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ORIGINAL ARTICLE

The potential of *Hypoxis hemerocallidea* for herb–drug interaction

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Abstract

Context: Aqueous decoction of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae) (*Hypoxis*) is widely consumed in Southern Africa by people living with HIV/AIDS, some of whom are on ARV and other medications.

Objective: The aim of this study was to investigate the potential of the crude aqueous extracts of *Hypoxis* to inhibit major forms of CYP450 and transport proteins.

Materials and methods: Corms of *Hypoxis* were water-extracted and incubated (in graded concentrations: 1–100 μg/mL) with human liver microsomes (20 min) to monitor the effects on phenacetin *O*-deethylation, coumarin 7-hydroxylation, bupropion hydroxylation, paclitaxel 6α-hydroxylation, diclofenac 4′-hydroxylation, *S*-mephenytoin 4′-hydroxylation, bufuralol 1′-hydroxylation, chlorzoxazone 6-hydroxylation, midazolam 1′-hydroxylation and testosterone 6β-hydroxylation as markers for the metabolic activities of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5, respectively. The generation of metabolites were monitored and quantified with the aid of LC-MS/MS. The potential of the extracts to inhibit human ATP-binding cassette transporter activity was assessed using recombinant MDCKII and LLC-PK1 cells over-expressing human breast cancer resistant protein and human P-glycoprotein , respectively (with Ko143 and cyclosporin A as positive controls). Similar assessment was performed with human organic anion transporting polypeptide (OATP1B1 and OATP1B3) using recombinant HEK293 cells over-expressing OATP1B1 and OATP1B3, respectively (with rifamycin and 10 μM atorvastatin as positive controls).

Results: Extracts of *Hypoxis* inhibited the production of the metabolites of the substrates of the following enzymes (as compared to controls) with the indicated IC_{50} values (μg/mL): CYP1A2 (120.6), CYP2A6 (210.8), CYP2B6 (98.5), CYP2C8 (195.2), CYP2C9 (156) and CYP3A4/5 (185.4). The inhibition of the uptake activity of OATP1B1 and OATP1B3 were also observed with IC_{50} values of 93.4 and 244.8 μg/mL, respectively.

Discussion: Extract concentrations higher than the estimated IC_{50} values are achievable in the gastrointestinal tract when traditional doses of *Hypoxis* are considered. This may have profound effects on presystemic metabolism of the drug substrates. If absorbed, systemic inhibition of metabolic enzymes/transporters by *Hypoxis* may be expected.

Conclusion: The result suggests that there is the potential for HDI between *Hypoxis* and the substrates of the affected enzymes/transporters, if sufficient *in vivo* concentration of *Hypoxis* extracts is attained.

Keywords

Antiretroviral, cytochrome P450, drug metabolism, drug transport, inhibition, P-glycoproteins, traditional medicine, transport proteins

History

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Introduction

An estimated 80% of Africans are said to rely on traditional medicine for the management of various pathological conditions (UNAIDS, 2007). For the majority of this group of individuals, consultation with traditional health practitioners (THP) is the first call for health services (Babb et al., 2007). Sexually transmitted infections, cardiovascular disorders, psychiatry ailments, fever and skeletomuscular pain are some of the common reasons why THPs are consulted in Africa (Peltzer, 2001, 2003). The high burden of HIV/AIDS, especially in Africa, has attracted various government-backed policies designed to accommodate a holistic management approach including the use of traditional medicine. One such measure taken in South Africa is the recommendation of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae) (*Hypoxis*) among other herbal products, for people living with HIV/AIDS (Southern African Development Community, 2003). This is in addition to the introduction of free antiretroviral drugs (ARV). Thus, while the use of medicinal herb, a principal component of traditional medicine, predates the emergence of HIV/AIDS, there is a widespread consumption of herbal products for the management of HIV/AIDS either as complementary or

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alternative medicine (MacPhail et al., 2002; Malangu, 2007; Mills et al., 2005; Morris, 2002).

One other reported reason for the popularity and wide acceptability of herbal remedies by HIV/AIDS patients is the perceived adverse reactions to ARVs, especially in comparison to the herbal products that are erroneously believed to be free of side effects (Peltzer & Mngqundaniso, 2008). Cultural and traditional beliefs have advanced traditional health practices so that medicinal herbs are consumed as alternatives to ARVs. It has been reported that, although the South African National ARV Treatment Guidelines require patients to disclose intake of any other medication including traditional medicines to their healthcare providers, most patients do not (South African Department of Health, 2004). Thus, the number of HIV/AIDS patients who consume herbal medicine alone or concomitantly with ARVs may have been underestimated. Peltzer et al. (2011) reported in a cross-sectional study that up to 90% of respondents who were taking herbal therapies for HIV did not disclose this to their health care providers. Additionally, information on the therapeutic benefit and/or toxicology of herb–drug combination is sparse. One of the most important clinical consequences of such combinations is herb–drug interaction (HDI). This can result from herb-induced inhibition/induction of the metabolic enzymes and drug transporters leading to the alteration in the pharmacokinetic profiles of the ARVs. HDI in HIV/AIDS management may significantly compromise the goal of antiretroviral therapy and enhance toxicity and/viral resistance to ARV.

