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Research Article

Assessing Drug Interaction Potential of Herbal Medicine Liv.52, Smokeless Tobacco Snuff and Edible Camphor using Human Liver Microsomes

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Abstract

Cytochrome P450 inhibition potential of Liv.52, snuff and camphor was assessed using human liver microsomes for any clinical consequences if taken together with other medications. They were screened for potential to inhibit 9 drug metabolizing cytochrome P450 (CYP 450) isoforms. Samples were analyzed by liquid chromatography mass spectrometry (LC-MS/MS) using stable labeled internal standards of metabolites. Liv.52 did not inhibit CYP2A6, CYP2D6, and CYP2E1 up to the highest tested concentration of 1.13 mg/mL. Its IC_{50} value ranged from 0.08 mg/mL to 0.160 mg/mL with CYP1A2, CYP2B6, CYP2C8, and CYP2C19. With CYP2C9, the IC_{50} value was 0.32 mg/mL, with CYP3A4 using midazolam as substrate, the mean IC_{50} value was 0.63 mg/mL and 0.86 mg/mL with testosterone as substrate. Snuff did not inhibit any of the tested CYPs up to the highest tested concentration of 500 µg/mL, except CYP2B6 and CYP2C8 with mean IC_{50} values of 381 µg/mL and 399 µg/mL, respectively. Camphor did not inhibit CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 up to the highest tested concentration of 100 µg/mL. Camphor inhibited CYP2A6 and CYP2C19 with mean IC_{50} values of 60 µg/mL and 74 µg/mL, respectively. It showed the most potent inhibition with CYP2B6 with mean IC_{50} of 3.2 µg/mL. Generally, test items with IC_{50} values below 0.5 µg/mL are considered as potent CYP inhibitors and liable for further drug interaction studies. Liv.52 showed the lowest IC_{50} of 80 µg/mL with various CYPs, snuff showed IC_{50} values greater than 350 µg/mL with all the tested CYPs confirming no major CYP interaction liabilities. As not many marketed drugs are substrates of CYP2B6, the liability of camphor as CYP2B6 inhibitor is limited.

Keywords: *CYP Inhibition; Human Liver Microsomes; LC/MS/MS*

Introduction

Metabolism and transporter dependent drug-drug interactions (DDIs) are one of the major reasons why several drugs are withdrawn from market, have black box warnings, refusal for approval or early termination of development. Interactions occur when absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of drug are affected by the co-administered drugs or dietary ingredients taken together. Interactions between dietary agents and co-administered drugs may increase or decrease pharmacological and toxicological effects of either component. Polypharmacy is common nowadays and patients add various over-the-counter vitamins, herbs and foods to the prescribed medicines. Herbal medicines are nowadays gaining increasing popularity and make it important to understand potential interactions between herbs and prescribed drugs. The likelihood of herb-drug interaction is more than drug-drug interaction, as drugs contain only single chemical entity, while most herbal medicines contain mixtures of pharmacologically active constituents [1,2].

Herbs and co-administered drugs may interact either by inhibiting or inducing the drug metabolizing cytochrome P450s responsible for elimination of components from body. CYP inhibition leads to increase in the plasma and tissue concentration of the drug, which may lead to toxic side effects. This may be dangerous with compounds which have narrow therapeutic index. In CYP induction, the clearance of drug is increased to such an extent that it is no longer efficacious. Classic examples of well-known diet-drug interactions are terfenadine and grapefruit juice by CYP inhibition and St John's wort with cyclosporine due to CYP induction.

Drug interaction studies are conducted to assess if the interactions are possible, to what extent and any dosage adjustment is required while prescribing. Understanding of DDI potential of drug and dietary components is very important as it helps to mitigate clinical risks upfront. Liver is the major organ where cytochrome P450 enzymes catalyze the biotransformation of many xenobiotics. It should be noted that many enzymes that catalyze drug metabolism in humans have different catalytic properties than their animal counter-parts. Therefore it is preferred to use human derived systems like human liver microsomes (HLM), S9 fractions and hepatocytes to study DDIs.

Further, quantitative predictions for understanding the clinical significance of DDIs require parameters like concentration of inhibitor *in-vivo*, plasma protein binding (unbound concentration of inhibitor), blood to plasma concentration ratio, hepatic contribution to total clearance (f_h), inhibitory concentration (K_i), gut metabolism, drug efflux and uptake by liver and intestine. These parameters are then incorporated into physiologically based pharmacokinetic models (PBPK) models to yield quantitative predictions for interaction potential. As this method is very resource intensive, a qualitative and high throughput *in-vitro* method for determining IC_{50} or inhibitor constant (K_i) values is often used [3,4].

As it is not possible to assess the potential of each drug and herb for CYP inhibition or induction in humans, various *in-vitro* techniques have been adopted. USFDA guideline has suggested list of *in-vitro* and *in-vivo* cytochrome P450 specific probe substrates for assessing CYP interactions [5]. For determining inhibitory concentration 50 (IC_{50}), a fixed concentration of CYP-specific probe substrate is co-incubated with various concentrations of the potential inhibitor. Based on IC_{50} values, new chemical entities (NCEs) or dietary ingredients are classified as weak or potent inhibitors. In addition, an understanding of the inhibition mechanism definitely helps in planning further *in-vivo* studies.

Liv.52: Liver is the major organ in the body, where all metabolism happens. Liv.52 exhibits hepatoprotective properties against chemically induced hepatoxic agents like paracetamol [6], ethanol [7] and carbon tetrachloride by improving metabolism by liver. Liv.52 is not known to have any side effects if taken as per the prescribed dosage of 3 tablets, three times a day and contains Himsra (*Capparis Spinosa*, 65 mg), Kasani (*Cichorium intybus*, 65 mg), Mandura bhasma (33 mg), Kakamachi (*Solanum nigrum*, 32 mg), Arjuna (*Terminalia arjuna,* 32 mg), Ksamarda (*Cassia occidentalis,* (16 mg), Biranjasipha (*Achillea millefolium*, 16 mg), Jhavuka (*Tamarix gallica*, 16 mg). It has excipients like carboxymethylcellulose, boricin and magnesium aluminum silicate [8].

Snuff: Snuff is a smokeless tobacco (tobacco administered without burning) made from pulverized tobacco leaves and is considered least harmful among different tobacco products available. For preparing such snuff, different varieties of tobacco are blended to achieve desired nicotine content, pH, flavor and aroma [9]. Tobacco contains more than 4000 chemicals of which 28 are known to be carcinogenic [10]. It is documented that an expert in snuffing would take 50 spoons of snuff in 85 seconds and French emperor Napolean reportedly sniffed over 0.5 kg of snuff in a month [11].

