International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 3 (10), pp. 001-008, October, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

In vitro inhibitory effects of *Hypoxis obtusa* and *Dicoma anomala* on cyp450 enzymes and p-glycoprotein

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Accepted 21 July, 2012

Herbal medicines have been shown to cause clinically significant interactions with conventional medicines. Herbal medicines can alter the activity of drug- metabolizing enzymes and transporters, potentially resulting in herb -drug interactions. However, such information is generally not yet available for herbal medicines used only in developing nations. The inhibitory effects of crude extracts of *Hypoxis obtusa* and *Dicoma anomala* on cytochrome P450 (CYP) isoforms 1A2, 2C9, 2C19, 2D6 and 3A4 were evaluated in vitro using human liver microsomes. Herbal extracts at concentrations of 50 and 250 g/ml were tested for inhibition of P-glycoprotein (Pgp) transport across Caco-2 cell monolayers using 4.3 nM [3H]-digoxin as the substrate. Extracts of Hypoxis inhibited CYPs strongly, while extracts of Dicoma were moderate CYP inhibitors. The IC50 values for methanol and water extracts on CYP3A4 were 0.20 and 1.0 mg/ml for *Hypoxis* and 1.2 and 2.4 mg/ml for *Dicoma*. In the Pgp inhibition experiments, the (basolateral to apical) / (apical to basolateral) digoxin efflux ratios did not change significantly in the presence of the herbal extracts. Whilst, the extrapolation of the data to in vivo is rather speculative, there is need for assessment of the clinical significance of Hypoxis - drug interactions.

Key words: Herb-drug interactions, cytochrome P450, P-glycoprotein, *Hypoxis obtusa*, *Dicoma anomala*.

INTRODUCTION

Use of herbal medicines and conventional medicines in combination poses the risk of herb-drug interactions. Drug interactions can result in therapeutic failure due to inhibition or induction of metabolism or transport. CYP3A-metabolized drugs such as mibefradil, terfenadine, astemizole and cisapride have been remo-ved from the pharmaceutical market due to inhibition-based metabolic interactions which may result in life-threatening “torsades de pointes” (Huang, 2004). Drugs which are CYP3A substrates are often also substrates of the efflux transporter P-glycoprotein (Pgp) found in the gut and liver (Benet et al., 2004). Intestinal Pgp limits absorption of its substrates; thus, inhibiting or inducing it will increase or decrease their systemic availability.

It is estimated that 65 - 80% of the people in developing nations use herbal medicines as a primary source of healthcare (Mahady, 2001; Kim et al., 2004; Kim, 2005). In resource- poor settings in the face of the HIV/AIDS pandemic and lack of access to antiretroviral drugs, many people living with HIV and AIDS have resorted to using herbal medicines as an alternative.

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South Africa's Ministry of Health recommends use of African potato (*Hypoxis hemerocallidea* and *Sutherlandia*) in HIV and AIDS management along with anti-retroviral drugs (Mills et al., 2005b). In addition, *H. hemerocallidea* has been shown to have a wide range of pharmacological activities from anti-inflammatory, anti-neoplastic, antioxidant, anti-diabetic to anti-infective properties (Owira and Ojewole, 2009). Members of the Southern African Development Community have also advocated for the use of herbal medicines to combat HIV (Giraldo, 2002). This situation has increased the risk of safety issues arising from potential herb-drug interacttions. *Dicoma anomala* is used medicinally as a root decoction for blood disorders, colic, diarrhoea, dysentery, toothache, fever, haemorrhoids and purgative for intestinal worms. The plant parts are used for coughs, respiratory conditions and the root can be chewed to induce vomiting (Mnengwane and Koekemoer, 2007).

Considerable research has been done on herbal medicines used in Western countries and some have been found to interact significantly with conventional medicines, including life-saving antiretroviral drugs e.g. St John's Wort, associated with increased elimination of indinavir (Piscitelli et al., 2000) and grapefruit juice which causes significant interactions with CYP3A4-substrate drugs (Lundahl et al., 1995; Bailey et al., 1998). *Ginkgo biloba* extracts significantly increased area under the plasma concentration-time curve of talinolol, a Pgp substrate, after multiple dosing (Fang et al., 2009). However, such information is generally not yet available for herbal medicines used only in developing nations, although a companion study from our laboratories investigating the inhibition of *Moringa oleifera* leaf extracts on CYP3A4 metabolism was recently published (Monera et al., 2008). The aim of the present study was to evaluate the effects on drug metabolizing enzymes and transporters *in vitro* by two further selected African herbal medicines.