Widely known as African potato, *Hypoxis* is one of the most popular herbal remedies consumed among people living with HIV/AIDS in South Africa to boost immunity and enhance general well-being. It is a well-known genus of the Hypoxidaceae family and referred to variously in local languages as Afrika patat (Afrikaans), inkomfe (Zulu) and ilabatheka (Xhosa) (Marandola et al., 1997).

A geophyte with bright yellow flowers giving it its common name ''yellow stars'', *Hypoxis* has found to be used traditionally in the treatment of benign prostatic hyperplasia in South Africa (Buck, 1996; Lowe & Ku, 1996; Marandola et al., 1997); as purgative and delirium remedy among the Zulus of South Africa (Msonthi & Magombo, 1983); for the management of abdominal pain, fever, anorexia and vomiting by the Karanga people of Southern Africa (Watt & Breyer-Brandwijk, 1962); for uterine cancer in Swaziland (Amusan et al., 1995); and for sexual-related disorders in Mozambique (Banderia et al., 2001). Its major phytochemicals, beta-sitosterol, hypoxoside and rooperol, have been investigated clinically for cancer therapy (Albrecht et al., 1995; Berges et al., 1995; Klippel et al., 1997; Smit et al., 1995). Various other studies of the extracts in animal models have suggested antidiabetic (Ojewole, 2006), anticonvulsant (Ojewole, 2008), antidiarrheal (Ojewole et al., 2009a), anti-inflammatory (Ojewole, 2002), antimicrobial (Ncube et al., 2012), antioxidant (Laporta et al., 2007; Nair et al., 2007), bronchorelaxant (Ojewole et al., 2009b), hypoglycaemic (Mahomed & Ojewole, 2003), hypotensive (Ojewole et al., 2006) and uterolytic activity (Nyinawumuntu et al., 2008).

Currently, the consumption of *Hypoxis* among HIV/AIDS patients, most of who are on ARV, is popular (Malangu, 2007). The safety of such concomitant administration has not been demonstrated. This is necessary to ascertain the presence or absence of the potential for HDI. This may be mediated by the inhibition/induction of the metabolic enzymes and/or drug transporters by the phytochemical constituents of *Hypoxis*. HDI may be potentially deleterious in people living with HIV/AIDS because of the high number of medications used in antiretroviral therapy and its co-morbidity. *In vitro* liver-based technologies have been utilized to assess the potential for drug interactions with other drugs, food or herbs. Such investigations provide predictive mechanism for *in vivo* reality (Umehara & Camenisch, 2011). Earlier investigation by Mills et al. (2005) using microsome-based *in vitro* fluorometric microtitre plate assay had suggested that extracts of *Hypoxis* are capable of inhibiting the activity of CYP3A4 and P-glycoproteins. Information on the influence of *Hypoxis* on other CYP isoforms and transport proteins are generally lacking.

The current study therefore investigates the potential of the crude extracts of *Hypoxis* to inhibit the nine major cytochrome P450 (CYP) isozymes employing human liver microsomes (HLM), two efflux and two uptake proteins using cell lines expressing the transporter proteins.

Materials and methods

Plant materials

Fresh corms of *Hypoxis* were obtained in September 2010, from two South African traditional health practitioners (THPs) in Cape Town who identified the plant as *ilabatheka* in local Xhosa language. The samples were identified with the help of Kwaleta Sibuyile and Viola Kalitz of the Stellenbosch University botanical garden. The sample specimens were prepared and housed at the Division of Pharmacology, University of Stellenbosch. Information on the mode of use, dosage and specific HIV/AIDS-related indications were obtained from the same THPs and documented through semi-structured interview at the time of collection.

Ethical approval (as required because of the involvement of the THPs) was obtained from the University of Stellenbosch Health Research Ethics Committee with Ethics reference number N10/09/307.

Chemical compounds

The chemical and bio-reagents used in this study were obtained as follows: 1′-hydroxybufuralol maleate, 4′-hydroxymephenytoin, 6β-hydroxytestosterone and bufuralol hydrochloride from Ultrafine Chemicals (Pty) Ltd (Manchester, UK); 6-hydroxychlorzoxazone, 7-hydroxycoumarin, acetaminophen, bupropion, chlorzoxazone, coumarin, furafylline, NADPH, estradiol-17β-D-glucuronide (sodium salt), paclitaxel, tranylcypromine, troleandomycin (TAO), warfarin, rifamycin, thioconazole, digoxin, cyclosporin A and testosterone from Sigma-Aldrich (Pty) Ltd (St. Louis, MO); dimethylsulfoxide (DMSO), di-potassium hydrogen phosphate (K_2HPO_4), acetonitrile, formic acid and methanol from Merck Chemicals (Pty) Ltd (Darmstadt, Germany); [$^{13}C_6$] 4′-hydroxydiclofenac, [$^{13}C_6$] 7-hydroxycoumarin, [$^{2}H_3$] 4′-hydroxymephenytoin, diclofenac and paroxetine

Table 1. Probe substrates, microsomal protein concentrations and incubation times.