Camphor (Borneol): Camphor has borneol and is a bicyclic terpenoid, a transparent white crystal and is present in the oils extracted from various medicinal plants. It stimulates digestive system by increasing production of gastric juices. In addition, it is extensively used in Indian, Chinese and Japanese traditional medicines as an analgesic, to treat cough and colds, for relieving stress, in cardiovascular diseases, cerebrovascular diseases and for abdomen infections [12,13]. Camphor penetrates skin easily and has a cold effect and therefore used for topical application during injuries, burns, and skin diseases. Camphor is known to enhance the pharmacodynamic effects of a co-administered drug(s) [14].

Many geriatric patients take 5 or more concomitant medications which can result in severe and fatal DDI. Increased knowledge of drug-drug, food-drug and herb-drug interactions and of genetic factors affecting pharmacokinetics and pharmacodynamics is expected to improve drug safety and will enable drug therapy tailored to the individual patients' needs. To the best of our knowledge, this is the first report studying the CYP inhibition potential of Liv.52, snuff, and camphor.

Materials and Methods

Human liver microsomes (mixed gender, pool of 50 donors) were procured from XenoTechLLC (Kansas, USA; protein content: 20 mg/mL; catalogue number: H0610). Standard substrates and inhibitors were procured from Sigma-Aldrich, US Biologicals and Acros Organics as previously reported [15]. All the stable labeled internal standard(s) (IS) used during analysis were from Toronto Research Chemicals, Canada. NADPH, formic acid, ammonium formate, sodium dihydrogen phosphate and disodium hydrogen phosphate, and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, St Louis, MO. Acetonitrile (ACN) was procured from Merck and Milli-Q® water was used for preparation of buffer. 96-well plates of 1 mL capacity were purchased from Axygen Scientific, USA. All other reagents used in the assay were of analytical grade. Liv.52 tablets (Himalaya Drug Company), snuff (Umbrella brand) and camphor (SVP brand) was procured from local market, Bengaluru.

Preparation of Inhibitor Stock Solutions

Liv.52 tablets (3 x 500 mg tablet), snuff (100 mg) and camphor (20 mg) were finely grounded and extracted with 10 mL of acetonitrile:DMSO (80:20). The contents were vortex mixed and sonicated for 5 minutes. The microfuge tube was centrifuged at 5000g for 5 minutes and supernatant was removed. Again 10 mL of acetonitrile:DMSO (80:20) was added to the same microfuge tube and extracted. The first and second extract were pooled and used for the experiments. Test dilutions of Liv.52 were 1.13, 0.56, 0.28, 0.14, 0.70, 0.035, 0.018, 0.009, 0.004, 0.002 and 0.001 mg/mL, Snuff were 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49 μg/mL and for camphor were 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.20, 0.10 μg/mL.

Positive Control Inhibitor Stock Solutions

Positive control inhibitor stock solutions were prepared in ACN:DMSO mixture (80:20). α-naphthoflavone (CYP1A2), Tranylcypromine (CYP2A6 and CYP2E1), Ticlopidine (CYP2B6), Quercetin (CYP2C8), Sulfaphenazole (CYP2C9), (±)-N-3-Benzylnirvanol (CYP2C19), Quinidine (CYP2D6), Ketoconazole (CYP3A4) were used as positive control inhibitors. The CYP inhibition assay was considered acceptable if the IC_{50} values of positive control inhibitors were within ± 2.5-fold of the in-house generated average values [15].

Substrate Stock Solutions

Phenacetin (CYP1A2), Coumarin (CYP2A6), Bupropion (CYP2B6), Amodiaquine (CYP2C8), Diclofenac (CYP2C9), (S)-mephenytoin (CYP2C19), Dextromethorphan (CYP2D6), Chlorzoxazone (CYP2E1), Midazolam and Testosterone (CYP3A4) were used as enzyme specific substrates. Stock solutions of substrates were prepared in acetonitrile or 50% aqueous acetonitrile.

Assay Incubations

A microsome-buffer-substrate mixture (MBS mix) was prepared for each isozyme by pre-mixing appropriate volumes of sodium phosphate buffer (pH 7.4, 50 mM), microsomes (2 - 14 μL) and substrate (5.6 μL). MBS mix (179 μL) was transferred to 96-well reaction plate to which 1 μL of inhibitor stock solution was added to achieve the final target inhibitor concentration. The reaction plate was pre-incubated for 10 minutes at 37˚C (New Brunswick air incubator with shaker) and the reaction was initiated by addition of 20 μL of 10 mM nicotinamide adenine dinucleotide phosphate (NADPH) solution. The reaction plate was further incubated at 37˚C for a predetermined time period (5 - 40 minutes based on CYP being investigated). The reaction was quenched with 200 μL ACN for all CYPs and 200 μL 1% formic acid: ACN (70:30) for CYP1A2. In all assays, the percentage of organic content (ACN and DMSO) contributed by substrate and inhibitor was less than 1% (v/v). All incubations were performed in duplicate for test and positive control inhibitors. All assays were performed using the same protocol as reported earlier [15].

Bioanalysis

All samples were processed by addition of acetonitrile containing stable labeled internal standard and analyzed by employing positive (for all CYPs) or negative (for CYP2A6, 2C19 and 2E1) electron spray ionization mode in liquid chromatography tandem mass spectrometry (API4000, Applied Biosystems, USA) as reported earlier [15].

Data Analysis

The IC_{50} value was estimated from the percentage reduction in CYP activity at eleven inhibitor concentrations. The area ratio of the metabolite in the sample without inhibitor was considered as 100%, and the percentage reduction in the CYP activity at each inhibitor concentration was determined relative to the no-inhibitor area as reported earlier [15].

Results and Discussion

Metabolic drug–drug interactions (M-DDI) have been identified as a key reason in drug development resulting in restricted use, withdrawal or non-approval by regulatory agencies. The most common mechanism responsible for M-DDIs is inhibition of cytochrome P450s mediated drug oxidation as many drugs administered show relatively high affinity for CYP superfamily of enzymes. The use of *in-vitro* technologies to evaluate the potential for M-DDI has become routine in the drug discovery and development process. The use of *in-vitro* predictive approaches offers several advantages including minimum compound requirement, assessing the risk of DDI during discovery phase, with associated cost and time savings, as well as minimization of human risk due to the rational design of clinical drug-drug interaction studies.

To understand the drug interaction potential of a compound, a high throughput *in-vitro* study called CYP inhibition assay is often performed. For this study, human liver microsomes are used which has all the panel of CYPs that are responsible for biotransformation. IC_{50} values provide a qualitative information and are normally compared to arbitrary threshold values, to classify the compound as weak (IC_{50} > 10 μM) or potent inhibitor (IC_{50} value < 1 μM).

