

The Induction of CYP1A2, CYP2D6 and CYP3A4 by Six Trade Herbal Products in Cultured Primary Human Hepatocytes

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(Received June 20, 2006; Accepted September 4, 2006)

Abstract: The aim of this study was to evaluate the *in vitro* inductive potential of six commonly used trade herbal products on CYP1A2, CYP2D6 and CYP3A4 metabolic activities. Herbal components were extracted from the trade products in a way that ensured a composition equal to that present in the original product. Primary human hepatocytes and specific CYP substrates were used. Classic inducers were used as positive controls and herbal extracts were added in *in vivo*-relevant concentrations. Metabolites were determined by high performance liquid chromatography (HPLC). St. John's wort and common valerian were the strongest inducing herbs. In addition to induction of CYP3A4 by St. John's wort, common valerian and *Ginkgo biloba* increased the activity of CYP3A4 and 2D6 and CYP1A2 and 2D6, respectively. A general inhibitory potential was observed for horse chestnut, *Echinacea purpurea* and common sage. St. John's wort inhibited CYP3A4 metabolism at the highest applied concentration. Horse chestnut might be a herb with high inhibition potentials *in vivo* and should be explored further at lower concentrations. We show for the first time that *G. biloba* may exert opposite and biphasic effects on CYP1A2 and CYP2D6 metabolism. Induction of CYP1A2 and inhibition of CYP2D6 were found at low concentrations; the opposite was observed at high concentrations. CYP2D6 activity, regarded generally as non-inducible, was increased by exposure to common valerian (linear to dose) and *G. biloba* (highest concentration). An allosteric activation is suggested. From the data obtained, *G. biloba*, common valerian and St. John's wort are suggested as candidates for clinically significant CYP interactions *in vivo*.

The use of herbs as alternative and/or complementary therapy in the Western world is on the rise and gaining increasing popularity. As people often take different herbs in combination with prescribed Western medication [1], there is a potential for both pharmacokinetic and pharmacodynamic herb–drug interactions. In addition to doctor's recommendations [2], patients are also self-medicating with several different herbs and herbal preparations, thinking it is safe [3,4], and often without informing their primary physician.

It is important that possible interactions are discovered in order to avoid clinical implications, as shown for example between oral contraceptives and St. John's wort [5], cyclosporine and St. John's wort [6], and between *Ginkgo* and warfarin [7]. These are just a few of many [8], and we need to identify such harmful combinations in order to avoid serious and negative effects of concurrent use.

Cytochrome P450 (CYP) is a superfamily of enzymes, predominantly expressed in the liver, but also in the respiratory tract, lungs, brain and the small intestine [9,10]. CYP isoenzymes are the most important phase 1 enzyme system in the metabolism of xenobiotics, including Western medicines, endogenous compounds and herbal components as effective substrates [11]. Herb–drug interactions can appear when herbs and chemical drugs are co-administered and

the herbal preparation (one or more components) modulates the metabolism of the chemical drug by induction or inhibition of specific CYP enzymes. Also the metabolism of herbal components can be changed. In light of the increasing consumption of different herbal medicines in the Western world, where many people also take conventional drugs, the potential for herb–drug interactions also rises.

Functionally, CYP1A2, CYP2D6 and CYP3A4 are the major human CYP enzymes metabolizing a large majority of currently known drugs. Most scientific reports have so far presented inhibition data on these CYP enzymes, and their activity can all be inhibited, to a different extent, by many different herbs. Data on CYP induction by *in vitro* methodology are more rare. However, it has been shown that St. John's wort has an inhibiting effect on all three CYPs *in vitro* [12], but this herb is also a potent inducer of CYP3A4, both *in vitro* and *in vivo*, when taken over a period of time [13,14]. The traditional Chinese medicine 'Wu-chu-yu-tang', containing the herb *Evodia ruteacarpa*, has been shown to induce CYP1A2 in mice [15] and rats [16], and other investigations of dietary supplements have revealed both inhibition and induction of CYP1A2 and other CYPs [17,18]. Gurley et al. have reported a modest increase in CYP2D6 activity in humans after an intake of St. John's wort for 28 days [19], but argue that this can be attributed by other factors. On the contrary, one report found no effect at all in man of St. John's wort on either CYP3A4 or CYP2D6 [20]. Different herbs have been reported to inhibit CYP2D6; for example, goldenseal in humans [21], isolated compounds

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Table 1.