Enzyme	Probe substrate	Microsome concentration (mg protein/mL)	Probe substrate concentration (μM)	Literature Km value (μM)	Internal standard
CYP1A2	Phenacetin	0.2	10	9.0[a], 14[b], 31[c], 54[d]	[$^2H_4$] acetaminophen
CYP2A6	Coumarin	0.2	2.5	0.5	[$^{13}C_6$]7-hydroxy-coumarin
CYP2B6	Bupropion	0.1	25	76[e], 89[f],130[g]	[$^2H_6$] hydroxybupropion
CYP2C8	Paclitaxel	0.2	10	4.0[h], 15[i]	warfarin
CYP2C9	Diclofenac	0.1	5	3.4[j], 9.0[k]	[$^{13}C_6$] 4′-hydroxy-diclofenac
CYP2C19	*S*-mephenytoin	0.5	30	51[l], 42[m], 31[n]	[$^2H_3$] 4′-hydroxy-mephenytoin
CYP2D6	Bufuralol	0.2	5	13[o], 44[p]	[$^2H_9$] 1′-hydroxybufuralol
CYP2E1	Chlorzoxazone	0.5	10	22–49[q]	[$^2H_3$] 4′-hydroxy-mephenytoin
CYP3A4/5	Testosterone	0.2	30	50–60[r], 51[s]	[$^2H_3$] 6β-hydroxy-testosterone

[a]Tassaneeyakul et al., 1993; [b]Brøsen et al., 1993; [c]Venkatakrishnan et al., 1998; [d]Rodrigues et al., 1997; [e]Faucette et al., 2000; [f]Hesse et al., 2000; [g]Li et al., 2003; [h]Rahman et al., 1994; [i]Cresteil et al., 1994; [j]Transon et al., 1996; [k]Bort et al., 1999; [l]Coller et al., 1999; [m]Venkatakrishnan et al., 1998; [n]Schmider et al., 1996; [o]Boobis et al., 1985; [p]Yamazaki et al., 1994; [q]Peter et al., 1990; [r]Draper et al., 1998; [s]Kenworthy et al., 2001.

from Novartis Pharma AG (Basel, Switzerland); hydroxybupropion, [$^2H_6$] hydroxybupropion and [$^2H_9$] 1′-hydroxybufuralol from BD Biosciences (Pty) Ltd (San Jose, CA); 6α-hydroxypaclitaxel from Gentest BD Biosciences (Woburn, MA); [$^2H_3$] 6β-hydroxytestosterone from Cerilliant Chemicals (Pty) Ltd (Round Rock, TX); phenacetin, magnesium chloride hexahydrate, ticlopidine hydrochloride and potassium dihydrogen phosphate (KH_2PO_4) from Fluka Chemicals (Pty) Ltd (Buchs, Switzerland); 4′-hydroxydiclofenac from Calbiochem (Pty) Ltd (La Jolla, CA), (3 S,6 S,12aS)-1,2,3,4,6,7,12,12 a-Octahydro-9-methoxy-6 -(2-methylpropyl)-1,4-dioxopyrazino[1′,2′:1,6]pyrido[3,4-b]indole-3-propanoic acid 1,1-dimethylethyl ester (Ko143) from Tocris Bioscience (Pty) Ltd (Ellisville, MI); 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-2 (PhIP) and its radio-labeled form (1.85 MBq/nmol, radiochemical purity >99%) from Toronto Research Chemicals (Pty) Ltd (North York, Canada); radio-labeled [^{3}H] digoxin (1.103 MBq/nmol, radiochemical purity >97%) and [^{3}H]estradiol-17β-D-glucuronide (1.72 MBq/nmol, radiochemical purity>97% from Perkin Elmer Radiochemicals (Pty) Ltd (Waltham, MA); zosuquidar trihydrochloride (LY335979) from Chembiotek (Pty) Ltd (Kolkata, India). Other reagents were of analytical grade.

Assay enzymes and cells

Pooled mixed gender HLM prepared from 50 individual donors with total CYP and cytochrome b_5 content of 290 pmol/mg protein and 790 pmol/mg protein, respectively, was obtained from Gentest BD Biosciences (Woburn, MA). The catalytic activities of enzymes were provided by the manufacturer.

Extraction of plant material

The air-dried and powdered corms of *Hypoxis* were extracted with water in a round bottom flask. After the initial constant stirring for 2 h, the mixture was allowed to extract for 24 h, decanted, and centrifuged (20 000 rpm, 5 min). The supernatant was filtered (0.45 μL; Whatman International LTD, Maidstone, England) and freeze dried. The dried extract, henceforth referred to as HP, was reconstituted in water and stored at − 20 °C for further use.

Incubation in HLM

Thawed HLM were diluted with potassium phosphate buffer (50 mM; pH = 7.4) and incubated in 96-well plate format. Graded concentrations of HP were prepared in water such that the addition of 1 μL to 200 μL incubation mixture yielded a final extract concentration of 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL, respectively. Appropriate CYP substrate (Table 1) was added and the mixture pre-incubated at 37 °C for 10 min using an IS89 96-well plate incubator (Wesbart, Leimuiden, The Netherlands). Metabolic reactions were initiated by adding NADPH (1 mM) and magnesium chloride (5 mM) solution, and terminated after 20 min through the addition of formic acid (10 μL; 50%). All incubations were performed in duplicate. Control incubations contained CYP-specific inhibitor, and incubations without inhibitors. The final concentration of organic solvents in the incubations was less than 0.5% (v/v). Probe substrate concentrations used were less or equal to published K_m values.