MATERIALS AND METHODS

NADPH, testosterone, 6ß-hydroxy testosterone and other specific probe substrates and metabolite standards were purchased from Sigma-Aldrich (St. Louis, MO). Ketoconazole was obtained from the U. S. Pharmacopeia (Rockville, MD). [3H] -digoxin (21.8 Ci/mmol) and [3H]-mannitol (53.7 mCi/mmol) were obtained from PerkinElmer Life Sciences (Boston, MA). N-(4-[2-(1, 2, 3, 4-tetrahydro-6, 7-dimethoxy-2-isoquinolinyl) ethyl] -phenyl)-9, 10-dihydro-5- methoxy-9-oxo-4-acridine carboxamide (GG918, also known as GF120918; GlaxoSmithKline, Research Triangle Park, NC) was kindly supplied by the manufacturer. NADPH regenerating system reagents were obtained from BD Biosciences (San Jose, CA). High performance liquid chromatography-grade methanol was purchased from Thermo Fisher Scientific (Waltham, MA). All other reagents used were of analytical or HPLC grade. Pooled mixed- sex human liver microsomes (HLM) were obtained from Celsis *in Vitro* Technologies (Baltimore, MD). Recombinant human CYP1A2 expressed in *Escherichia coli* was obtained from Cypex (Dundee, United Kingdom). Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD). Minimum essential medium (Eagles), 2 mM L-glutamine, Earles BSS, 1.5 g/l sodium bicarbonate, 15% heat inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 1.0 mM sodium pyruvate, Hanks BSS without phenol red, 25 mM pH 7.4 N-(2- hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES) buffer and 1% non-essential amino acids were all obtained from the UCSF Cell Culture Facility.

Hypoxis obtusa (African potato) capsules made from the corm (underground stem) were obtained from Chiremba Herbs (Harare) and dried *D. anomala* (chifumuro) from the root was obtained from a traditional open market (Mbare) in Harare.

Extraction of herbal medicines

Extracts of dried herbal medicines were prepared using water and methanol as extraction solvents. Briefly, the herbs were ground to a fine powder using mortar and pestle and 100 mg/ml suspensions of crude powder were prepared by adding ~0.8 g of powder to an appropriate volume of solvent. The mixtures were sonicated for a total of 5 min using a Thermo Fisher Scientific (Waltham, MA) 550 Sonic Dismembrator equipped with a Misonix (Farmingdale, NY) 20 kHz model CL4 Ultrasonic Converter. Sonicating was conducted for intervals of 30 s, followed by immersion of the samples in ice water to cool them back to room temperature. Samples were next stirred for 2 h in the dark at room temperature. The mixtures were centrifuged at 10,000 x g for 5 min and the supernatant re-centrifuged using micro-centrifuge tubes at 13000 x g for 5 min.

The pH of aqueous extracts was adjusted to 7.4 using dilute NaOH and samples were stored at -80^{O}C. Extract concentrations were calculated on the basis of the original suspensions extracted.

Single concentration inhibition of CYP enzymes

Water and methanol extracts of *H. obtusa* and *D. anomala* at a starting powder concentration of 1 mg/ml crude extract were tested for inhibitory effects on the activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 using phenacetin o-deethylation, diclofenac 4-hydroxylation, s -mephenytoin 4-hydroxylation, bufuralol 1-hydroxylation and midazolam 1-hydroxylation, respectively, as marker reactions. Appropriate concentrations of recombinant CYP enzymes and substrates (summarized in Table 1) were used for each specific marker reaction, in mixtures containing 1 mM NADPH and 0.1 M phosphate buffer (pH 7.4). Methanol extract aliquots were evaporated under nitrogen (N_2) before re-dissolving in water and buffer with vortexing and sonicating.

CYP3A4 testosterone assay

The CYP3A4-inhibitory properties of crude water and methanol extracts of the herbal medicines were tested at a starting powder concentration of 2 mg/ml at 37°C in reaction mixtures consisting of 0.4 mg/ml HLM, 50 mM pH 7.4 sodium/potassium phosphate buffer, 100 µM testosterone and 1 mM NADPH (total volume 700 µl). Ketoconazole (2.5 µM) was used as a positive control. Testosterone and methanol extract aliquots were evaporated under nitrogen (N_2) before re- dissolving in water and buffer with vortexing and sonicating. Prior to addition of NADPH, the other reaction mixture components were pre-incubated 5 min at 37°C. Aliquots (100 µL) were removed 0, 10, 20, 40 and 60 min after the start of the reaction, mixed with 50 µL of cold methanol, placed on dry ice, then centrifuged at 13000 × g for 5 min, with the supernatant collected. Samples were analyzed with an Agilent Technologies (Santa Clara, CA) 1100 HPLC employing a Phenomenex (Torrance, CA) Prodigy 5 micron ODS (3) column

Table 1. Incubation and reaction conditions for single-point CYP inhibition experiments.

Isoform	pmol rCYP	µM substrate	Min incubated	Positive control	Stop solution
CYP1A2	3	Phenacetin (45)	20	-naphthoflavone (10 M)	100 µl MeOH
CYP2C9	10	Diclofenac (100)	10	sulfaphenazole (10 M)	50 µl 94:6 ACN:glacial acetic acid
CYP2C19	50	s-mephenytoin (100)	45	ticlopidine (10 M)	100 µl 6% perchloric acid
CYP2D6	3	Bufuralol (50)	10	quinidine (10 M)	10 µl 60% perchlorate
CYP3A4	3	Midazolam (5)	5	ketoconazole (10 M)	100 µl MeOH

The CYP3A4 inhibition of serial dilutions of herbal extracts were tested using a method similar to that described above except that the final volume was 200 and 12 µl of NADPH regenerating system was used to start the reaction. Aliquots (50 µl) were removed 0, 10, and 20 min after the start and the reaction was stopped with a half volume of cold methanol as above. The samples were then processed and analyzed as before.