Weights before and after herbal solvent extraction, solvents used and concentrations of herbal stock solutions.

Herb	Weight, one pill or capsule (mg)	Weight, dried EtOH extract (mg)	EtOH for extraction and stock (%)	Volume for extraction (ml)	Concentration, stock solutions (mg/ml)
Common valerian	506	318	60	25+8	75.7
St. John's wort	534	472	60	25+8	47.8
Horse chestnut	386	300	50	20+6	125
<i>Echinacea purpurea</i>	3 ¹	95.3	20	–	146.6
<i>Ginkgo biloba</i>	264	210	50	15+5	56.8
Common sage	296	154	0	20+6	55

¹ml liquid.

from *Ginkgo biloba* [22] and *Serenoa repens in vitro* [23], to name but a few.

This study focuses on St. John's wort (*Hypericum perforatum*), common sage (*Salvia officinalis*), common valerian (*Valeriana officinalis*), horse chestnut (*Aesculus hippocastanum*), *Echinacea purpurea* and *Ginkgo biloba*. They are all high-selling herbal medicines worldwide and are also ingredients in 'one-herb' products classified as herbal medicinal products by the Norwegian Medicines Agency [24]. The advantage of investigating crude herbal extracts, as in this study, is that you will pick up inter-constituent interactions for different herbal constituents, as shown for instance for ginseng, where the compounds ginsenoide Rc and Rf increase the activity of c-DNA expressed CYP2C9 and CYP3A4, respectively, while ginsenoide Rd inhibits the same CYP enzymes [25].

To evaluate the magnitude of CYP induction by these herbs, primary human hepatocytes were used. This is considered by many a reliable and relevant *in vitro* system for evaluating interactions involving P450 enzymes [26–28]. The main purpose of this study is to identify the existence of possible invidious interaction effects of six frequently used herbal products on central CYP enzymes and to evaluate possible clinical significances of this in order to ensure and facilitate patient safety when these herbs are co-administered with Western medicines. Horse chestnut is not earlier investigated for its CYP interaction potential and common valerian is not earlier investigated for *in vitro* CYP induction.

Materials and Methods

Chemicals and reagents. Phenacetin (Sigma A-2500), testosterone (Sigma T-1500), rifampicin (Sigma R-5777), omeprazole (Sigma O-104), dextromethorphan (Sigma D-9684), collagenase (Sigma C-0130), hyaluronidase (Sigma H-3506), gentamicin (Sigma G-3632), amikacin (Sigma A-2324), albumin (Sigma A-8022), HEPES (Sigma H-3375), penicillin-streptomycin (Sigma P-0781), collagen (Sigma C-7661), β -NADPH (Sigma N-1630), Dulbecco's modified eagle's medium (Sigma D-2902), and insulin (Sigma I-6634) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetaminophen (product of phenacetin demethylation), dextroprhan (product of dextromethorphan O-demethylation) and 6 β -hydroxy-testosterone (product of testosterone hydroxylation) were provided by Daiichi Pure Chemicals (Tokyo, Japan).

St. John's wort (Hypericum Stada[®], Stada Arzneimittel AG, batch no. 4523), *Ginkgo biloba* (Seredrin[®], Bioplanta Arzneimittel GmbH, batch no. 3090103), common sage (Nosweat[®], Biokraft Pharma AB, batch no. JH561), common valerian (Valerina[®] Forte, Pharbio

Medical Int. AB, batch no. 010411), horse chestnut (Venastat[®], Boehringer Ingelheim International GmbH, batch no. 40230906) and *Echinacea* (Echinagard[®], Madaus AG, batch no. F0241165) were purchased at a local pharmacy.

