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Short communication

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

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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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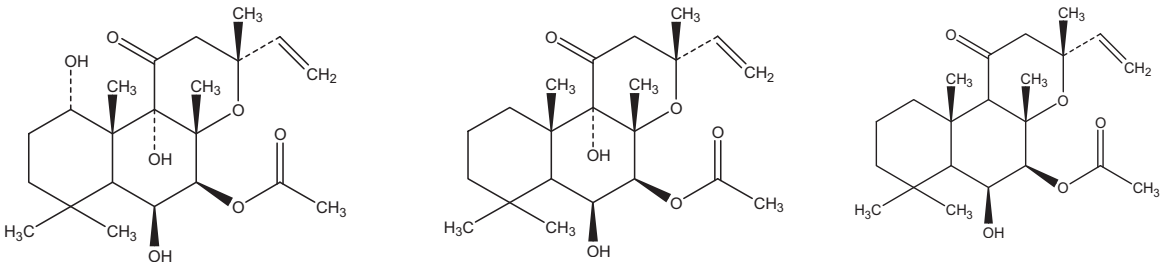

(1) (2) (3)

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 × 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

Table 1
RP-HPLC quantification of forskolin (1), 1-deoxyforskolin (2), and 1, 9-dideoxyforskolin (3) in extracts and fractions.

Sample	% of 1 (W/W)	% of 2 (W/W)	% of 3 (W/W)
CCF	11.03 ± 1.41	0.21 ± 0.04	4.60 ± 0.28
CEE	10.00 ± 0.81	1.09 ± 0.05	10.33 ± 1.08
12.5% EAF	0.00 ± 0.00	4.57 ± 0.20	16.35 ± 2.17
20% EAF	34.68 ± 1.46	18.45 ± 1.93	0.00 ± 0.00

Values are represented as mean ± SEM ($n = 3$).
CCF = 10% standardized commercial *C. forskohlii* extract; CEE = *C. forskohlii* ethanolic extract; EAF = ethyl acetate fraction; RP-HPLC = reversed-phase high-performance liquid chromatography; SEM = standard error of mean.

RNA was estimated using the 260/280 absorbance ratio, and samples were frozen at −80°C for further analysis.

2.9. Reverse transcription polymerase chain reaction

From the total RNA isolated from treated hepatocytes, cDNAs were prepared. The reaction mixture consisted of 1× cDNA synthesis buffer, dithiothreitol (0.5M), RiboLock RNAse inhibitor (20 U), deoxynucleotide mix (1.6mM), oligo dT (100 ng), reverse transcriptase (25 U), and total RNA. Primers for CYP3A and CYP2B were selected as per the method of Park et al.[18] Primers for CYP2C and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were selected following the method of Wauthier et al[19] and using the Gene runner software, ver. 3.05 (Hasting Software Inc., Hasting, NY, USA; Eurofins Bangalore, Karnataka, India), respectively.[19] The forward primers for CYP3A, CYP2B, CYP2C, and GAPDH were 5′ CCACCAAACCCATGCATACC 3′, 5′ AGCCGGGGTGTCTTCTATCT 3′, 5′ TGCCCCCTTTTTACGAGGCT 3′, and 5′ GTGAAGGTCGGTGTGAACGG 3′, respectively. Reverse primers were 5′ CAGTATCATGCTGCGGCTTC 3′, 5′ CAAAGTGGGCTTTTGCTCAT 3′, 5′GGAACAGATGACTCTGAATTCT 3′, and 5′ CACGCCACAGCTTTCCAGAG 3′ for CYP3A, CYP2B, CYP2C, and GAPDH, respectively. Polymerase chain reaction (MJ mini thermocycler; Bio Rad, Hercules, CA, USA) conditions were initial denaturation at 94°C for 5 minutes, followed by 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing of primers at 62°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 2 minutes.

2.10. Analysis of amplified sequences

The amplified samples were analyzed through 1.8% agarose gel electrophoresis. The gel was scanned with UV illumination using digital imaging (Alpha Digi DOC, Upland, CA, USA). Relative sample expression levels were calculated using Alpha View, version 3.3.1.0. (Cell Biosciences Inc., Santa Clara, CA, USA).