Caco-2 cell bidirectional transport experiments

Caco-2 cells were seeded onto semi-permeable polycarbonate Transwell filter membranes in 6-well plates and cultured for 21 days at 37°C and 5% CO_2 in a standard cell culture incubator. The cells were grown in Eagle's minimum essential medium with 2 mM L-glutamine and Earles BSS plus 2.2 g/l sodium bicarbonate and 1.0 g/l glucose supplemented with 15% heat- inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 1.0 mM sodium pyruvate and 1% non-essential amino acids mixture. The medium was changed twice a week for 3 weeks, allowing the formation of a monolayer with tight junctions.

For the transport assays, the culture medium was removed and cells washed twice with 25 mM pH 7.4 HEPES buffer for 1 min at 37°C. The transendothelial electrical resistance (TEER) values were measured after the second wash before removing the wash buffer. All measurements of transport in the apical (A) to basolateral (B) direction and in the B A direction were made in triplicate. Transport assays were started by replacing the wash buffer with transport medium (Hanks BSS without phenol red, 25 mM pH 7.4 HEPES buffer and 1% FBS) in the receiving compartment and with medium plus the Pgp substrate [3H]-digoxin (0.0115 nmol/ml, 0.25 Ci/ml initial concentration) in the donor compartment. The inhibitors were added to both compartments. At each time point (60, 120 and 180 min), 200 µL aliquots were removed from the receiving compartment and replaced with an equal volume of transport medium.

After taking the 180 min aliquot, the Transwell filters were removed and rinsed three times in ice cold phosphate buffered saline. Intracellular concentrations were measured by removing the filter membranes with the cell monolayer and sonicating in 5 ml liquid scintillation fluid for 10 min. Radioactivity was measured using an LS6000-TA liquid scintillation counter (Beckman Coulter, Fullerton, CA).

Evaluation of Caco-2 cell monolayer integrity

In the Caco- 2 cell transport experiments, an increase in apparent permeability (Papp) values was observed with some of the herbal extracts, with the water extract of *D. anomala* showing the greatest increase. To test whether this might have been due to damage to the monolayer integrity, a transport experiment using [3H]-mannitol (7.45 nmols/ml, 0.04 Ci/ml) as the substrate in the presence and absence of 250 g/ml of the water extract of *D. anomala* was carried out. TEER values were measured before and after running the transport assays.

Data analysis

IC50 values for inhibition of CYP3A4 by serial dilutions of plant extracts were obtained by using nonlinear regression (Kaleida-Graph 3.5, Synergy Software, Reading, PA) to fit % inhibition versus extract concentration data to a variant of the Hill equation:

$$\% \text{ inhibition} = 100/(1 + 10\text{^}((IC_{50} - C_{PE})*HC))$$

Where:

C_{PE} = the concentration of plant extract
and HC = the Hill coefficient.

Apparent permeability values (Papp) for Caco-2 monolayer cell transport studies were calculated using the equation:

$$P_{app} = (dCr/dt)*Vr /(A*Cd) = (dQr/dt)/(A*Cd)$$

Where:

dCr/dt = the rate of increase of drug concentration in the receiver chamber,
Vr = the volume of the receiver chamber in cm3,
A = the surface area of the cell culture insert membrane in cm2, Qr = the total amount of drug in the receiver chamber, and
Cd = the initial donor chamber drug concentration.

RESULTS AND DISCUSSION

Effects of crude extracts on CYPs 1A2, 2C9, 2C19, 2D6 and 3A4

At a concentration of 1 mg/ml of crude extracts, both methanol and water extracts of *H. obtusa* generally inhibited the activity of the major CYP enzymes (Figure 1) more than the *D. anomala* extracts. In most cases, *Dicoma* extracts inhibited the CYP isoforms less than 50%, while *Hypoxis* extracts inhibited more than 50% and thus *Hypoxis* appears more likely to cause potentially significant interactions. Methanol extracts inhibited more than the corresponding water extracts in every case. The methanol extract of *Hypoxi* had the strongest inhibitory effects on all the CYP isoforms, comparable with the positive controls, with 95% or more inhibition of