For any *in-vitro* study, it is critical to ensure the solubility of test inhibitor. We either extracted or dissolved the test inhibitors in acetonitrile: DMSO mixture (80:20). We spiked the stock solution at the highest concentration into the assay buffer and observed under focused lamp to ensure that the test inhibitor is soluble. Before conducting the CYP inhibition study, the production of metabolite was validated for protein and time linearity (data not shown). It was observed during preliminary studies that if a structural analog is used as IS, the area counts of metabolite and/or IS may get suppressed or enhanced due to co-elution of test inhibitor. It is difficult to develop a bioanalytical method for all the test inhibitors and screen them upfront for co-elution with metabolite or internal standard. Therefore, stable labeled internal standard of metabolite produced was used for analysis of samples to offset any mass related artifacts.

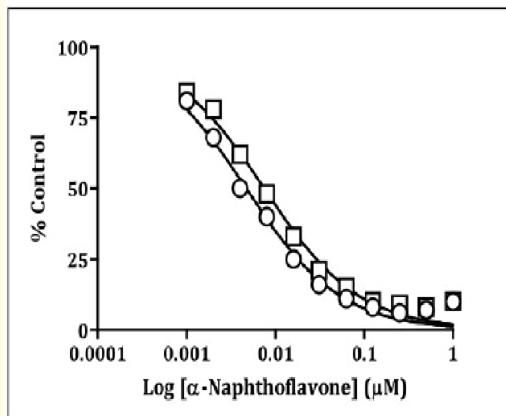
CYP inhibition potential for Liv.52, snuff and camphor was assessed using human liver microsomes to understand the potential drug-interaction liabilities. Liv.52 did not inhibit CYP2A6, CYP2D6, CYP2E1 up to the highest tested concentration of 1.13 mg/mL. Its IC_{50} value varied from 0.08 mg/mL to 0.160 mg/mL with CYP1A2, CYP2B6, CYP2C8, and CYP2C19, with CYP2C9 the IC_{50} value was 0.32 mg/mL, with CYP3A4 using midazolam as substrate, the mean IC_{50} value was 0.63 mg/mL and 0.86 mg/mL with testosterone as substrate. Snuff did not inhibit any of the tested CYPs up to the highest tested concentration of 500 μg/mL, except CYP2B6 and CYP2C8 with mean IC_{50} values of 381 μg/mL and 399 μg/mL, respectively. Camphor did not inhibit CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 up to the highest tested concentration of 100 μg/mL. Camphor inhibited CYP2A6 and CYP2C19 with mean IC_{50} values of 60 μg/mL and 74 μg/mL, respectively. It showed the most potent inhibition with CYP2B6 with mean IC_{50} of 3.2 μg/mL. All the tested positive control inhibitors IC_{50} values were within the acceptable in-house historical range. The results summary is presented in table 1 and CYP profiles are provided in figure 1.

Inhibitor	IC_{50} (mg/mL for Liv.52, μg/mL for snuff, camphor and μM for positive controls)									
	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4[a]	CYP3A4[b]
Liv-52	0.08, 0.11	> 1.13, > 1.13	0.09, 0.09	0.09, 0.09	0.30, 0.34	0.12, 0.16	> 1.13, > 1.13	> 1.13, > 1.13	0.56, 0.69	0.80, 0.92
Snuff	> 500, > 500	> 500, > 500	391, 371	394, 404	> 500, > 500	> 500, > 500	> 500, > 500	> 500, > 500	> 500, > 500	> 500, > 500
Camphor	> 100, > 100	62, 58	3.4, 3.1	> 100, > 100	> 100, > 100	66, 82	> 100, > 100	> 100, > 100	> 100, > 100	> 100, 80
α-Naphthoflavone	0.01, 0.01	-	-	-	-	-	-	-	-	-
Tranylcypromine	-	0.080, 0.079	-	-	-	-	-	-	-	-
Ticlopidine	-	-	0.014, 0.015	-	-	-	-	-	-	-
Quercetin	-	-	-	0.25, 0.26	-	-	-	-	-	-
Sulfaphenazole	-	-	-	-	0.21, 0.20	-	-	-	-	-
N-3-Benzylnirvanol	-	-	-	-	-	0.87, 0.54	-	-	-	-
Quinidine	-	-	-	-	-	-	0.12, 0.14	-	-	-
Tranylcypromine	-	-	-	-	-	-	-	4.5, 6.9	-	-
Ketoconazole[a]	-	-	-	-	-	-	-	-	0.03, 0.02	-
Ketoconazole[b]	-	-	-	-	-	-	-	-	-	0.03, 0.03