2.11. Statistical analysis

Experimental data were expressed as the mean ± standard deviation or standard error of mean. The results were subjected to one-way analysis of variance, followed by Dunnett's multiple-comparison test by fixing the significance level at $p < 0.05$, $p < 0.01$ and $p < 0.001$. The statistical analyses were performed using GraphPad Prism Version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results and discussion

Interactions of drugs with herbal preparations or foodstuffs present unique challenges due to the involvement of a number of unidentified chemical entities. Therefore, standardization parameters and marker profiling are highly important tools in maintaining batch-to-batch reproducibility of herbal drugs.[20–22] *C. forskohlii* extracts were subjected to column chromatography. Different fractions were collected, and finally four fractions were selected for a CYP450 induction study based on the TLC profile. Compounds 1, 2, and 3 in extracts and fractions were identified by comparing with the respective retention times of reference standards. Calibration curves showed good linearity in the concentration range of 100–800 μg/mL for compounds 1 and 3. In the case of standard 2,

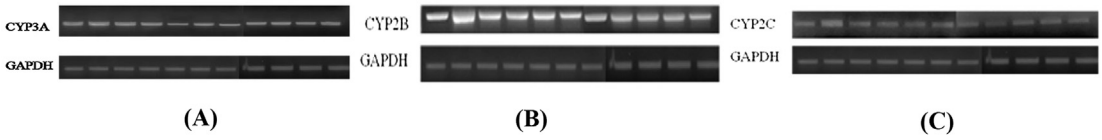

Fig. 2. Relative (A) CYP3A, (B) CYP2B, and (C) CYP2C mRNA expressions by CC, RIF, 1, 2, 3, CCF, 12.5% EAF, 20% EAF, 50% EAF, and MEF. CC = control; CCF = 10% standardized commercial *C. forskohlii* extract; CEE = *C. forskohlii* ethanolic extract; DEX = dexamethasone; EAF = ethyl acetate fraction; MEF = methanol fraction; RIF = rifampin; 1 = forskolin; 2 = 1-deoxyforskolin; 3 = 1,9-dideoxyforskolin.

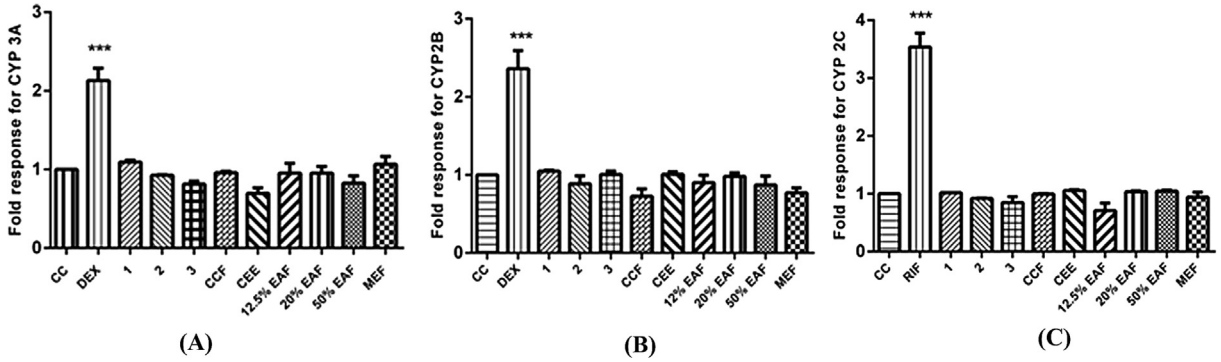

Fig. 3. Fold induction of (A) CYP3A, (B) CYP2B, and (C) CYP2C mRNA by RIF, 1, 2, 3, CCF, 12.5% EAF, 20% EAF, 50% EAF, and MEF; mean ± standard deviation; $n = 3$. One-way ANOVA was carried out, followed by Dunnett's multiple comparison test ($p < 0.001$ and above). ANOVA = analysis of variance; CC = control; CCF = 10% standardized commercial *C. forskohlii* extract; CEE = *C. forskohlii* ethanolic extract; DEX = dexamethasone; EAF = ethyl acetate fraction; MEF = methanol fraction; RIF = rifampin; 1 = forskolin; 2 = 1-deoxyforskolin; 3 = 1,9-dideoxyforskolin.