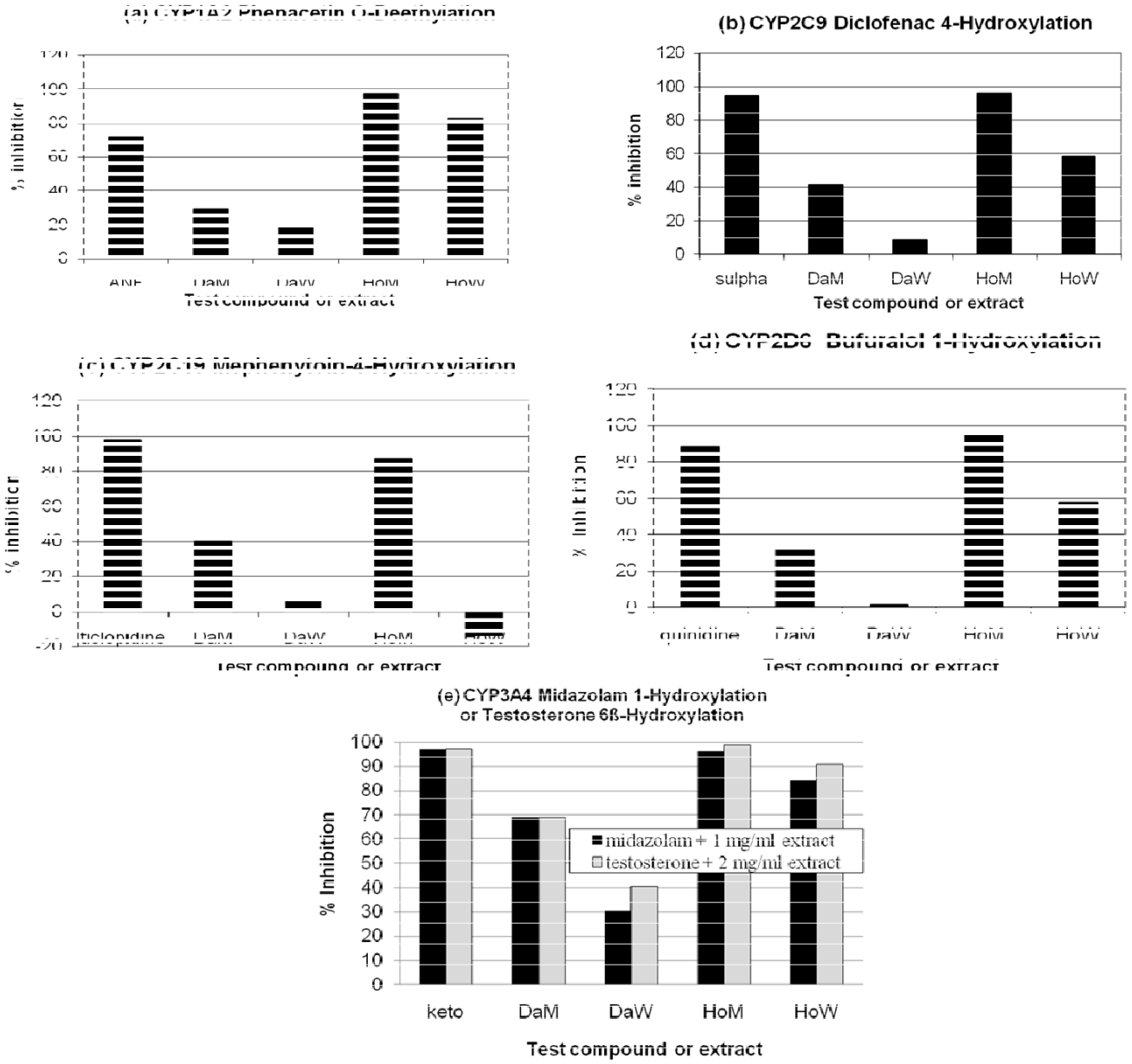

Figure 1. Inhibition of CYP enzymes by 1 and 2 mg/ml of herbal extracts. A crude extract concentration of 2 mg/ml was used in CP3A4 testosterone 6ß-hydroxylation tests (as part of the serial dilution experiment), and 1 mg/ml was used in all other cases. A concentration of 10 µM was used for positive controls (as indicated in Table 1) except with CP3A4 testosterone 6ß-hydroxylation, where 2.5 µM ketoconazole was used.

inhibition of CYPs 1A2, 2C9, 3A4 and 2D6. Aqueous *Hypoxis* extracts inhibited CYPs 1A2 and 3A4 in the 80 - 85% range and CYPs 2C9 and 2D6 around 60%. *Hypoxis* extracts were least inhibitory to CYP2C19. The methanol extract of *Dicoma* inhibited CYP3A4 68%, CYPs 2C9 and 2C19 about 40% and CYPs 1A2 and 2D6 about 30%. The aqueous extract of *Dicoma* inhibitted about 30% CYP3A4 and the others 20% or less. CYP3A4 inhibition by the herbal extracts was similar whether using midazolam or testosterone as probe substrates (Figure 1e).

Since CYP3A4 is the most important CYP isoform in drug metabolism (Evans and Relling, 1999) and one of the most inhibited enzymes by the herbal extracts, further studies were done to determine the IC_{50} value (inhibitor concentration causing 50% loss of activity) using serial dilutions of each of the herbal extracts. A twelve-fold difference was observed between the most potent inhibitor, the methanol extract of *H. obtusa* and the least potent, the water extract of *D. anomala*, with IC_{50} values of 0.20 and 2.4 mg/ml, respectively (Figure 2). In this case, the single point inhibition assays were