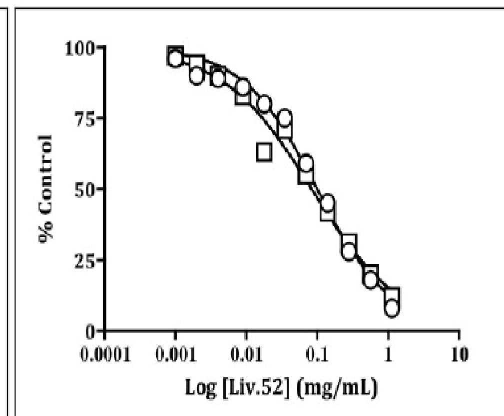
[a]Midazolam, [b]Testosterone

***Table 1:** CYP Inhibition Potential of Liv.52, Snuff and Camphor.*

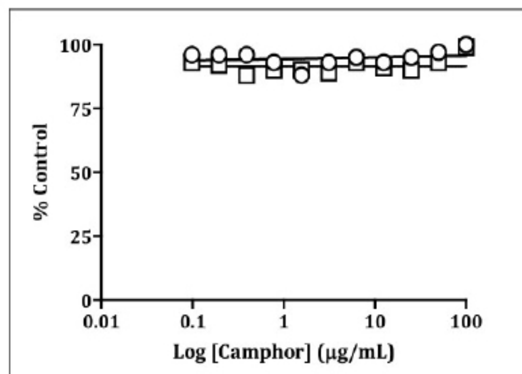
a) α-Naphthoflavone



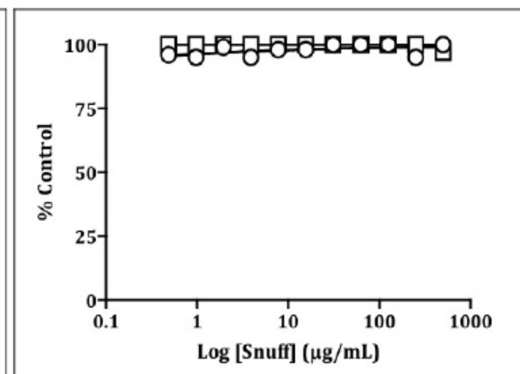
b) Liv.52



c) Camphor

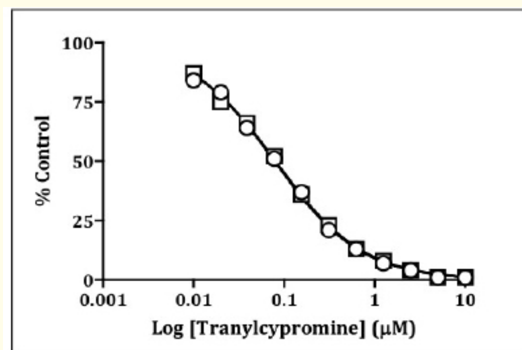


d) Snuff

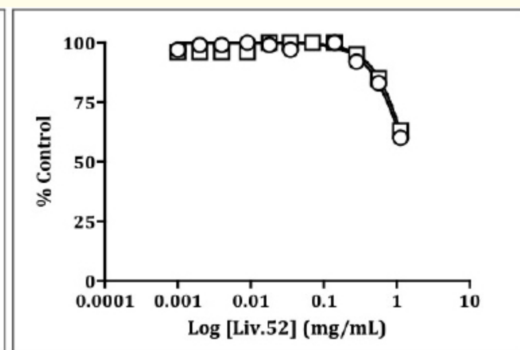


A) Inhibition of CYP1A2 (Substrate-Phenacetin)

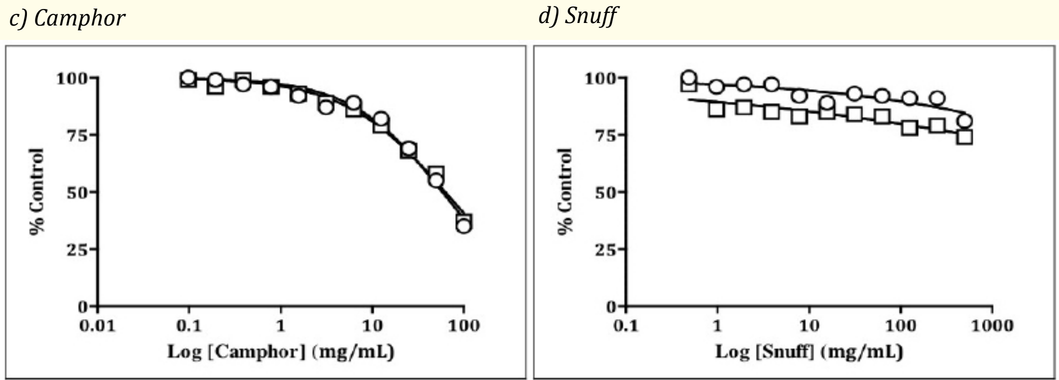
a) Tranylcypromine



b) Liv.52

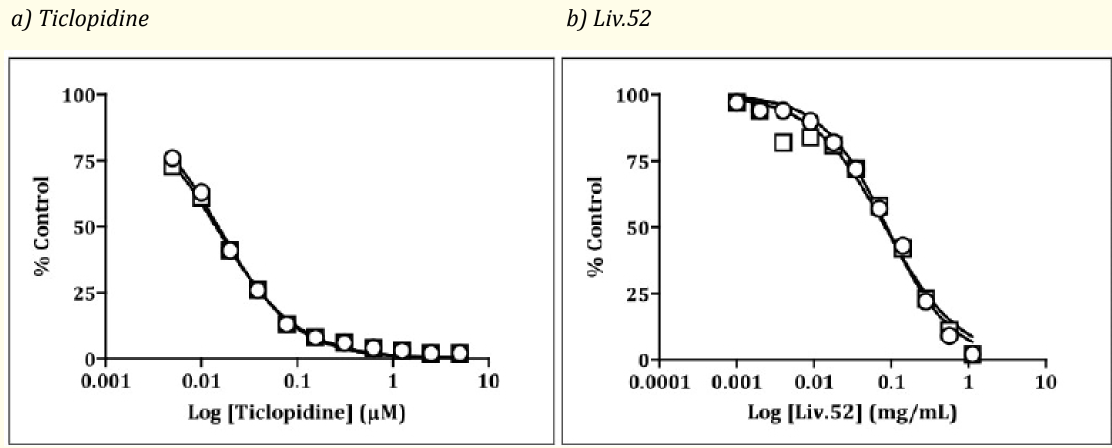


c) Camphor *d) Snuff*

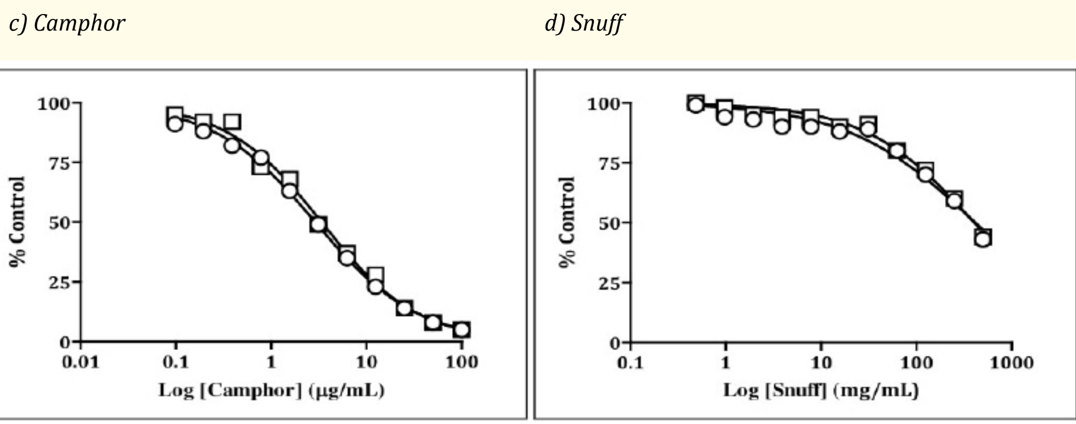


B) Inhibition of CYP2A6 (Substrate-Coumarin)

a) Ticlopidine *b) Liv.52*

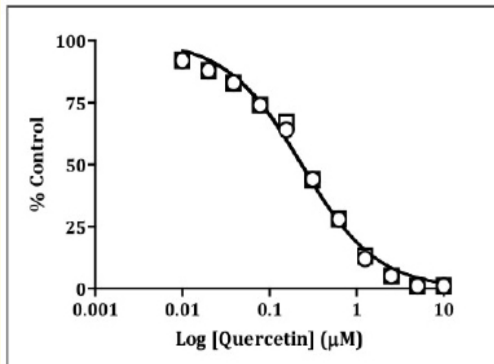


c) Camphor *d) Snuff*

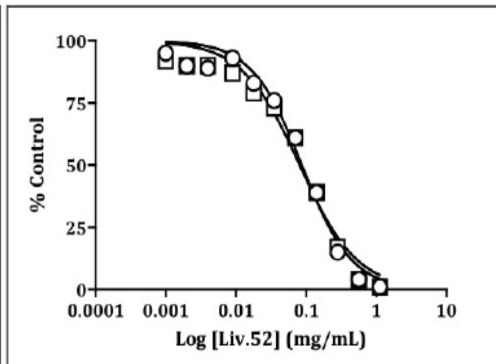


C) Inhibition of CYP2B6 (Substrate-Bupropion)