Quantitative analysis of metabolites

LC-MS methods were developed to simultaneously analyze the metabolites of bupropion, paclitaxel, diclofenac and bufuralol (Group A), phenacetin, coumarin and testosterone (Group B); and *S*-mephenytoin with chlorzoxazone (Group C) with the appropriate internal standard (Table 1). Total separation and elution of the analytes were achieved within 10 min retention time, using the Luna Phenyl-Hexyl [3 μm, inner dimensions 50 × 1 mm (Phenomenex, Torrance, CA)] column (30 °C), in a dual mobile phase of water and acetonitrile (each containig 1% v/v formic acid), with a gradient (group A) and isocratic (group B and C, 70:30) flow set at 60 μL/min.

Before chromatographic analysis, samples were pooled according to the groups and subjected to solid-phase extraction using OASIS HLB 96-well plate 30 μm (30 mg) elution plate (Waters, Milford, MA). This was performed by sequential washing with 1 mL each of water and water-methanol (95/5; v/v) followed by two-time elution with 1 mL methanol. The elutes were dried employing the 96-well Micro-DS96 evaporator (Porvair Sciences Ltd., Shepperton, UK) at 37 °C and reconstituted in 100 μL of 10% acetonitrile containing 0.1%

formic acid for LC-MS analysis. Enzyme activity was measured in terms of the production of the CYP-specific metabolite. Relative activity (100%) was defined in terms of metabolite production in the absence of inhibitor. The enzyme inhibition parameter (IC_{50}) was calculated by employing the kinetic equation for sigmoid curves (Equation 1) where x = concentration; y = relative enzyme activity; and s = slope factor.

$$y = \frac{100\%}{1 + \left(\frac{x}{IC_{50}}\right)^2}. \quad (1)$$

Investigation of the inhibitory effects of *Hypoxis* on P-gp and BCRP

HP was soluble up to a final concentration of 500 µg/mL in P-gp and BCRP uptake buffers. LLC-PK1 cells stably transfected with human P-gp were grown and maintained in Medium 199 supplemented with 10% FBS, 50 ng/µL gentamycin and 100 ng/µL hygromycin B at 37°C under an atmosphere of 5% CO_2. MDCKII cells stably transfected with human BCRP were grown and maintained in DMEM supplemented with 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin at 37°C under an atmosphere of 5% CO_2. Cells ($\sim 0.6 \times 10^5$ cells per well for LLC-PK1-P-gp and MDCKII-BCRP transfectants) were seeded into Falcon® clear bottom 96-well plates (Becton Dickinson) with 200 µL of culture medium and the assay was performed after 24 h.

Graded concentrations of HP were prepared in HBSS containing 12.5 mM HEPES adjusted with HCl to pH 7.4 (for P-gp) and OPTIMEM containing 12.5 mM HEPES adjusted to pH 7.8 (for BCRP). The assay procedure was begun by the aspiration and replacing the culture medium with pre-incubation uptake buffer system containing HP or the specific inhibitors as positive control (10 µM cyclosporin A for P-gp and 1 µM Ko143 for BCRP). Plates were subsequently incubated at 37 °C for 10 min. Transporter-mediated uptake was initiated by the addition of the radiolabeled probe substrates (1 µM digoxin for P-gp and 1 µM PhIP for BCRP). The uptake reaction was terminated after 40 min by removing the incubation solution. The cells were then washed twice with ice-cold PBS buffer and monolayer integrity was assessed optically. This is followed by the dissolution of the cells by the addition on 0.2 N NaOH (200 µL per well) and 20 min at 37°C incubation. Dissolved cells were then transferred to scintillation vials containing scintillation cocktail and uptaken substrates were determined using the scintillation counter.

All uptake studies were performed in triplicate in an incubator without shaking. Methanol/DMSO was always below $1\%^{v}/_{v}$ of the total volume and all solutions used in cell growth were maintained at the appropriate pH.

Investigation of the inhibitory effects of *Hypoxis* on OATP1B1 and OATP1B3

HEK293 cells stably transfected with human OATP1B1 were grown and maintained in DMEM supplemented with 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin, and 50 ng/µL hygromycin B at 37°C under an atmosphere of 5% CO_2. HEK293 cells stably transfected with human OATP1B3 were grown and maintained in DMEM supplemented with 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin and 0.8 mg/mL G418 at 37°C under an atmosphere of 5% CO_2. Cells ($\sim 0.2 \times 10^6$ cells per well) for overexpressing OATP1B1 and OATP1B3 were seeded into pre-coated (poly-L-lysine, poly-L-ornitin, 0.1 mg/mL) clear bottom 96 well plates (Corning Product No 734-1795) with 200 µL of culture medium. Graded concentrations of HP were prepared in HBSS containing 12.5 mM HEPES adjusted with HCl to pH 7.4. Uptake studies, performed 72 h after seeding was started by aspirating and replacing the culture medium with the final incubation buffer system containing HP or the positive control inhibitor (20 µM rifamycin and 10 µM atorvastatin). The uptake reactions were terminated after 5 min by removing the incubation solution. Subsequently, the wells were washed twice with ice-cold PBS buffer and monolayer integrity was assessed optically. Radioactive samples were analyzed by liquid scintillation counting in a similar procedure as described above. All uptake studies were performed in triplicate in an incubator without shaking.