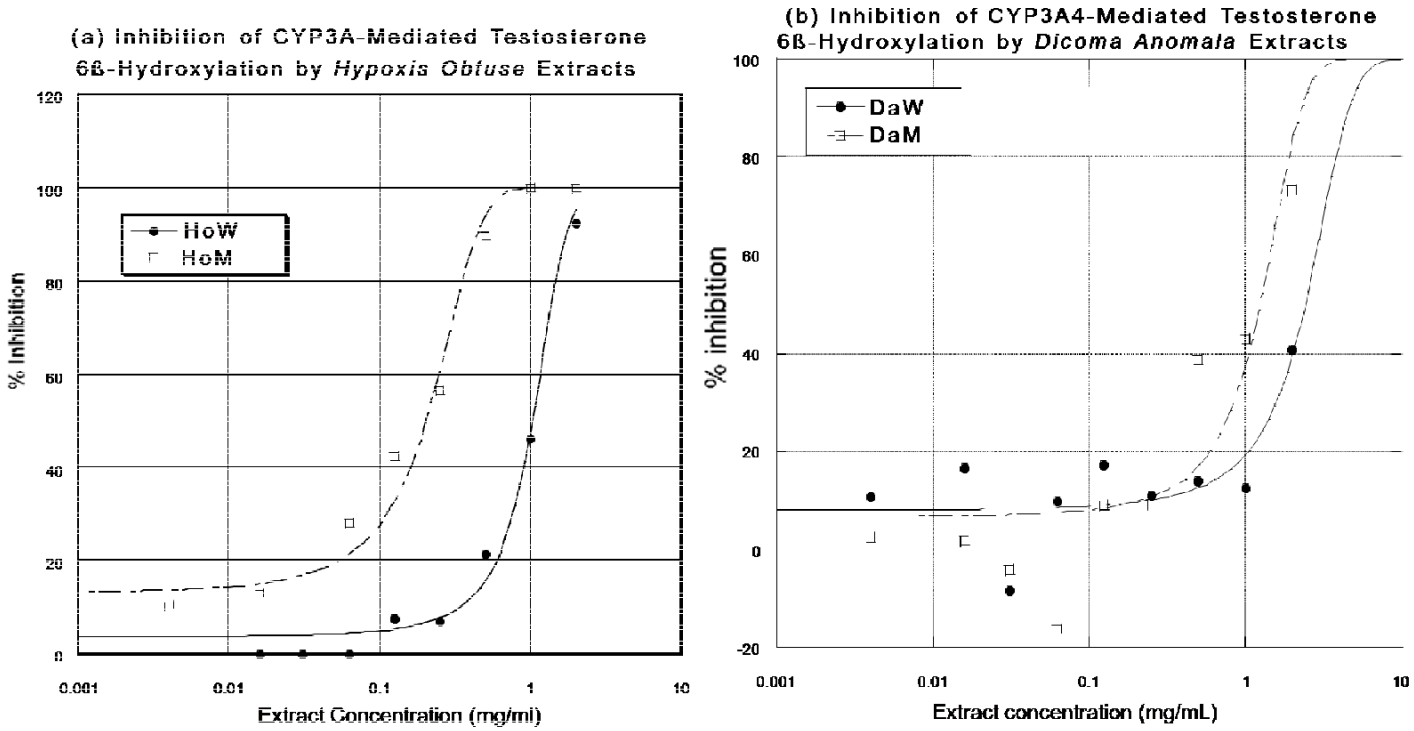

Figure 2. Determination of IC_{50} of crude herbal extract inhibition of CYP3A4. Serial dilutions of herbal extracts were prepared and tested for CYP3A4 inhibition. (a) IC_{50} values for *H. obtusa* were 1.0 and 2.4 mg/ml for the water and methanol extracts, respectively. (b) IC_{50} values for *D. anomala* were 0.2 and 1.2 mg/ml for water and methanol extracts, respectively.

accurate in determining rank order of inhibitory effects and are therefore useful for screening purposes. However, single point data is less useful for prediction of likelihood of potential interactions *in vivo*. We observed stronger CYP3A4 inhibition by the methanol extract of *H. obtusa* than (Mills et al., 2005a) observed with *H. hemerocallidea*. The large variation, that is, our observation of more than 90% inhibition at 1 mg/ml compared to less than 80% inhibition at 100 mg/ml extract, may be attributable to differences in the species studied and in experimental procedures.

CYP3A4 is a promiscuous enzyme believed to have multiple binding sites in the active site which account for its metabolism and inhibition by structurally diverse compounds, making the selection of probe substrate(s) very important. Using 3 different probe substrates to measure the activity of CYP3A4 *in vitro*, Foti et al. (2007) found inhibition of midazolam and testosterone metabolism by an herbal product was approximately the same (60%) whereas there was no inhibition when nifedipine was used as the probe drug. Our study also showed no significant difference in inhibition profiles when using midazolam or testosterone as probe substrate.

Our *in vitro* results suggest that for single-isoform-metabolized drugs, *Hypoxis* has a potential for CYP2D6- and CYP2C9-mediated interactions comparable to its potential for CYP3A4-mediated interactions. CYP2C9 substrates which are mainly eliminated by one isoform and have a narrow therapeutic index and thus have greater potential for interactions include phenytoin and S-warfarin.