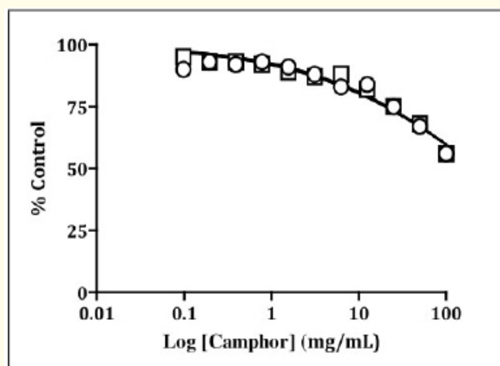
a) Quercetin



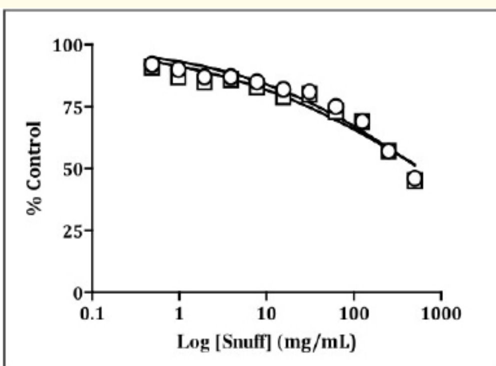
b) Liv.52



c) Camphor

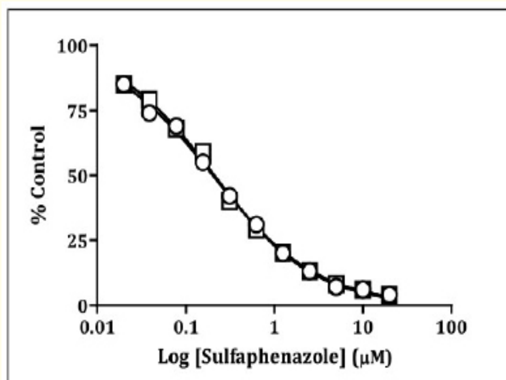


d) Snuff

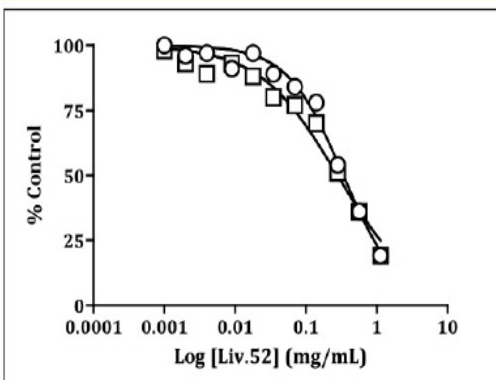


D) Inhibition of CYP2C8 (Substrate-Amodiaquine)

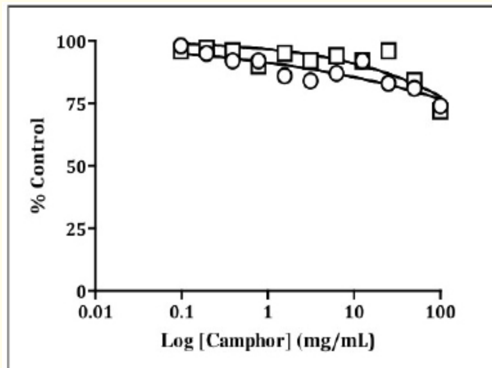
a) Sulfaphenazole



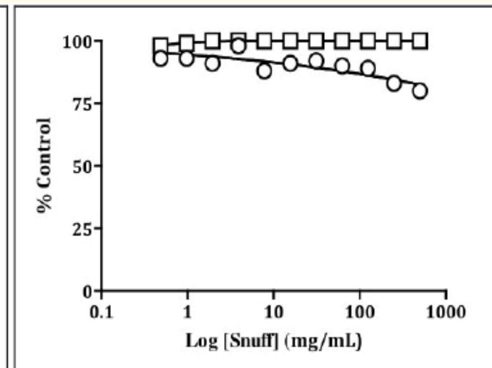
b) Liv.52



c) Camphor

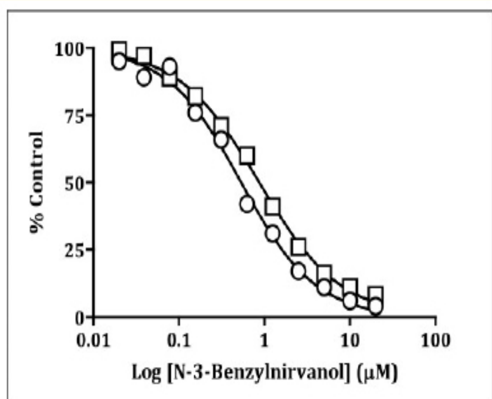


d) Snuff

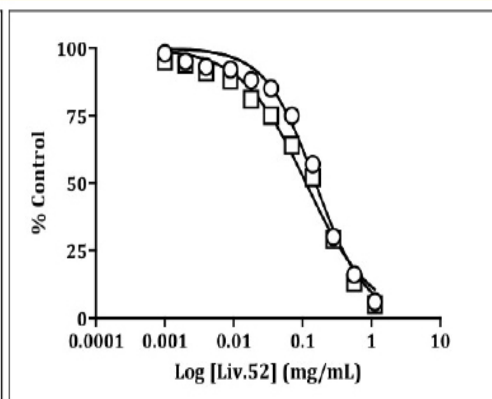


E) Inhibition of CYP2C9 (Substrate-Diclofenac)