Table 2
Cytotoxicity assay of phytoconstituents, extracts, and fractions.

Sample	CTC_{50} value (μg/mL)
1	>1000 ± 0.93
2	>1000 ± 0.80
3	>1000 ± 0.67
CCF	346.66 ± 0.52
CEE	200.66 ± 0.73
12.5% EAF	215.49 ± 1.22
20% EAF	295.46 ± 1.30
50% EAF	360.93 ± 1.91
MEF	480.10 ± 4.37

Values are represented as mean ± SD ($n = 3$).
CTC_{50} = 50% cytotoxic concentration; CCF = 10% standardized commercial *C. forskohlii* extract; CEE = *C. forskohlii* ethanolic extract; EAF = ethyl acetate fraction; MEF = methanol fraction; SD = standard deviation; 1 = forskolin; 2 = 1-deoxyforskolin; 3 = 1,9-dideoxyforskolin.

the calibration range was 1–625 μg/mL. The percentages of compounds 1, 2, and 3 in CCF, CEE, and EAF are given in Table 1. The maximum quantity of compounds 1 and 2 was found in 20% EAF. The maximum yield of compound 3 was observed in 12.5% EAF (Table 1). In 50% EAF and MEF, the quantity of compounds 1, 2, and 3 was 0.

Relative mRNA expressions of CYP3A, CYP2B, and CYP2C by *C. forskohlii* extracts, fractions, and phytoconstituents on agarose gel are represented in Figs. 2A, 2B, and 2C, respectively. Values are depicted as arbitrary units normalized to (GAPDH) mRNA. The induction effects of extracts, fractions, and phytoconstituents on CYP3A, CYP2B, and CYP2C are shown in Figs. 3A, 3B, and 3C, respectively. Based on cytotoxicity results (Table 2), the test dose (100 μg/mL) was confirmed for mRNA expression study. Extracts and fractions did not show any significant expression of CYP3A, CYP2B, and CYP2C mRNA compared to the control. Positive controls such as dexamethasone (10μM) and rifampin (50μM) showed significant fold induction ($p < 0.001$) compared to the control. Results revealed that none of the studied phytoconstituents was involved in CYP3A, CYP2B, and CYP2C mRNA induction. CCF; CEE; 12.5%, 20%, and 50% EAF; and MEF did not show any significant mRNA expression against CYP3A, CYP2B, and CYP2C, compared to positive inducers.

Induction of CYP450 is of maximum concern during drug development due to their potential role in the metabolism of drugs. The mRNA expression assay is a widely accepted and reliable tool for determining the induction potential of test substances.[23] If a drug produces a change in the catalytic activity or mRNA expression that is >40% of that of the positive control, then it is considered an effective inducer. Moreover, the Food and Drug Administration (FDA) has validated the CYP450 induction study and recommended that induction value of the positive control should be twofold times greater than the blank.[24] Yokotani et al[25] reported that *Coleus* extract showed induction potential against CYP2C *in vivo*, as well as inhibition potential against CYP2C *in vitro*. In both *in vivo* and *in vitro* assays, the effect of main bioactive compound 1 was negligible. In the present *in vitro* assay, *C. forskohlii* extracts, fractions, and compounds 1, 2, and 3 did not show any significant CYP3A, CYP2B, and CYP2C induction potential compared to positive controls. Fold induction values of positive controls were two times more compared to the control, which confirmed the assay precision. The detail mechanisms of induction are not within the scope of the present study.

4. Conclusion

Based on the study results, it can be concluded that *C. forskohlii* and its major bioactive constituents may not be involved in CYP450 mRNA expression-based drug interaction, and safe to consume. Further studies using human hepatic mRNA and clinical trials are need.

Conflicts of interest

The authors have declared that there is no conflict of interest.

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