Preparation of herbal extracts. The dry commercial herbal products (pills or capsules) were ground using a mortar, if needed, and dissolved in solvent for extraction (table 1). To make herbal solutions, the same extraction solvent was applied as that used by the producer when they extracted the actual herbal constituents from specific plants or roots. All solutions were incubated for 60 min. at 30°C with constant stirring. After decanting off the solvent, containing dissolved herbal constituents, additional volumes of solvent was added to the residue, followed by a new 60 min. of incubation. The two fractions containing extracted herbal constituents were pooled evaporated to dryness at 40°C and weighed (weight, dried EtOH extract; table 1). The residue was then dissolved in a small amount of the appropriate solvent to make herbal stock solutions of known concentrations (table 1). To make the stock solution of *E. purpurea*, 3 ml of the original herbal product was evaporated until dry, weighed and dissolved in a small amount of 20% ethanol. All stock solutions were kept at 4°C, avoiding light. Shelf life was set to 2 weeks.

Herbal concentrations. The herbal concentrations used in our *in vitro* metabolic system are expected to cover the range of herbal concentrations occurring *in vivo*. Our estimates are based on the total recommended daily intake of the different herbs (St. John's wort 1 capsule, 425 mg; common valerian, 5 capsules, 1000 mg; horse chestnut, 2 capsules, 540 mg; common sage, 1 capsule, 450 mg; *G. biloba*, 2 pills, 120 mg; *E. purpurea*, 60 drops, 265 mg) dissolved in 53 l, 5.3 l and 0.53 l, respectively. These are roughly representing, first, the concentration in the extra cellular fluid (and plasma), and, second and third, an extreme concentration range anticipated to appear in the small intestine. We are anticipating a 100% bioavailability, minor protein binding and minor accumulation. Final ethanol concentrations in all incubations were <1%, which did not interfere with the experiment (data not shown).

Hepatocyte isolation and induction. Human primary hepatocytes were prepared from donation following the Shanghai Donation Regulation (China) and informed consent (2001). The donor was a 32-year-old Mongolian man (Research Institute for Liver Diseases donor number GFHQ). Trauma was the cause of death. All serology data [human immunodeficiency virus, hepatitis B virus, hepatitis C virus and rapid plasma reagin (RPR)] were normal, and the donor was homozygous for the CYP2D6 wild type *1/*1. No data were available on smoking habits.

Human hepatocytes were prepared by a collagenase perfusion technique previously described by others [29]. Viability of prepared human primary hepatocytes was determined by the trypan blue exclusion method and hepatocytes were accepted for experimental use if viability was more than 70%. Primary human hepatocytes were plated on Falcon 24-well culture plates (0.5 ml, 0.35 \times 10⁶ cells/well) coated with collagen in Dulbecco's modified eagle's medium,

Table 2.

Effects¹ of selected herbs and inducers on CYP activities in human hepatocytes.