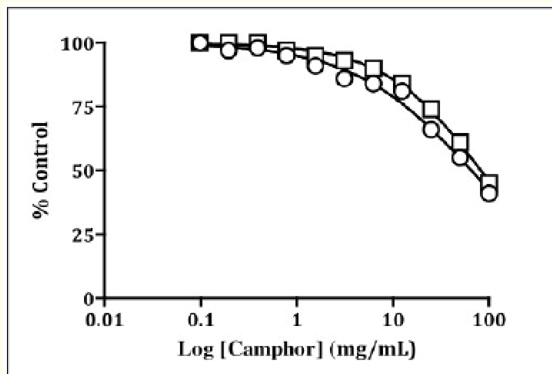
a) N-3-Benzylnirvanol



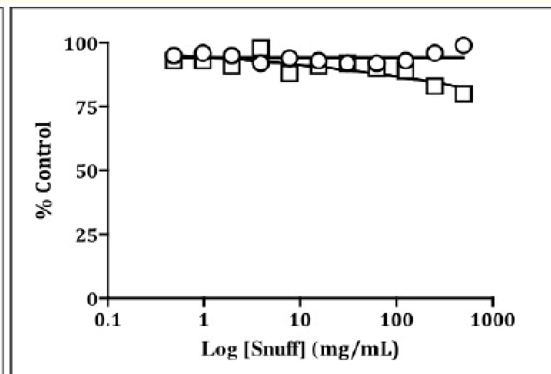
b) Liv.52



c) Camphor

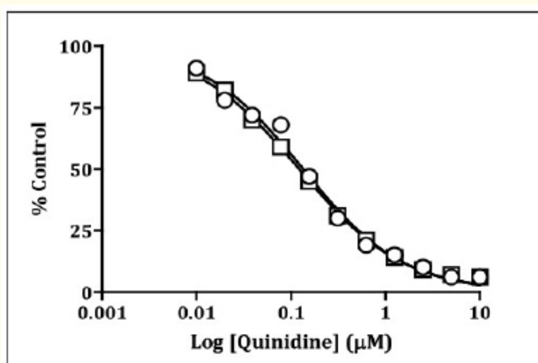


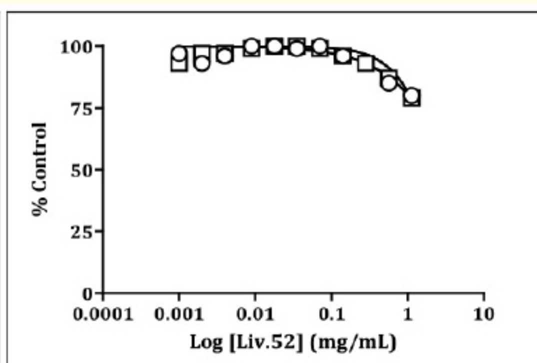
d) Snuff



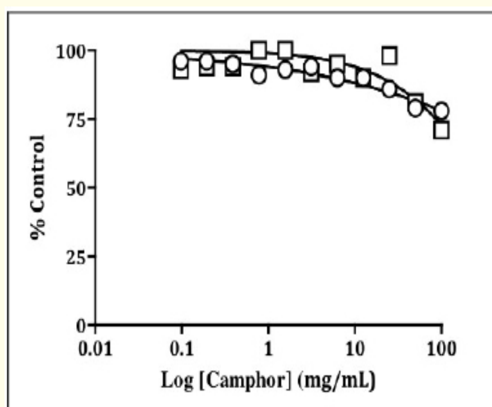
F) Inhibition of CYP2C19 (Substrate- (S)-Mephenytoin)

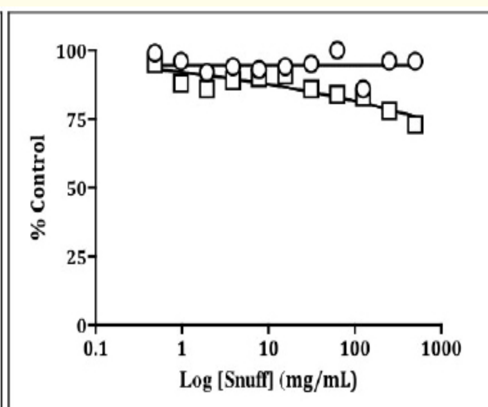
a) Quinidine *b) Liv.52*





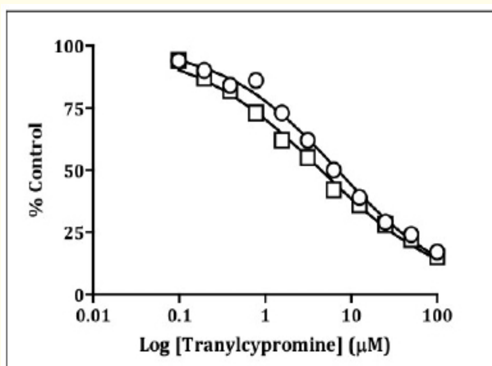
c) Camphor *d) Snuff*

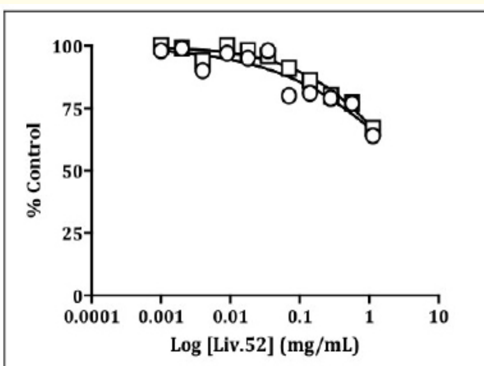




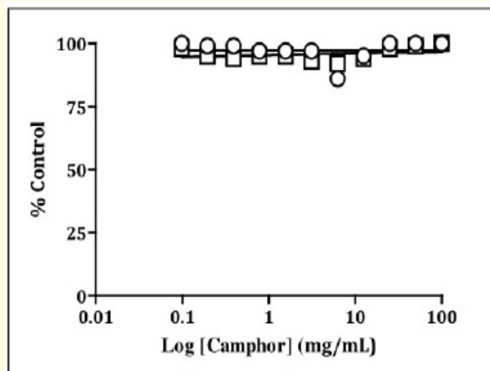
G) Inhibition of CYP2D6 (Substrate-Dextromethorphan)

a) Tranylcypromine *b) Liv.52*

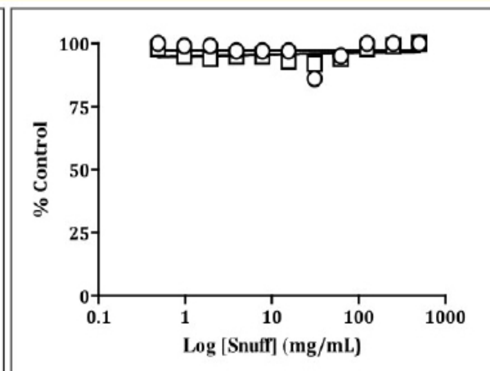




c) Camphor

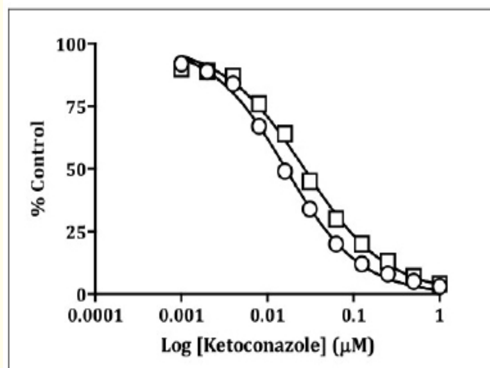


d) Snuff

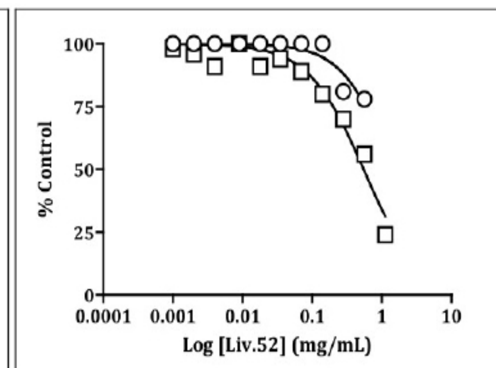


H) Inhibition of CYP2E1 (Substrate-Chlorzoxazone)