Drug uptake clearance calculations

Probe substrate uptake clearance (nL/min/mg protein) by the stably transfected cells expressing the transporter proteins was determined from the specific amount of radiolabeled probe substrate inside the cells divided by the concentration in the incubation medium and normalized to the incubation time and the mean protein concentration measured in test wells.

Absolute transporter uptake data were converted into relative inhibition values by defining membrane permeability of the probe substrate in the absence of inhibitor as 0% inhibition while the positive control exerted 100% transporter inhibition. Relative inhibition (%) was profiled against inhibitor concentration and the IC_{50} calculated using the nonlinear-regression method.

The IC_{50} values (inhibitor concentration that causes 50% inhibition of the maximal drug effect) were calculated using the following equation:

$$y = y0 + \frac{a \cdot I^n}{IC_{50}{}^n + I^n} \quad (2)$$

where n is the slope factor (Hill coefficient), $y0$ is the relative baseline inhibition and a is the maximal transporter inhibition (%).

Results

Influence of HP on the metabolic activity of CYPs

A concentration-dependent inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5 was observed with HP. The extract concentration profiled against relative enzyme activity is shown in Figures 1 and 2. The estimated IC_{50} values are presented in Table 2. Within the concentration tested (100 µg/mL), no significant inhibition of CYP2C19, CYP2D6 and CYP2E1 was observed.

