Journal of Traditional and Complementary Medicine 6 (2016) 130–133

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Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Short communication

Effect of *Coleus forskohlii* and its major constituents on cytochrome P450 induction

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ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form
2 July 2014
Accepted 13 August 2014
Available online 29 January 2015

Keywords:
Coleus forskohlii
Cytochrome P450
Drug interaction
mRNA expression
Standardization

ABSTRACT

Coleus forskohlii Briq. has been used traditionally for the treatment of several ailments since antiquity in Ayurveda. In the present study, an approach has been made to evaluate the effect of *C. forskohlii* and its major constituents on cytochrome P450 (CYP3A, CYP2B, and CYP2C) mRNA expression in rat hepatocytes. To gain better understanding of the herb–drug interaction potential of the chemical constituents present in *C. forskohlii*, the extract was subjected to column chromatography followed by standardization with respect to forskolin, 1-deoxyforskolin, and 1,9-dideoxyforskolin using reversed-phase high-performance liquid chromatography (RP-HPLC). Hepatocytes were treated with extracts, fractions, and phytoconstituents, followed by extraction and purification of total mRNA. Study of mRNA expression was carried out through reverse transcription polymerase chain reaction, followed by agarose gel electrophoresis. Results revealed that the test substances did not show any significant mRNA expression compared to the control against CYP3A, CYP2B, and CYP2C. Positive controls such as dexamethasone and rifampin showed significantly high ($p < 0.001$) induction potential compared to the control. It can be concluded that *C. forskohlii* and its major constituents may not be involved in CYP450 induction-based drug interaction.

1. Introduction

Coleus forskohlii Briq. (family: Lamiaceae) has a very long history of use in many traditional herbal medicines, with special reference to Ayurveda. It is considered to possess antianaphylactic, antiobesity, amebicidal, gastroprotective, bronchodilating, antiaging, antioxidant, anti-inflammatory, and anticancer activities. It is being used exclusively for weight management and hypotension.[1–3] Forskolin (1), 1-deoxyforskolin (2), and 1,9-dideoxyforskolin (3) are structurally related bioactive diterpenoids from *C. forskohlii* (Fig. 1). Compound 1 is exclusively known as a fat burner, commonly used for the treatment of obesity, hypothyroidism, allergies, asthma, eczema, psoriasis, and glaucoma.[4] *C. forskohlii* is one of the commercially important herbal ingredients for weight loss dietary supplements in the global market.[5] Its favorable effects on body fat management have been well established.[6,7]

Herb–drug interaction is important for assessing the safety of the use of herbal products. Most interactions are pharmacokinetic, and a major concern would be its effect on drug metabolism through interfering with CYP450.[8] More than 50% of clinically used drugs are metabolized by CYP4503A (CYP3A). CYP4502B (CYP2B) is capable of metabolizing 25–30% of substrates metabolized by CYP3A4, whereas CYP4502C (CYP2C) metabolizes 20% of the marketed drugs.[9–11] Induction of CYP450 leads to increases in the rate of metabolite production and hepatic biotransformation of coadministered drugs, and decreases in serum half-life and drug response.[9] There is now increasing literature reference to the actions of isolated bioactive constituents, and fractions or standardized whole plant extracts on the regulation of CYP450.[12,13] In the present study, commercial extracts and fractions of *C. forskohlii* were standardized by reversed-phase high-performance liquid chromatography (RP-HPLC) based on compounds 1, 2, and 3. The quantity of compound 1, 2, and 3 in the extracts and fractions were

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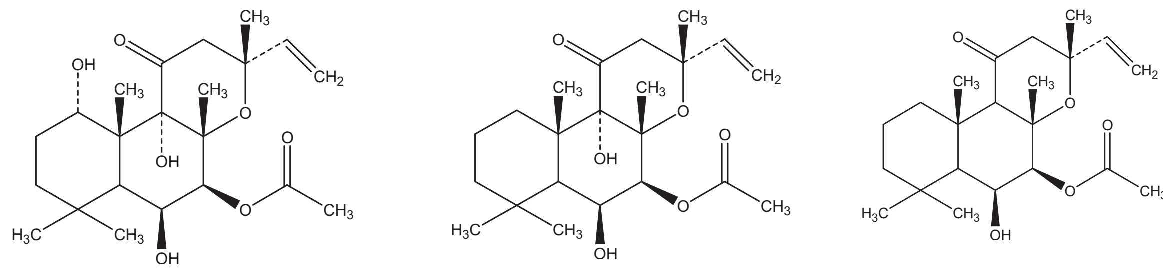
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.11.027



(1) (2) (3)

Fig. 1. Structure of forskolin (1), 1-deoxyforskolin (2), and 1,9-dideoxyforskolin (3).

determined. Further, the effect of extracts, fractions, and phytoconstituents on CYP3A, CYP2B, and CYP2C mRNA expression were evaluated in primary cultures of rat hepatocytes.