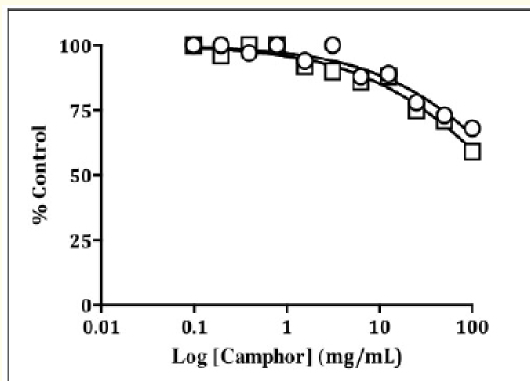
a) Ketoconazole



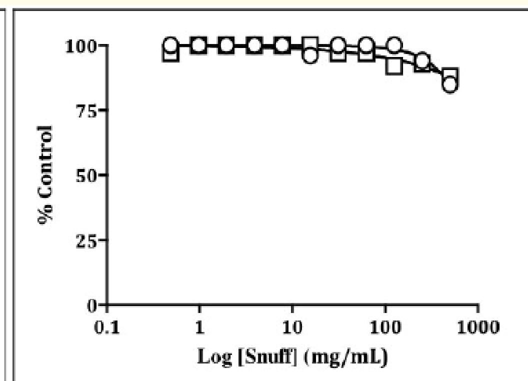
b) Liv.52



c) Camphor

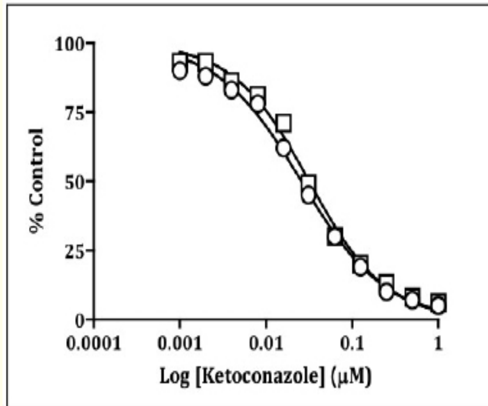


d) Snuff

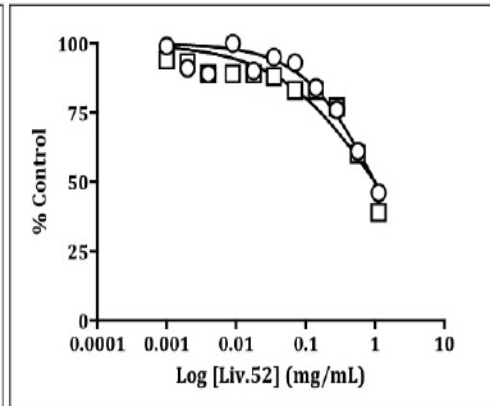


I) Inhibition of CYP3A (Substrate-Midazolam)

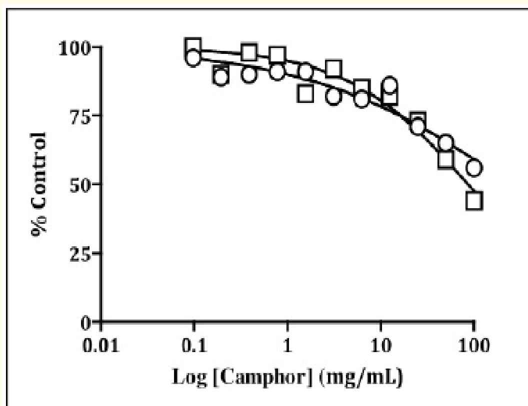
a) Ketoconazole



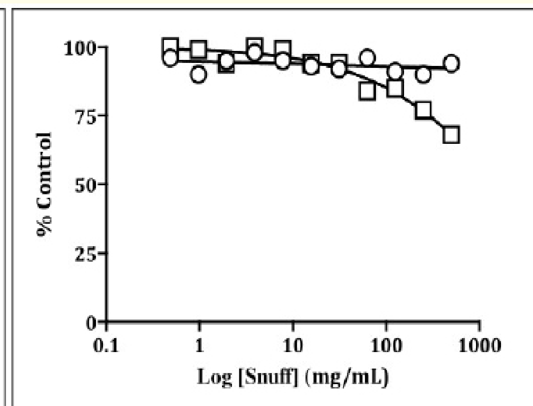
b) Liv. 52



c) Camphor



d) Snuff



J) Inhibition of CYP3A (Substrate-Testosterone)

Figure 1: *CYP Inhibition Profile of Liv.52, Camphor, Snuff and Positive Control Inhibitors.*

Typically, if IC_{50} of a compound is less than 10 μM, then Ki (inhibition constant) is determined and correlated with *in-vivo* concentrations. This is due to the fact that IC_{50} values may vary between labs but Ki value is a constant. Based on the substrate concentration chosen for the study with respect to K_m value (Michaelis-Menten constant), K_i values typically range from $IC_{50}/2$ to $2IC_{50}$ [18].

Due to an increased understanding of drug metabolism process, enzymes responsible for it and the key role they play in biotransformation of a drug, a more mechanistic approach to assessing DDI is considered now-a-days. Generally, it is accepted that inhibitors possessing *in-vitro* potency values (IC_{50}) below 1 μM demonstrate drug interactions of at least 2-fold clinically; however exceptions are there to this rule. In addition, it has also been observed that *in-vitro* inhibition potency rank order is similar to *in-vivo* potency rank order [19].

Moreover, the results of clinical DDI studies with one drug can be extrapolated to other drugs that are cleared by the same enzyme. In addition, physiologically based pharmacokinetic (PBPK) modeling and simulation is being used to predict the pharmacokinetics of drugs in humans and evaluate the effects of intrinsic (e.g. organ dysfunction, age, genetics) and extrinsic (e.g. drug-drug interactions) factors, alone or in combinations on drug exposure. Of the extrinsic factors, DDI potential is most important. These predictions aid in the selection of optimal dosing regimens as magnitude of DDI depend on it. Based on the pre-clinical pharmacokinetic data and using prediction tools, *in-vivo* concentration of suspected inhibitor [I] is determined. Using the Ki value, the clinical relevance is calculated as per USFDA guidance document [20].