Herbs/inducers	Concentration ² (µg/ml)	Percentage of control		
		CYP1A2 ^{3†}	CYP2D6 ^{3‡}	CYP3A4 ^{3§}
Common valerian	18.75	57.1 ± 35.3	123.6 ± 4.4	131.5 ± 28.3
	187.5	123.1 ± 1.9	133.6 ± 3.8*	174.3 ± 5.8*
	1875	95.7 ± 6.1	141.4 ± 3.2*	210.5 ± 5.0*
St. John's wort	8	96.9 ± 10	107.1 ± 1.9	660.1 ± 1.7*
	80	120.4 ± 2.2*	117.1 ± 6.3	1075.8 ± 7.0*
	800	3.9 ± 22.7*	BLQ	61.8 ± 83.1
<i>Echinacea purpurea</i>	4.735	BLQ	29.7 ± 7.8*	56.2 ⁴
	47.35	BLQ	24.4 ± 13.0*	36.0 ⁴
	473.5	BLQ	BLQ	BLQ
Common sage	8.15	82.0 ± 4.4*	78.9 ± 13.7	33.3 ± 39.6*
	81.5	68.9 ± 2.2*	83.1 ± 3.1*	50.9 ± 22.4*
	815	31.7 ± 5.0*	67.7 ± 7.9*	42.5 ± 14.6*
<i>Ginkgo biloba</i>	2.19	139.1 ± 8.8*	55.0 ± 14.9*	73.8 ± 7.3*
	21.9	57.4 ± 20.7*	85.7 ± 10.8	65.0 ± 3.9*
	219	21.6 ± 30.6*	143.4 ± 6.0*	40.6 ± 22.1
Rifampicin (50 µM)	41.2	–	134.5 ± 4.3*	2163.8 ± 4.0*
Omeprazole (50 µM)	17.3	296.1 ± 7.6*	–	–

¹Results are given as mean ± S.D. for n = 4, *P ≤ 0.05; BLQ, metabolite below limit of quantification.²Concentration of total herb constituents or inducer in the incubation medium. The composition of constituents is equal to that found in the original product from the manufacturer.³Substrates: [†]phenacetin, [‡]dextromethorphan and [§]testosterone (all 100 µM).⁴Single measurements.

supplemented with insulin, albumin, D-fructose, gentamicin, amikacin and penicillin-streptomycin, and foetal calf serum. At 2–4 hr, the sample wells were inspected to ensure cell attachment. After 12 hr, a monolayer of cells was formed and unattached cells were removed by gentle agitation and the medium was replaced with a serum free medium (incubation medium) containing all the supplements previously mentioned. The incubation medium was changed every 24 hr. Culture plates with cells were maintained at 37°C in an atmosphere of 5% CO₂ and 95% air.

At 48, 72 and 96 hr, the cells were exposed to 0.5 ml incubation medium containing selective inducers (CYP1A2: omeprazole 50 µM, 17.3 µg/ml; CYP2D6 and CYP3A4: rifampicin 50 µM, 41.2 µg/ml), or herbal preparations with concentrations equal of that given in table 2.

Cytochrome P450 metabolism. At 24, 48, 72 and 96 hr, medium was aspired and non-induced cells were exposed to incubation medium containing probe substrates of CYP1A2 (phenacetin 100 µM, 17.9 µg/ml), CYP2D6 (dextromethorphan 100 µM, 37.0 µg/ml) or CYP3A4 (testosterone 100 µM, 28.8 µg/ml) for 1 hr at 37°C to establish basal levels of CYP activities. Incubations were stopped by adding 0.5 ml ice-cold methanol. Samples were transferred to centrifuge tubes, centrifuged at 1400 g for 5 min. and 500 µl of the supernatants were transferred to high performance liquid chromatography (HPLC) vials.

At 120 hr, incubation medium containing probe substrates were added to the cells exposed to inducers or herbal preparations, and incubated as described above.

HPLC measurement. The activity of CYP1A2 was determined by analysing the production of acetaminophen (phenacetin demethylation) by a validated HPLC method under the following conditions: column – Luna 5 µ phenyl-hexyl (150 × 4.60 mm, Phenomenex); mobile phase: solvent A – 10% CH₃CN, 15% CH₃OH, 75% H₂O; solvent B – 10% CH₃CN, 60% CH₃OH, 30% H₂O; flow rate – 1 ml/min.; gradient programme – initially 100% solvent A, ramp to 25% solvent B (and 75% solvent A) at 7.5 min. and back to 100% solvent A at 17.6 min. Total run time 23 min. Injection volume was 50 µl. Ultraviolet detection at 245 nm. Limit of quantification was 0.195 µM.