2. Materials and methods

2.1. Materials

HPLC-grade anhydrous potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), orthophosphoric acid (H_3PO_4), water, sodium carbonate (Na_2CO_3), analytical-grade dimethyl sulfoxide (DMSO) propanol, silica gel (60–120 mesh), and silica gel 60 F_{254} plates were procured from E. Merck Ltd. (Mumbai, India). Membrane filters (0.45 μm) were obtained from Millipore (Billerica, MA, USA). Standard 1 (assay ≥98%), 2 (assay ≥98%), 3 (assay ≥97%), 3-(4,5–dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide, fetal bovine serum, phosphate buffered saline, Dulbecco's modified Eagle's medium—high glucose, Ham's F-10 medium, and trypsin were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). EDTA, glucose, penicillin, and streptomycin were obtained from Hi-Media Laboratories Ltd, (Mumbai, India). Nuclease-free water, dithiothreitol, RiboLock RNAse inhibitor, deoxynucleotide mix, oligo dT, and Revert Aid reverse transcriptase were procured from Thermo Scientific (Waltham, MA, USA). Tri reagent from G Biosciences (St. Louis, MO, USA) was used for the study.

2.2. Plant material

Three different batches of dried *C. forskohlii* roots were purchased from a regular vendor (Olive Lifesciences Pvt. Ltd., Bangalore, Karnataka, India). Voucher specimens were deposited (VU/H/BD/07/14a, VU/H/BD/07/14b, and VU/H/BD/07/14c) at the Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal, India.

2.3. Extraction and fractionation

Roots (2.5 kg) were chopped and extracted with hot ethanol to produce reddish brown oleoresin (batch no. PM13038). The extract yield was 9.14% (W/W). This ethanol extract (CEE) was subjected to column chromatography (60–120 mesh silica) to obtain four different fractions [12.5%, 20%, and 50% ethyl acetate fraction (EAF) and 100% methanol fraction (MEF)]. Column chromatography was started with hexane, followed by ethyl acetate and methanol. Fractions were collected and combined based on the thin layer chromatography (TLC) profile. Another two batches (batch numbers PM13039 and PM13040) of extracts [yield: 8.77% (W/W) and 9.68% (W/W), respectively] were subjected to column chromatography. Ten percent standardized commercial *C. forskohlii* root extract (CCF) (batch no. OL130769) was prepared at the production unit of Olive Lifesciences Pvt. Ltd., and followed the standard operating procedure (SOP no. MMR-21044).

2.4. RP-HPLC analysis of extracts and fractions

The RP-HPLC system (Shimadzu, Kyoto, Japan) consisted of two LC-20AP controller pumps; an SPD-M20A PDA detector; and a SIL-10AP autosampler with a 20-μL loop and integrated LC solution software. A Phenomenex ODS2 (Phenomenex, Hyderabad, Andhra Pradesh, India) ($250 \times 4.6\ mm^2$; 5 μm) column (USA) was used for the stationary phase. Separation was achieved using a gradient elution program for pump A (0.136 g of KH_2PO_4 and 0.5 mL of H_3PO_4 in 1000 mL water) and pump B (acetonitrile) for 50 minutes, with a flow rate of 1.5 mL/min. Ideal resolution of chromatogram was achieved at 205 nm. Test samples were filtered through a Whatman NYL 0.45 μm syringe filter (Millipore) prior to injection. The amount of the phytoconstituents present in the respective extracts and fractions were determined from the calibration curve.

2.5. Cytotoxicity study

Cytotoxicity of the test samples was assessed based on the method described by Denizot and Lang.[14] The monolayer culture was incubated with test solution in Dulbecco's modified Eagle's medium (high-glucose medium) at 37°C for 3 days in a 5% CO_2 atmosphere.

2.6. Isolation of rat hepatocytes

Hepatocytes were isolated from male Sprague–Dawley rats using the modified collagenase digestion method, as described by LeCluyse et al.[15]

2.7. Treatment of hepatocytes

Hepatocytes were washed and treated with commercial extracts, fractions, and bioactive constituents within nontoxic doses (100 μg/mL) for 24 hours in Ham's F-10 medium. Cultures were processed for total RNA extraction. Dexamethasone was used as a positive inducer of CYP3A and CYP2B.[15,16] Rifampin was used as a positive control against CYP2C.[17]

2.8. Extraction and purification of total mRNA

Total mRNA was extracted from treated hepatocytes using Tri reagent, according to the protocol described by the manufacturer. The mRNA pellet was dissolved in nuclease-free water. Purity of