC were extracted using liquid-liquid extraction method [20]. Briefly, 100 µL volume of plasma was transferred to a 1.5 mL polypropylene microcentrifuge tube. Extraction was performed by adding an IS (20 µL of 30 µg/mL of ranitidine), 100 µL of 8 M NaOH and 1.3 mL of 1-butanol/n-hexane (50:50, v/v) to the tube followed by 2 min. shaking. After centrifugation at 10,000 g for 2 min., complete organic layer was separated and transferred to another tube. Then, 100 µL of 1% acetic acid was added and mixture was vortex mixed followed by centrifugation for 2 min. The organic phase was removed and 50 µL volume of aqueous phase was injected into the HPLC system.

2.6. Method validation

2.6.1. Specificity and selectivity

The specificity of the method was evaluated by analyzing rat plasma samples from at least six different lots to investigate the potential interferences at the chromatogram peak region for analytes and IS.

2.6.2. Linearity and lower limit of quantification (LLOQ)

The linearity of the method was generated by analysis of five calibration curves containing seven non-zero concentrations ranging from 0.8 to 51.2 µg/mL. Each calibration curve was analyzed individually by fitting the area ratio response for analyte/IS as a function of standard concentration.

2.6.3. Accuracy and precision

Plasma samples with MET (at a concentration of 0.8, 6.4 and 51.2 µg/mL) were prepared. Intra- and inter-day assays were carried out using these plasma samples at six timepoints of the same day and for three different days, respectively. Analytical precision was evaluated by calculating the % CV of intra- and inter-day assays while accuracy was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows: accuracy (bias, %) = $((C_{nom} - C_{obs}) / C_{nom}) \times 100$.

2.6.4. Extraction recovery

The percentage extraction recoveries of MET were determined at three QC levels (0.8, 6.4 and 51.2 µg/mL) by calculating the ratio of the mean peak area of MET spiked before extraction (R1) to the mean peak area of MET spiked post-extraction (R2) × 100.