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Evaluation of herb-drug interaction of a polyherbal Ayurvedic formulation through high throughput cytochrome P450 enzyme inhibition assay

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ABSTRACT

Ethnopharmacological relevance: Arishtas are Ayurvedic formulation made with decoction of herbs. Arjunarishta formulation is being used in Ayurveda for cardio-protective activity. Ashwagandharishta formulation possesses antioxidant, anti-atherosclerotic and anti-stress properties. Ridayarishta, a novel empirical formulation was prepared using combination of selected ingredients from these two formulations to support healthy heart functions and to reduce stress.

Aim of the Study: Aim of the Study was to investigate herb-drug interaction (HDI) of Ridayarishta formulation through human hepatic cytochrome P450 (CYP450) enzyme inhibition assay.

Materials and methods: Ridayarishta formulation was phyto-chemically standardized against arjunolic acid, arjunetin, berberine, piperine, resveratrol and withaferin-A using high performance thin layer chromatography (HPTLC) analysis. The formulation was standardized with respect to ethanol by gas chromatographic (GC) analysis. HDI was evaluated with Ridayarishta formulation and amlodipine besilate, atenolol, atorvastatin, metformin, glipizide glimepiride cocktail using high throughput CYP450 enzyme inhibition assay; against CYP1A2, 2C19, 2D6 and 3A4 isozymes.

Results: Contents of arjunolic acid, arjunetin, berberine, piperine, resveratrol and withaferin-A in Ridayarishta formulation were found to be 1.76 ± 0.12, 1.51 ± 0.09, 1.85 ± 0.05, 3.2 ± 0.12, 1.21 ± 0.08, and 2.16 ± 0.09 ppm, respectively. Quantity of ethanol in Ridayarishta was found to be $7.95 \pm 0.023\%$ (V/V). Ridayarishta showed significantly higher ($P < 0.001$) IC_{50} value against CYP1A2 (IC_{50}–13.80 ± 1.96 μg/mL), 2C19 (IC_{50}–14.343 ± 2.28 μg/mL), 2D6 (IC_{50}–0.897 ± 0.28 μg/mL) and 3A4 (IC_{50}–32.057 ± 2.51 μg/mL) compared to positive controls such as furafylline, tranylcypromine, quinidine and ketoconazole respectively. Cocktail of herbal formulation and cardio protective, antihypertensive, anti-diabetic drugs showed significantly ($P < 0.001$and $P < 0.01$) less or negligible HDI.

Conclusion: Ridayarishta formulation alone and cocktail with amlodipine besilate, atenolol, atorvastatin, metformin, glipizide, glimepiride had negligible or insignificant effect on CYP450 inhibition. It may be concluded that consumption of Ridayarishta along with selective cardio protective, antihypertensive and anti-diabetic conventional medicine is safe with negligible or without any significant CYP450 (CYP1A2, 2C19, 2D6 and 3A4) inhibition mediated HDI.

1. Introduction

Arishtas are classical Ayurvedic formulation made with decoction of herbs. These unique liquid dosage forms contain self-generated alcohol which improves the efficacy of extraction of alcohol soluble molecules along with water soluble molecules from the herbs; to enhance drug delivery into human body site (Sekar and Mariappan, 2008). Arjunarishta is an Arishta formulation supports normal cardiac functions, loss of appetite and immune system traditionally (The Ayurvedic formulary of India, 2003). Ashwagandharishta formulation is being used in Ayurveda to relieve stress, reduces the chances of cardiovascular risk factors and mental disorders (The Ayurvedic formulary of India, 2003;

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Tiwari and Patel, 2010). Important precursors of Arjunarishta and Ashwagandharishta were selected based on Ayurvedic literature and blend them to get a proprietary formulation for two associated indications. Ridayarishta, an empirical novel formulation having cardio protective and stress reliever properties was developed by decoction of 25 major Ayurvedic plants (Supplementary Table S1), followed by self-fermentation process. Principal ingredients of Arjunarishta having cardioprotective properties and Ashwagandharishta having anti-stress activity were selected for preparation of this formulation.

Standardization parameter for Ayurvedic medicine with respect to bioactive compounds is vital to maintain quality control and batch to batch reproducibility (Pandit et al., 2011a). Standardization of Ayurvedic Arishtas formulation with respect to ethanol is foremost important to maintain its quality and efficacy. Among these herbs *Terminalia arjuna* W. and A., *Vitis vinifera* Linn. and *Withania somnifera* Dunal. were used in higher quantity. Arjunolic acid of *T. arjuna* is proven for prevention of myocardial necrosis, platelet aggregation and lowering of blood pressure, heart rate and cholesterol levels (Hemalatha et al., 2010). Cardio protective and antimicrobial activities of arjunetin have also been reported (Aneja et al., 2012). Cardio protective effect of *V. vinifera* derived polyphenol resveratrol has been well proven (Wu et al., 2011). Withaferin A, an active compound from *W. somnifera*, has been endorsed its usefulness for anti-inflammatory, cardio protective and vascular inflammatory diseases (Lee et al., 2012). Principle marker compounds such as Arjunolic acid, arjunitin, berberine (*Berberis aristata*), piperine (*Piper longum and Piper nigrum*), resveratrol and withaferin-A of these plants were selected in order to phytochemical standardization of Ridayarishta formulation.