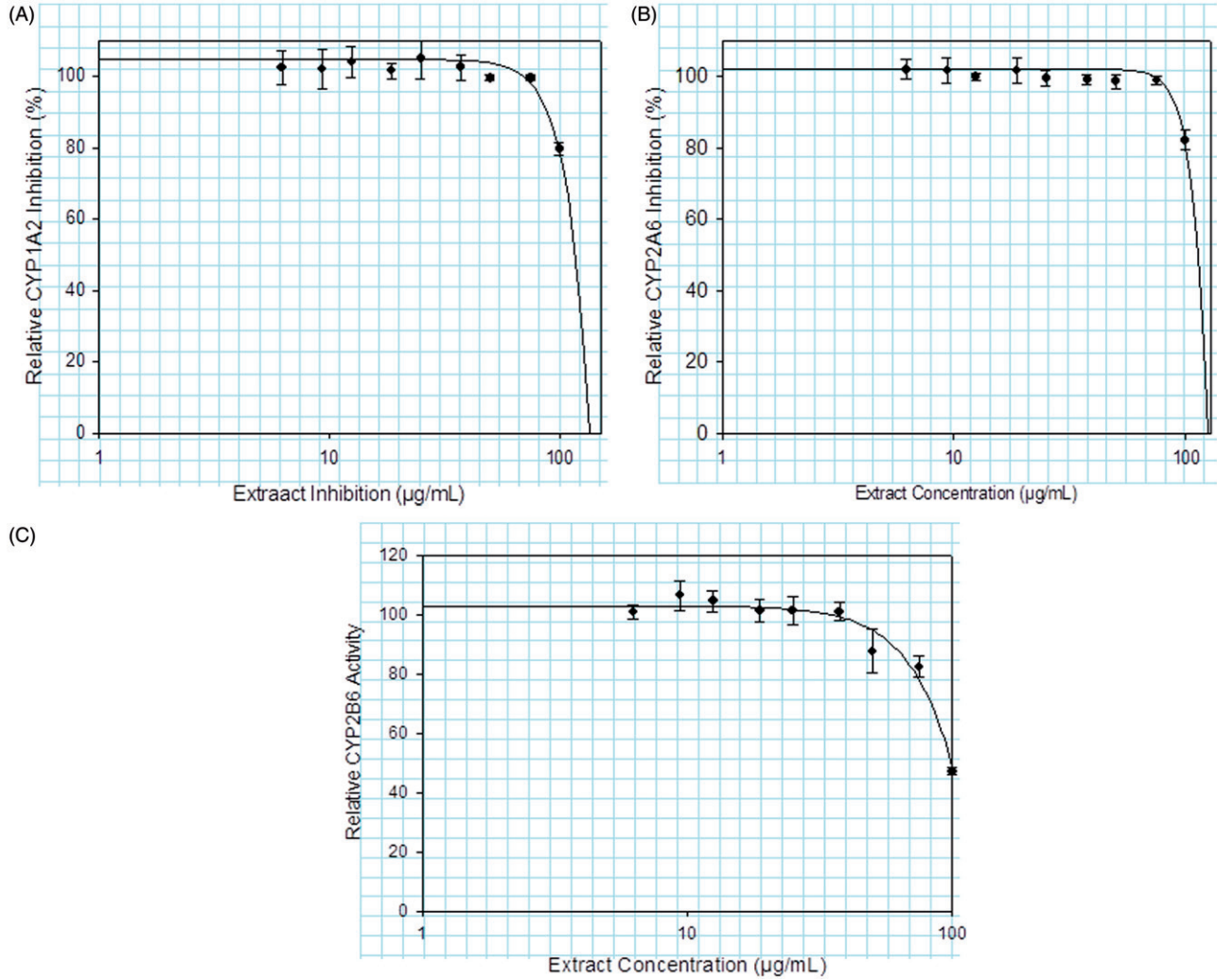

Figure 1. Effect of the extracts of *Hypoxis hemerocallidea* on (A) CYP1A2-mediated phenacetin deethylation; (B) CYP2A6-mediated coumarin 7-hydroxylation, and (C) CYP2B6-mediated bupropion hydroxylation.

Influence of *Hypoxis* on transport proteins

The uptake activity of OATP1B1 and OATP1B3 was inhibited by HP in a concentration-dependent manner. No inhibitory activity of P-gp and BCRP was observed with up to 500 µg/mL of HP. The effect of increasing concentration of HP on the activity of the transport proteins is illustrated in Figures 3 and 4. An overall summary is provided in Table 3.

Discussion

The popularity of *Hypoxis hemerocallidea* as a medicinal herb in Africa, especially among people living with HIV/AIDS, most of who are on ARV, increases the likelihood for its combination with ARV. Additionally, its other traditional indications may encourage its use with orthodox medicines used for the treatment of such diseases. The obvious risk of HDI in herb-drug combination is worth considering especially with *Hypoxis*; as such information is not available. Thus, this study presents the first comprehensive evaluation of the effects of *Hypoxis* on the activity of important P450s and transport proteins.

Hypoxis was obtained from THPs for this study. THPs are considered representative source of *Hypoxis* to reflect the actual practice of patients' patronage. Although, some products of *Hypoxis* are now available in open shops, the majority of Africans, for cultural and traditional reasons, consult THP to obtain it. The samples were water-extracted. This is because, as obtained from the consulted THPs, the popular mode of *Hypoxis* use is as aqueous decoction. Thus, the patients are exposed to the water-extracted constituents.

While various liver-based technologies are available for assessing drug–drug and HDI, HLM is the most common, and well-accepted. It has been widely used to assess the HDI potential of a number of herbal products. The outcome of such studies provides an indication for clinically significant interactions. Results from *in vitro* metabolic studies have been extrapolated for *in vivo* correlation with high degree of predictability (Tassaneeyakul, 1993). The use of HLM with CYP-specific substrates, while monitoring the production (through identification and quantification) of metabolites from CYP-specific probe reactions as we did, is considered closer to humans (in terms of results interpretation) than the methods of Mills et al. (2005). Our study is therefore significantly different in scope, methodology and comprehensiveness than earlier similar studies.