The activity of CYP2D6 was determined by analysing the production of dextropran (dextromethorphan O-demethylation) by a validated HPLC method under the following conditions: column – Nucleosil 5 µ micron C18 100A (250 × 4.60 mm Phenomenex); mobile phase: solvent A – 35.3% CH₃CN, 1.2% CH₃COOH, 63.4% H₂O, 0.1% C₂H₅F₃O₂; flow rate – 1 ml/min. Isocratic 100% solvent A, total run time 35 min. Injection volume was 50 µl. Fluorescence detection at 270 nm excitation and 310 nm emission. Limit of quantification was 0.081 µM.

The activity of CYP3A4 was determined by analysing the production of 6β-hydroxy-testosterone (6β-hydroxylation of testosterone) by a validated HPLC method under the following conditions: column – Luna 5 µ phenyl-hexyl (150 × 4.60 mm, Phenomenex); mobile phase: solvent A – 10% CH₃CN, 15% CH₃OH, 75% H₂O; solvent B – 10% CH₃CN, 60% CH₃OH, 30% H₂O; flow rate – 1 ml/min.; gradient programme – initially 100% solvent A, ramp to 35% solvent B (and 65% solvent A) over 10 min., ramp to 90% solvent B over the next 13 min., 100% solvent B for 4 min. and back to 100% solvent A at 27 min. Total run time 31 min. Injection volume was 50 µl. Ultraviolet detection at 247 nm. Limit of quantification was 0.065 µM.

Calculation of CYP activity. To calculate the level of CYP activities, the peak areas of metabolite formations were compared with standard curves of pure compounds (CYP1A2: acetaminophen – range 0.195 µM–25 µM, r² = 0.99; CYP2D6: dextropran – range 0.041 µM–5.18 µM, r² = 0.99; CYP3A4: 6β-hydroxytestosterone – range 0.065 µM–4.125 µM, r² = 0.99) with known concentrations. The concentration of metabolite formation was calculated using regression lines from the standard curve, and the enzyme activity was then calculated and normalized on the basis of million cells (0.35) in and time (60 min.) of the incubations. Enzyme activity: picomole metabolite formed/10⁶ cells/min.

Statistics. Data are presented as means ± S.D. of four replicates. A two-sample t-test was used to test the effect of herbal preparations or inducers on enzyme activity, and analysis of variance was used

to compare the effects on each CYP by each herbal concentration. Statistical analysis was performed on SPSS (SPSS for Windows, Release 13.0. 2004, SPSS Inc., Chicago, IL, USA). A P -value <0.05 was considered to be statistically significant.

Results

To verify the inductive properties of the human hepatocyte system, the cells were incubated with known CYP inducers (positive inductive control). Omeprazole was used for CYP1A2 and rifampicin for CYP3A4 and CYP2D6. The results are given in table 2 as percentage of controls. High inductive potentials were observed for both inducers, with a close to 3 times increase in CYP1A2 activity, a close to 1.5 times increase in CYP2D6 activity, and a 20 times increase in CYP3A4 activity. However, special attention should be paid to the induction of CYP2D6. The basic activities for CYP1A2, CYP2D6 and CYP3A4 were 83.20 ± 4.2 , 11.74 ± 0.7 , and 11.84 ± 1.3 pmol/ 10^6 cells/min., respectively.

The CYP inductive properties of the six selected herbal products were investigated at three different concentrations, as shown in table 2. All concentrations are estimated to be relevant for *in vivo* extrapolated biological concentrations. The effects of the positive inductive controls (data not shown) were not significantly different from those presented in table 2.

Effects of the selected herbs on CYP activities in the human hepatocytes are shown in detail in table 2. The main statistically significant inductive properties were found for the following herbs: common valerian (CYP2D6 and CYP3A4), St. John's wort (CYP3A4) and *G. biloba* (CYP1A2 and 2D6). The herbal preparations *E. purpurea*, common sage and horse chestnut (data not shown) showed inhibitory properties towards the CYPs investigated. The metabolite formation in incubations containing horse chestnut (at 9.85, 98.5 and 985 $\mu\text{g/ml}$) was below limit of quantification for all enzymes.