Herb-drug interaction (HDI) study is the key marker for determination of adverse drug reaction (ADR) of herbal medicine and conventional pharmaceuticals. Among the Cytochrome P450 (CYP450) superfamily, 1, 2 and 3 are majorly responsible for xenobiotic and drug metabolism in human liver (Anzenbacher and Anzenbacherova, 2001). 5 human CYP isoforms (CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4) are responsible for 80% of the drug metabolism (Arora et al., 2015). CYP450 inhibition or induction is the most commonly studied mechanism for the HDI (Mukherjee et al., 2011; Shivaprasad et al., 2014). Intestinal and hepatic CYP450 is responsible for metabolism of numerous structurally unrelated compounds. Multi-drug combination therapy is now a common practice for numbers of diseases and the interaction between herbal and conventional drugs are inescapable (Mukherjee et al., 2011; Pandit et al., 2012). Thus, it is prime important to evaluate hepatic CYP450 mediated HDI of Ridayarishta formulation to get knowledge about any ADR. Based on above context, CYP1A2, 2C19, 2D6 and 3A4 inhibition mediated HDI of Ridayarishta was studied with cocktail of selective cardioprotective (atenolol), anti-hypertensive (amlodipine besilate, atorvastatin) and anti-diabetic (metformin, glipizide and glimepiride) drugs. High-performance thin layer chromatography (HPTLC) was used in order to standardize the Ridayarishta formulation against arjunolic acid, arjunetin, resveratrol and withaferin-A. Moreover, gas chromatographic (GC) analysis was also carried out for standardization of Ridayarishta formulation with respect to ethanol.

2. Material and methods

2.1. Chemicals

CYP1A2 (Catalogue no.: 459500), CYP2C19 (Catalogue no.: 459400), CYP2D6 (Catalogue no.: 459200) and CYP3A4 (Catalogue no.: 459100) high throughput inhibitor screening kit were procure from CORNING (Discovery labware, Inc., Woburn, MA, U.S). Kit components contains cDNA expressed recombinant human CYP1A2 (Cat. No. : HTS-703) using baculovirus infected insect calls, potassium phosphate (pH 7.4) buffer; Tris base, $NADP^+$, $MgCl_2$, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, CEC (3-Cyano-7-Ethoxycoumarin), Furafylline, CHC (3-cyano-7-hydroxycoumarin); CYP2C19 (Cat. No. : 04-80759), CEC, Tranylcypromine, CHC; CYP2D6 (Cat. No.: 04-80717), AMMC (3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarine), quinidine and AHMC (3-[2-(N,N-diethylamino) ethyl]-7-hydroxy-4-methylcoumarine hydrochloride); ketoconazole, BFC (7-benzyloxy-trifluromethylcoumarin); HFC (7-hydroxy-trifluromethylcoumarin). Fluorimetric screening was performed using the 96 well black-microplates (NUNC, Roskilde, Denmark). Tablets of amlodipine besilate (Stamlo), atenolol (Aten), atorvatatin calcium (Atocor), metformin hydrochloride (Glyciphage), glipizide (Glide), glimepiride (Glimulin) were purchased from local vendor. All the solvents and chemicals for standardization and sample preparation were of analytical grade and purchased locally. Arjunolic acid (purity $\geq$95%, LC/MS-ELSD), berberine (purity $\geq$97%, HPLC), piperine (purity $\geq$97%, HPLC), resveratrol (purity $\geq$99%, HPLC) and withaferin-A (purity $\geq$95%, HPLC) were procured from Sigma (Steinheim, Germany). Standard arjunetin (purity $>$95%, HPLC) was obtained from Natural Remedies Pvt Ltd. (Bangalore, India). 0.2 μ Nylon syringe filter was procure from membrane Solution (USA). Silica gel $60F_{254}$ precoated plates for HPTLC analysis and all analytical grade solvents were bought from Merck (Mumbai, India).

2.2. Ridayarishta formulation

Ridayarishta formulation was obtained from commercial batch (batch No FJ001, manufacturing date: May 03, 2015), prepared at production unit of Emami Limited, VAPI, Gujarat, India. Specimen of the formulation was retained at real time stability study storage chamber at Research & Development Center, Emami Limited, Kolkata, India. List of plants used for preparation of Ridayarishta formulation have been represented in Supplementary Table S1. Plant ingredients used in commercial batch preparation were collected from VAPI unit. Entire plant specimens were authenticated by Mr. Amalesh Nanda, pharmacognosist, Research & Development Center, Emami Limited, Kolkata, India. Voucher specimen (Supplementary Table S1) has been deposited at Research & Development Center, Emami Limited, Kolkata, India for further references.

2.3. Preparation of test samples

Ridayarishta formulation was extracted in chloroform. Solvent was completely removed by rotary evaporator. Extract was reconstitutes in methanol and used for HPTLC analysis. Standard arjunetin (2.5 μg/mL), arjunolic acid (2.5 μg/mL), berberine (1 mg/mL), piperine (0.1 mg/mL), resveratrol (2.5 μg/mL) and withaferin-A (3.0 μg/mL) were prepared in methanol for HPTLC analysis. Samples were filtered through 0.20 μm membrane filter prior to application. Standard ethanol and Ridayarishta formulation was prepared in water and filtered through 0.20 μm membrane filter for GC analysis. Different concentrations ranging from 0.0002 to 2162.7 μg/mL of Ridayarishta was prepared for fluorescence screening assay. Stock amlodipine besilate (25 μg/mL), atenolol (266 μg/mL), atorvastatin (60 μg/mL), metformin (165 μg/mL), glipizide (44.5 μg/mL) and glimepiride (49.2 μg/mL) solutions were prepared in mobile phase consist of acetonitrile and methanol, based on published C_{max} value (Liu et al., 2009, 2010; de Abreu et al., 2003; Zhi-yu et al., 2012, Kobylińska et al., 2000, Jovanović et al., 2006).