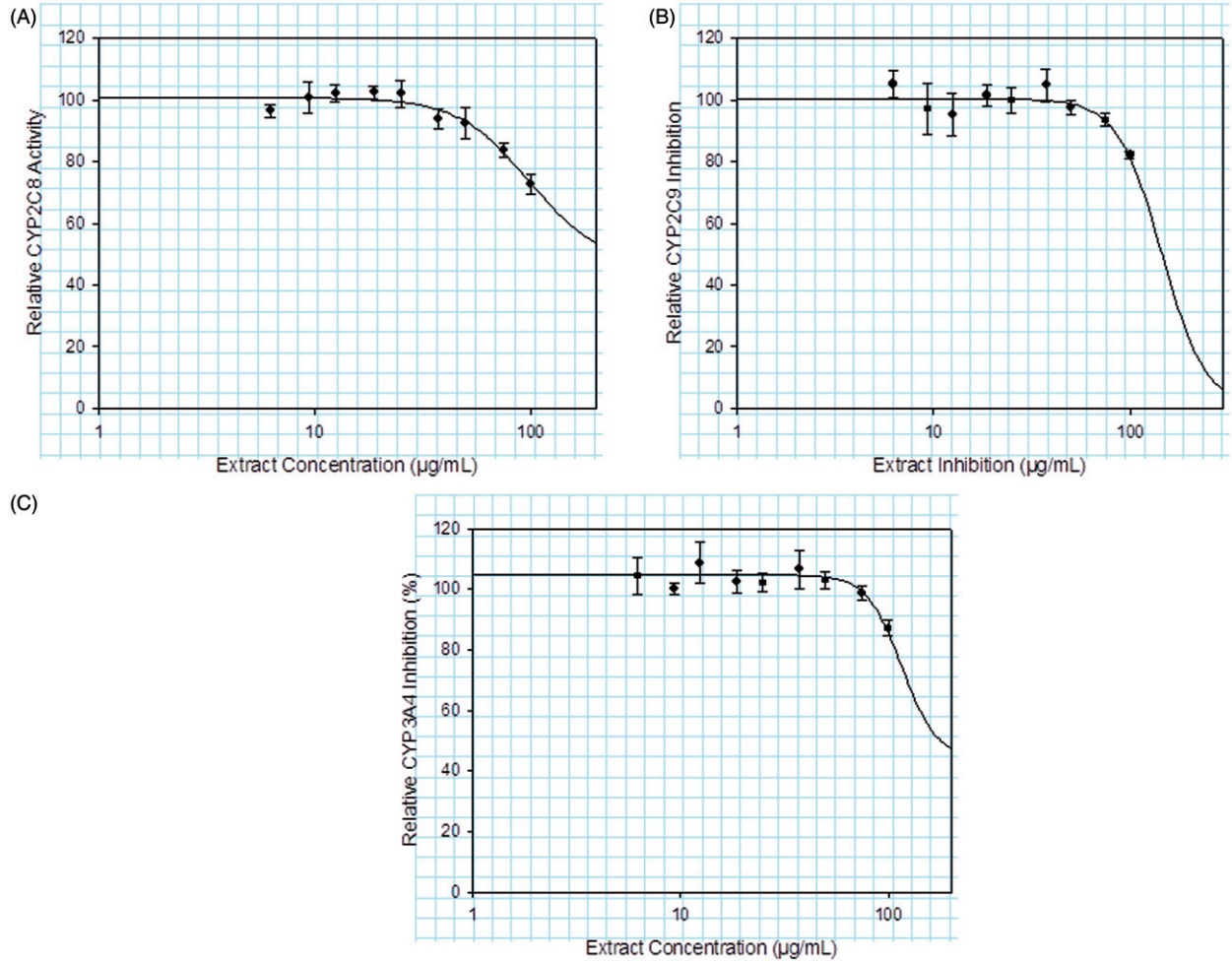

Figure 2. Effect of the extracts of *Hypoxis hemerocallidea* on (A) CYP2C8-mediated paclitaxel 6α-hydroxylation; (B) CYP2C9-mediated diclofenac 4′-hydroxylation, and (C) CYP3A4/5-mediated testosterone 6β-hydroxylation in pooled human liver microsomes.

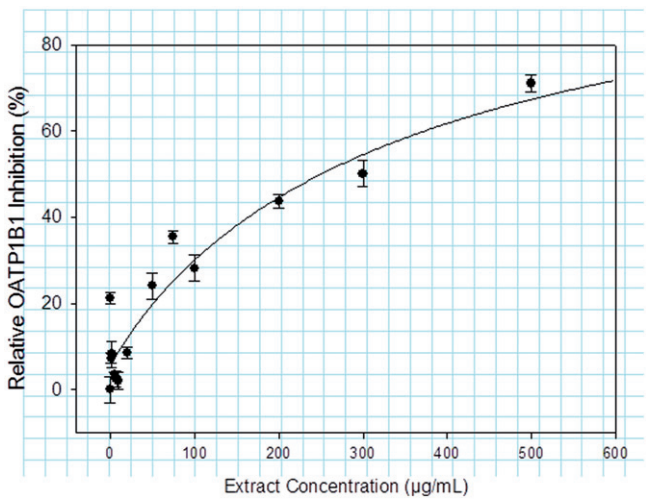

Figure 3. Effect of varying concentrations of the extracts of *Hypoxis hemerocallidea* on estradiol-17β-D-glucuronide uptake by OATP1B1 expressing HEK293 cells. The line represents the ''best fit'' of the data to the equation 2 ($R^2 = 0.99$) which are averages of triplicate determinations and the bars represent the standard deviations.

In the average, about 1000 mg of *Hypoxis* is extracted for singular consumption. The achievable gastrointestinal concentration is higher than the determined IC_{50} values for the enzymes/transported that were inhibited in this study (Table 4). This can have potential deleterious effects on intestinal CYP and transporters with attendant effects on drug absorption. Drugs like the viral protease inhibitors, calcium channel receptor blockers, various chemotherapeutic agents, statins, for example, undergo significant pre-systemic intestinal metabolism. The inhibition of the intestinal CYP may alter the pharmacokinetic profile of such drugs with the potential of exceeding safety margins. This will be more important for drugs with narrow therapeutic window.

The inhibitory effects observed in this study were based on singular exposure of the enzymes to the herbal extract. In reality, herbal products are consumed repetitively over a long

Table 2. The potency of inhibitory effects of HP on CYP expressed in IC_{50} values.

CYP enzyme	Probe reaction	IC_{50} value (µg/mL)
CYP1A2	Phenacetin *O*-deethylation	120.6 ± 5.1
CYP2A6	Coumarin 7-hydroxylation	210.8 ± 8.5
CYP2B6	Bupropion hydroxylation	98.5 ± 3.3
CYP2C8	Paclitaxel 6α-hydroxylation	195.2 ± 3.8
CYP2C9	Diclofenac 4′-hydroxylation	156.0 ± 4.5
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	n.i.o.
CYP2D6	Bufuralol 1′-hydroxylation	n.i.o.
CYP2E1	Chlorzoxazone 6-hydroxylation	n.i.o.
CYP3A4/5	Testosterone 6β-hydroxylation	185.4 ± 9.3

n.i.o. = no inhibition observed.

Table 3. Summary of inhibitory effects of HP on transporter proteins.