Effects of crude extracts on P-glycoprotein *in vitro*

The ability of the herbal extracts to inhibit the apical efflux transporter Pgp in Caco-2 cell monolayers was tested with the Pgp substrate digoxin (Tanigawara et al., 1992). Apparent permeability, Papp (Figure 3) is a measure of the permeation of the monolayer by a compound in a specified direction; the net efflux ratio (Figure 4) is the basolateral to apical divided by the apical to basolateral permeation ratio (B A/A B). In all Caco-2 transport experiments, permeation of digoxin was roughly linear over the 3 h experimental period. In the absence of any Pgp inhibitor, the transport of digoxin across Caco-2 monolayers gave a net efflux ratio close to 2 (1.94). The presence of the potent Pgp inhibitor GG918 caused a 28% decrease in B A transport and a 13% increase in A B transport (Figures 3 and 4) and reduced the net efflux ratio to close to unity (1.17, $p = 0.023$). Unlike GG918, crude methanol and water extracts of *H. obtusa* and *D. anomala* had no significant effect on the digoxin net efflux ratio when present at levels up to 250 µg/ml (Figure 4), indicating they had little or no Pgp inhibitory activity. Efflux ratios in the pre-

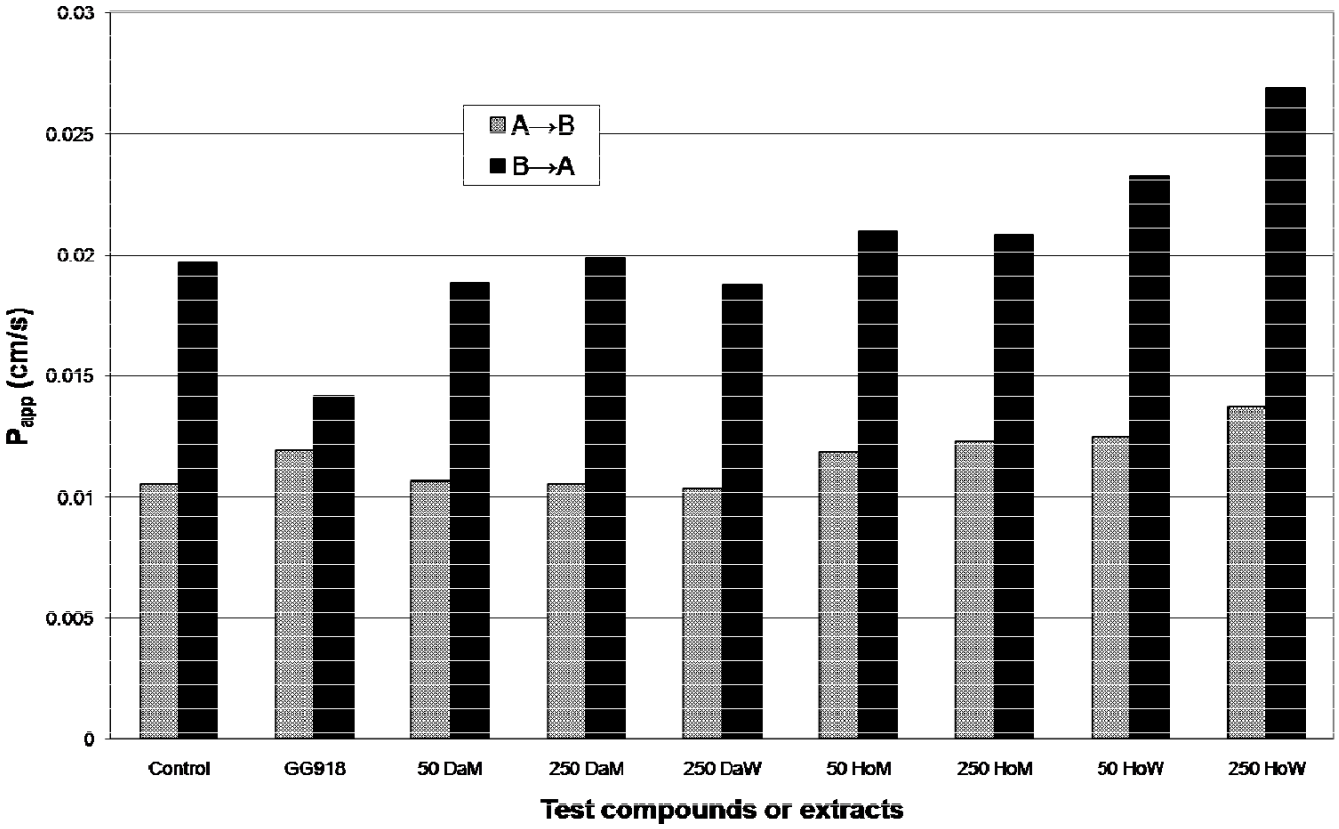

Figure 3. Effect of herbal extracts on the apparent permeability (Papp) of Caco-2 cell monolayers to digoxin. The methanol extract of Dicoma anomala (DaM), the water extract of Dicoma anomala (DaW), the methanol extract of Hypoxis obtusa (HoM), and the water extract of Hypoxis obtusa (HoW) were tested at 50 and 250 µg/ml after apical (A) or basolateral (B) dosing of digoxin. The Pgp inhibitor GG918 (0.5 µM) was used as a positive control.