Further, a study of the marketed drugs showed that around 20% of elimination happens through urine, bile, expired air or feces, 55% by CYP metabolism and 20% with all other metabolic processes. From the pharmaceutical industry's perspective, CYP1A2, CYP2C9, CYP2D6 and CYP3A4 address the majority of P450 issues and more than 50% of pharmacokinetic drug-drug interaction studies [21].

Based on the activity of various CYPs in HLM and participation in the clearance of marketed drugs, an impact table was prepared to understand the overall potential for CYP inhibition (Table 2) [22]. Due to abundance in intestine and liver and contribution to clearance of many marketed drugs, interaction with CYP3A4 seemed to have the highest impact for DDI. Due to low abundance in HLM and due to limited number of drugs that are cleared through them, CYP2A6 and CYP2C19 seemed to show the least impact due to DDIs. CYP2D6 is known to show the highest polymorphism followed by CYP2C9 and CYP2C19.

Isozyme	Activity in HLM/Hepatic abundance[#]	Participation in Drug Clearance of Marketed Drugs*	Polymorphism*	Overall Impact due to inhibition
CYP1A2	7	4	No	5
CYP2A6	5	8	No	Lowest, 7
CYP2B6	6	6	No	6
CYP2C8	3	7	No	4
CYP2C9	2	3	Yes	2
CYP2C19	Lowest, 9	4	Yes	Lowest, 7
CYP2D6	8	2	Yes	4
CYP2E1	4	5	No	3
CP3A4	Highest, 1	Highest, 1	No	Highest, 1

*[#]HLM data sheet, *as per list in http://medicine.iupui.edu/clinpharm/ddis/clinical-table.*

Table 2: *Prediction of Likely Impact of CYP Inhibition on Drug-Drug Interaction Studies with Major CYP Isozymes.*

Considering all these factors, snuff and camphor does not seem to have any major CYP inhibition liability. Liv.52 showed moderate inhibition with CYP1A2, CYP2B6, CYP2C8 and CYP2C19 and as per the impact table have very low liability. As CYP3A4 and CYP2C9 inhibition may have the highest liability and since Liv.52 IC_{50} values are more than 0.3 mg/mL, the likelihood of CYP inhibition is less from the *in-vitro* data. A comparison of IC_{50} values with the *in-vivo* concentrations would be ideal to understand the clinical drug-drug interaction relevance. Due to genetic polymorphism possible with CYP2C9, CYP2C19 and CYP2D6, the IC_{50} data for these CYPs has to be carefully interpreted.

Conclusions

Liv.52 and snuff are likely to show no drug interaction liability based on the CYP inhibition values. Camphor may have CYP2B6 interaction liability and needs to be correlated with *in-vivo* concentration to assess its impact.

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Conflict of Interest

None.

Bibliography

1. Fugh-Berman A. "Herb-drug interactions". *The Lancet* 355.9198 (2000): 134-138.
2. Fugh-Berman A and Ernst E. "Herb-drug interactions: review and assessment of report reliability". *British Journal of Pharmacology* 52.5 (2001): 587-595.
3. Blanchard N., *et al*. "Qualitative and quantitative assessment of drug-drug interaction potential in man, based on Ki, IC50, and inhibitor concentration". *Current Drug Metabolism* 5.2 (2004): 147-156.
4. Zhao P., *et al*. "Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review". *Clinical Pharmacology and Therapeutics* 89.2 (2011): 259-267.
5. U. S. Department of Health and Human services, Food and Drug Administration. Drug interaction studies, study design, data analysis, implications for dosing, and labeling recommendations (2012).
6. Singh B and Dhawan D. "Role of Liv.52 – A herbal formulation on 14C-ethaol metabolism and 14C-acetaldehyde accumulation in rat liver". *Journal of Nuclear Medicine* 15.1 (2000): 27-29.
7. Sandhir R and Gill KD. "Hepatoprotective effects of Liv.52 on ethanol-induced liver damage in rats". *Journal of Experimental Biology* 37.8 (1999): 762-766.
8. Kalab M and Krechler T. "Effect of the hepatoprotective drug Liv.52 on liver damage". *Casopis Lekaru Ceskych* 136.24 (1997): 758-760.
9. Sapundzhiev N and Werner JA. "Nasal snuff: historical review and health related aspects". *Journal of Laryngology and Otology* 117.9 (2003): 686-691.
10. Harrison DFN. "Dangers of snuff both "wet" and "dry"". *British Medical Journal* 293.6544 (1986): 405-406.
11. Russell MAH., *et al*. "Nicotine intake by snuff users". *British Medical Journal* 283.6295 (1981): 814-817.
12. Li YR., *et al*. "Pharmacokinetics of natural borneol after oral administration in mice brain and its effect on excitation ratio". *European Journal of Drug Metabolism and Pharmacokinetics* 37.1 (2012): 39-44.
13. Kumar MS., *et al*. "Antihypertensive and Antioxidant Potential of Borneol – A Natural Terpene in L-NAME – Induced Hypertensive Rats". *International Journal of Pharmaceutical and Biological Archive* 1.3 (2010): 271-279.
14. Zhang Q., *et al*. "Borneol, a novel agent that improves central nervous system drug delivery by enhancing blood-brain barrier permeability". *Drug Delivery* 24.1 (2017): 1037-1044.
15. Rao M., *et al*. "Modulation of Cytochrome P450 Metabolism and Transport across Intestinal Epithelial Barrier by Ginger Biophenolics". *PLOS ONE* 9.9 (2014): e108386.

16. Walsky RL and Obach RS. "Validated assays for human cytochrome P450 activities". *Drug Metabolism and Disposition* 32.6 (2004): 647-660.

17. Yao M., *et al*. "Development and full validation of six inhibition assays for five major cytochrome P450 enzymes in human liver microsomes using an automated 96-well microplate incubation format and LC–MS/MS analysis". *Journal of Pharmaceutical and Biomedical Analysis* 44.1 (2007): 211-223.

18. Burlingham BT and Widlanski TS. "An intuitive look at the relationship of Ki and IC50: A more general use for the Dixon plot". *Journal of Chemical Education* 80.2 (2003): 214-218.

19. Obach RS., *et al*. "The utility of in vitro cytochrome P450 inhibition data in the prediction of drug-drug interactions". *Journal of Pharmacology and Experimental Therapeutics* 316.1 (2006): 336-348.

20. U. S. Department of Health and Human services, Food and Drug Administration. In Vitro metabolism and transporter mediated drug-drug interaction studies (2017).

21. Paine MF., *et al*. "The human intestinal cytochrome P450 Pie". *Drug Metabolism and Disposition* 34.5 (2006): 880-886.

22. Clarke SE and Jones BC. "Human cytochrome P450 and their role in mechanism based drug-drug interactions". Ed. David Rodrigues A, Drug-drug interactions, 2nd edition. New York: CRC Press. 179 (2008): 53-85.

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