Transporter	Probe substrate	IC_{50} value[a,b] (μg/mL)	Ki[c] (μg/mL)	Max. inhibition[b,d] (%)
P-gp	Digoxin	Not observed	Not applicable	Not observed
BCRP	PhIP	Not observed	Not applicable	Not observed
OATP1B1	Estradiol-17β-D-glucuronide	118.7 ± 33.3	93.44	71.29[d]
OATP1B3	Estradiol-17β-D-glucuronide	290.1 ± 59.7	244.79	93.95[d]

[a]HP concentration estimated to inhibit transporter activity by 50%; [b]Data used for curve fitting are mean ± SD of $N = 3$.
[c]Calculated with $Ki = IC_{50}/(1 + S/Km)$, where S is the substrate concentration of the probe substrate and Km is the transporter affinity of the probe substrate according to Michaelis–Menten.
[d]Maximal observed inhibition with respect to positive control.

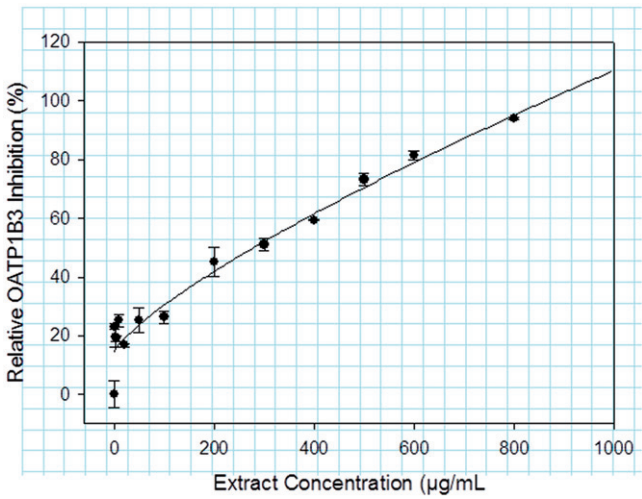

Figure 4. Effect of varying concentration of the extracts of *Hypoxis hemerocallidea* on estradiol-17β-D-glucuronide uptake by OATP1B3 expressing HEK293 cells. The line represents the ''best fit'' of the data to the equation 2 ($R^2 = 0.98$) which are averages of triplicate determinations and the bars represent the standard deviations.

period of time. The effects on metabolic enzymes may be more pronounced.

Information on the bioavailability of the extracts or constituents of *Hypoxis* is not available. Thus, it is difficult to predict the effects on systemic CYP and transporters. However, with claims of its systemic activity as immune booster and for other systemic indications, its phyto-constituents are expected to be absorbed for efficacy. Since OATP1B1 and OATP1B3 are located at the sinusoidal membrane of the liver, the inhibitory effects of *Hypoxis* on the OTA1B1 and OTP1B3 will only be important if there is absorption. Absorbed constituents may thus inhibit systemic CYPs, OATP1B1 and 1B3 with consequent alteration of the pharmacokinetic profile of co-administered drugs.

With the increasing popularity of herbs among people who also consume orthodox medications in Africa, it is important to understand the potential for HDI. Such information will be useful to health care professionals, the consuming public and policy makers.

There are few factors that may limit the clinical applicability of this study. *Hypoxis* was obtained from THPs just as their clients do. Aqueous extracts were also used to reflect the popular use of water-decoctions in reality. Thus, the approach adopted in this study represents the popular practice in source and use. Variations in phytochemical composition of herbs are known to occur depending on the place and time of harvest.

Table 4. Interpretation of the *in vitro* findings.

Part used in traditional practice	Leaves
Usual human dose (single; mg)	1000
Extraction yield ($\%^{w}/_{w}$)	12.6
Estimated extract per dose (mg)	126
Putative GIT concentration (μg/mL)[a]	504
In vitro IC_{50} range (μg/mL)	98–244
Potential for clinically significant HDI[b]	Yes

[a]Estimated GIT fluid = 250 mL, with the assumption that the 60% methanol-soluble extracts are extracted in the GIT.
[b]This refers to HDI in the GIT with possible effects on drug absorption.

Such variation is not accounted for in this study. In addition, there are commercial forms of *Hypoxis* including capsules of dried powdered corms. The consumption of such capsules may expose consumers to non-water-soluble constituents. The range of extract concentration tested is arbitrary and methodology-limited. This is because information on the achievable plasma levels of the extracts is not available. The exact active phytochemicals exerting the inhibitory effects is not known. Although *in vitro* HDI studies are the main source of information on the potential for *in vivo* HDI, *in vivo* human studies are the ultimate proof of clinically significant HDI.

Hypoxis may inhibit the metabolic clearance of co-medications metabolized by CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP3A4/5, OATP1B1 and OATP1B3 if sufficient *in vivo* concentration is achieved. This presents a potential for pharmacokinetic HDI if *Hypoxis* is consumed with drug substrates of these enzymes and/or transporters. Within the concentration tested, *Hypoxis* is not expected to inhibit human CYP2C19, CYP2D6, CYP2E1, P-gp or BCRP. It is therefore advisable to take caution in *Hypoxis*-ARV combination use.

Declaration of interest

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