Figure 1 demonstrates a statistically significant log–log linear relationship ($y = 2.3 - 0.4x$, $r^2 = 0.99$, $P < 0.05$) between a log increase in CYP1A2 activity and log decrease in *G. biloba* concentrations. It should be noted that the two

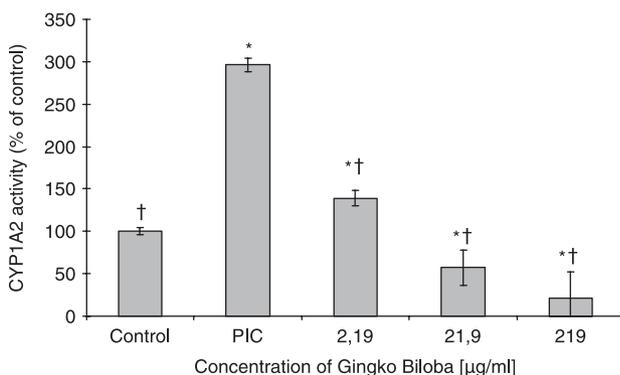


Fig. 1. CYP1A2 (phenacetin *O*-dealkylase) activity: hepatocytes incubated without (control) and with *Ginkgo biloba* (three concentrations) and with omeprazole as positive inductive control (PIC, 50 μM , 17.3 $\mu\text{g/ml}$), $n = 4$. *Different from control ($P < 0.05$); †different from PIC ($P < 0.05$).

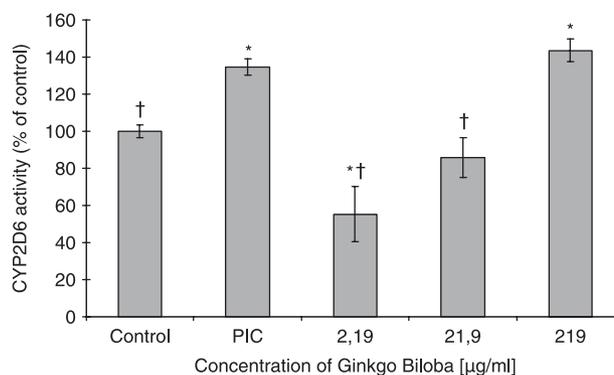


Fig. 2. CYP2D6 (dextromethorphan *O*-demethylation) activity: hepatocytes incubated without (control) and with *Ginkgo biloba* (three concentrations) and with rifampicin as positive inductive control (PIC, 50 μM , 41.2 $\mu\text{g/ml}$), $n = 4$. *Different from control ($P < 0.05$); †different from PIC ($P < 0.05$).

higher concentrations of *G. biloba* reduced CYP1A2 activity ($P < 0.05$), whereas the lower concentration increased the activity ($P < 0.05$) relative to control.

Figure 2 demonstrates a close to linear log dose–response relationship ($y = 0.4x + 0.35$, $r^2 = 0.97$, $P = 0.1$) between the log increase in concentration of *G. biloba* and increase in CYP2D6 activity. It should be noted that *G. biloba* increased CYP2D6 activity at the highest test concentration ($P < 0.05$) and decreased the activity at the lowest concentration ($P < 0.05$), relative to control.

Figure 3 demonstrates a statistically significant linear log dose–response relationship ($y = 0.09x + 1.13$, $r^2 = 0.99$, $P < 0.05$) between an increase in CYP2D6 activity and increase in common valerian concentrations. All concentrations of common valerian increased CYP2D6 activity ($P < 0.05$) in a dose-dependent manner relative to control.