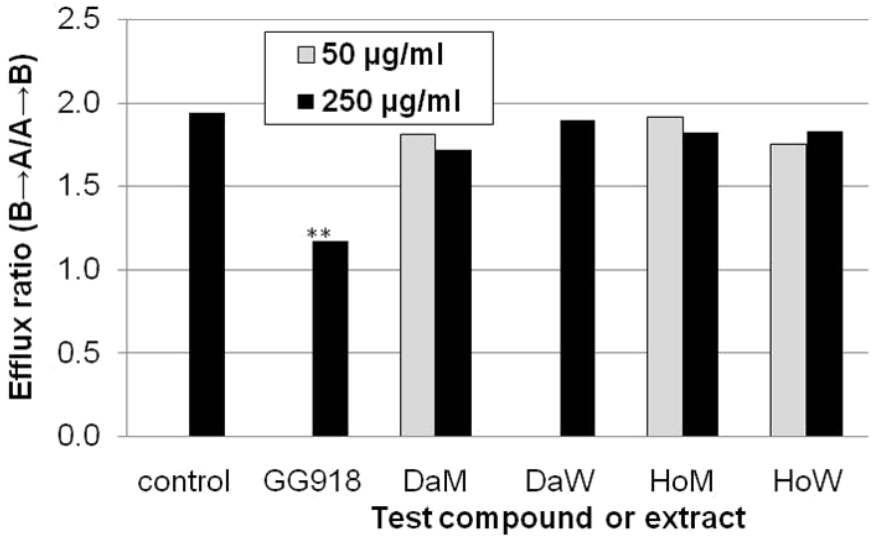

Figure 4. Effects of herbal extracts on the efflux ratios (B A)/(A B) for digoxin transport across Caco-2 cell monolayers. Methanol and water extracts of Dicoma anomala are denoted DaM and DaW, and those of Hypoxis obtusa are denoted HoM and HoW, respectively. The Pgp inhibitor GG918 (0.5 µM) was used as a positive control. The results are presented as mean ± standard deviation of triplicate measurements. **Significantly different from control ($p < 0.05$).

presence of the herbal extracts ranged from 1.7 to 1.9, similar to the ratio of 1.9 from the control experiment. *Hypoxis* extracts also had no effect on P_{app}. However, significant increases in P_{app} were observed with the water extract of *Dicoma* in both A B (30%) and B A (37%) directions (Figure 3), suggesting an increase in paracellular or transcellular passive diffusion across the monolayer. To investigate whether this resulted from a change in cell monolayer integrity, mannitol, a small hydrophilic molecule which is not a Pgp substrate was used as a marker for diffusion by the paracellular route in Caco-2 transport assays. The *Dicoma* extract had had no effect on permeation of [3H]-mannitol in either direction (data not shown).

Pgp is an efflux pump that is involved in drug disposition and protecting biological systems from xenobiotics. Its role in the gut is to limit absorption of Pgp substrates and it plays a significant role in pre-systemic metabolism though enzyme-transporter interplay with CYP3A4 in the enterocyte villi. Pgp inhibition in the liver and the gut has opposing effects on exposure (to decrease or increase it, respectively) of substances that are both Pgp and CYP3A4 substrates (Cummins et al., 2002; Benet et al., 2003; Benet et al., 2004). Extracts of *Hypoxis* and *Dicoma* showed no significant inhibition of Pgp in Caco-2 cells. However, it is possible that at higher concentrations than used in the assays, some effect on Pgp would be observed.

Experimental impact of the extraction procedure

Hydrophilic compounds extractable by water are less likely to pass through the lipid membranes in the intestines compared to hydrophobic compounds extractable by organic solvents, unless they are substrates of influx transporters. Hypoxoside, the main constituent characterized from *Hypoxis* products, is reported to be rea-dily soluble in methanol and its extraction by methanol from the commercial products was shown to be more than 90% (Nair and Kanfer, 2006); this may explain the observed higher CYP inhibition by methanol extracts compared to aqueous extracts. Therefore, choice of extraction solvent for *in vitro* studies is critical if the *in vitro* results are to be of clinical relevance. Methanol extracts have consistently shown larger inhibitory effects *in vitro* compared to water extracts in our experiments and in the literature (Mills et al., 2005a). Higher methanol concentrations (> 2%) have been shown to inhibit CYP enzymes. In our experiments, final methanol concentrations were reduced to less than 1% in the incubation assays, either through evaporation the extract under nitrogen and re-dissolving the residue in the incubation mixture or by using appropriate dilutions.

Despite their limitations, *in vitro* assays are the most practical means of screening for potential interactions for herbal medicines and to provide guidance on the selection of herbal medicines on which *in vivo* studies should be conducted. For example, the *H. obtusa* methanol extract has an IC_{50} for CYP3A4 inhibition equivalent to 0.2 mg/ml of the herb. The normal dose used according to the product label is 1600 mg of herbal medicine. Assuming complete absorption into the plasma (adult volume~ 3L) and minimal tissue distribution would yield a concentration of ~0.5 mg/ml.

Assuming further that uptake into the liver is efficient, significant inhibition of CYP3A4 would be possible. However, because of the importance of absorption, plasma uptake and clearance processes that are difficult to predict, significant *in vitro* inhibition may not necessarily result in major interactions *in vivo*. *In vivo* herb-drug interaction studies provide the ultimate information on the clinical significance of *in vitro* results.

Conclusion

Hypoxis extracts at 1 mg/ml strongly inhibited all the five major CYP isoforms 1A2, 2C9, 2C19, 2D6 and 3A4. CYP3A4 was among the most strongly affected, being inhibited by the methanol extract of *Hypoxis* with an IC_{50} of 0.20 mg/ml.

This suggests that *in vivo* testing for *Hypoxis*-drug interactions is desirable. The weaker inhibition by *Dicoma* (IC_{50} of 1.2 mg/ml for the methanol extract) is of less concern. Methanol extracts inhibited CYPs more than the corresponding water extracts in all cases. Methanol and water extracts of *Hypoxis* and *Dicoma* at levels up to 250 µg/ml did not significantly inhibit P-glycoprotein in bi-directional Caco-2 cell monolayer transport experiments.

ACKNOWLEDGEMENTS

The work presented here was done in collaboration with the School of Pharmacy, University of Zimbabwe, the African Institute of Biomedical Science and Technology (AiBST) and the School of Pharmacy, University of California, San Francisco (UCSF), United States. The authors would like to thank Joseph Custodio for the assistance with cell culture for the P-glycoprotein experiments. L. G. was a recipient of the Fogerty International Fellowship award for research training at UCSF in the United States. The African Institute of Biomedical Science and Technology (AiBST) also provided financial support to L. G. during the course of this research work.

REFERENCES

Bailey DGJ, Malcolm OA, Spence JD (1998). "Grapefruit juice-drug interactions." Br. J. Clin. Pharmacol. 46(2): 101-110.

Benet LZ, Cummins CL, Wu CY (2003). "Transporter-enzyme interactions: implications for predicting drug-drug interactions from *in vitro* data." Curr. Drug Metab. 4(5): 393-398.

Benet LZ, Cummins CL, Wu CY (2004). "Unmasking the dynamic

interplay between efflux transporters and metabolic enzymes." Int. J. Pharm. 277(1-2): 3-9.
Cummins CL, Jacobsen W, Benet LZ (2002). "Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4." J. Pharmacol. Exp.Ther. 300(3): 1036-1045.
Evans WE, Relling MV (1999). "Pharmacogenomics: translating functional genomics into rational therapeutics." Science 286(5439): 487-491.
Fang L, Tao GY, Wang G, Chen Y, Zhang W, He YJ, Li Q, Lei HP, Jiang F, Hu DL, Huang YF, Zhou (2009). "Effects of Ginkgo biloba extract ingestion on the pharmacokinetics of talinolol in healthy chinese volunteers". Ann. Pharmacother. 43: 944-949.
Foti RS, Wahlstrom JL, Wienkers LC (2007). "The in vitro drug interaction potential of dietary supplements containing multiple herbal components." Drug Metab. Dispos. 35(2): 185-188.
Giraldo R (2002). Southern African Development Community (SADC) meeting on Nutrition and HIVAIDS, Johannesburg, South Africa. http://www.laleva.cc/choice/aids/aids_giraldo.html
Huang SM, Toigo MMT, Chen MC, Sahajwala C, Lesko LJ, Temple R (2004). Evaluation of Drugs in Women: Regulatory Perspective. Principles of gender-specific Medicine. M. Legato, Academic Press. pp. 848-859.
Kim HS (2005). "Do not put too much value on conventional medicines." J. Ethnopharmacol. 100(1-2): 37-39.
Kim J, Phongsamran P, Park S (2004). "Use of antidepressant drugs in transplant recipients." Prog Transplant 14(2): 98-104.
Lundahl J, Regardh CG, Edgar B, Johnsson G (1995). "Relationship between time of intake of grapefruit juice and its effect on pharmacokinetics and pharmacodynamics of felodipine in healthy subjects." Eur. J. Clin. Pharmacol. 49(1-2): 61-67.
Mahady GB (2001). "Global harmonization of herbal health claims." J. Nutr. 131(3s): 1120S-1123S.
Mills E, Cooper C, Seely D, Kanfer I (2005). "African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology." Nutr. J. 4: 19.
Mills E, Foster BC, Heeswijk RV, Phillips E, Wilson K, Leonard B, Kosuge K, Kanfer I (2005). "Impact of African herbal medicines on antiretroviral metabolism." AIDS. 19(1): 95-97.
Mnengwane J, Koekemoer M (2007). Dicoma anomala. National Herbarium, Pretoria, SA National Biodiversity Institute. www.plantzafrica.com/plantcd/dicomanom.htm
Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J (2008). "*Moringa oleifera* leaf extracts inhibit 6 -hydroxylation of testosterone by CYP3A4." J. Infect. Dev. Ctries. 2(5): 379-383.
Nair VD, Kanfer I (2006). "High-performance liquid chromatographic method for the quantitative determination of hypoxoside in African potato (Hypoxis hemerocallidea) and in commercial products containing the plant material and/or its extracts." J. Agric. Food Chem. 54(8): 2816-2821.
Owira PMO, Ojewole JAO (2009). "'African Potato' (Hypoxis hemerocallidea corm); A plant-medicine for modern and 21st century diseases of mankind? - A review". Phytother. Res. 23: 147-152.
Piscitelli SC, Burstein AH, Chaitt D, Alfaro RM, Falloon J (2000). "Indinavir concentrations and St John's wort." Lancet 355(9203): 547-548.
Tanigawara Y, Okamura N, Hirai M, Yasuhara M, Ueda K, Kioka N, Komano T, Hori R (1992). "Transport of digoxin by human P-glycoprotein expressed in a porcine kidney epithelial cell line (LLC-PK1)." J. Pharmacol. Exp. Ther. 263: 840-845.