Figure 4 demonstrates a statistically significant linear log dose–response relationship ($y = 0.4x + 0.82$, $r^2 = 0.99$, $P < 0.05$) between an increase in CYP3A4 activity and log increase in common valerian concentrations. No significant increase was

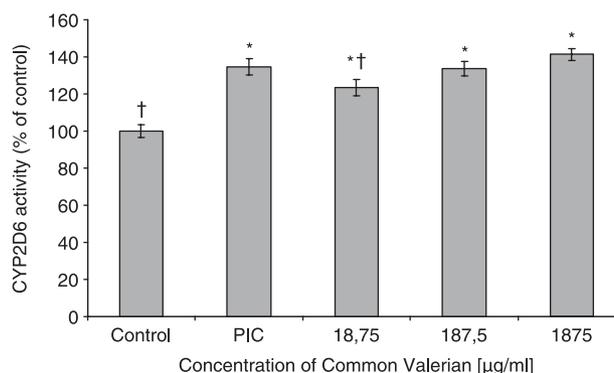


Fig. 3. CYP2D6 (dextromethorphan *O*-demethylation) activity: hepatocytes incubated without (control) and with Common valerian (three concentrations) and with rifampicin as positive inductive control (PIC, 50 μM , 41.2 $\mu\text{g/ml}$), $n = 4$. *Different from control ($P < 0.05$); †different from PIC ($P < 0.05$).

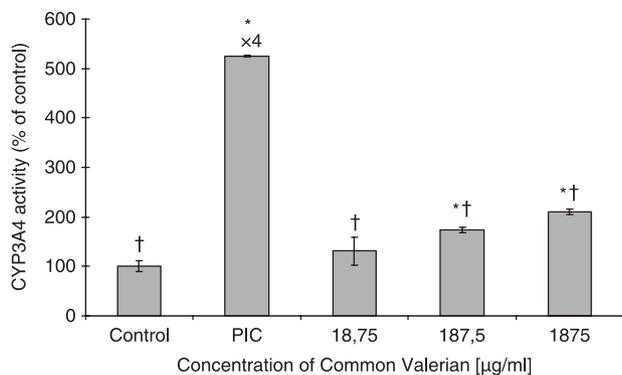


Fig. 4. CYP3A4 (testosterone 6 β -hydroxylation) activity: hepatocytes incubated without (control) and with Common valerian (three concentrations) and with rifampicin as positive inductive control (PIC, 50 μ M, 41.2 μ g/ml), $n = 4$. *Different from control ($P < 0.05$); †different from PIC ($P < 0.05$).

observed relative to control at the lowest level, but at the two high concentrations of common valerian significant higher values were observed ($P < 0.05$) compared to control.

Figure 5 shows the activity of CYP3A4 following exposure to increasing concentrations of St. John's wort. Activity increased in a dose-dependent manner at the two lower concentrations ($P < 0.05$), whereas it decreased dramatically at the highest concentration, down to an approximate control level.

Discussion

In this study, we investigated the CYP induction potential of six commonly traded herbal preparations manufactured in Norway. The selected herbal products are registered as herbal medicinal products by the Norwegian Medicines Agency. Each product contains, according to the manufacturer, only one main herb and not a mixture of different major herbs as for instance in products like Aqualette[®] (Chemist Brokers, Portsmouth, UK; contains horsetail and dandelion

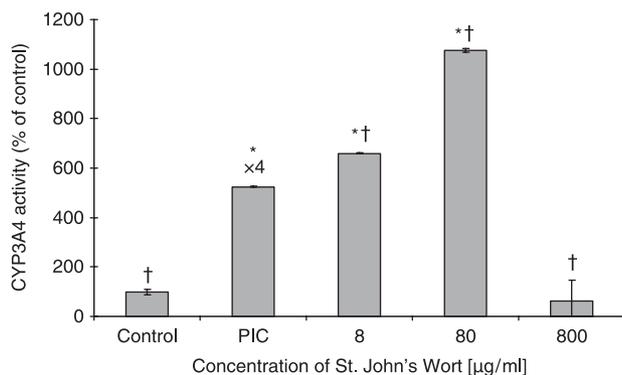


Fig. 5. CYP3A4 (testosterone 6 β -hydroxylation) activity: hepatocytes incubated without (control) and with St. John's wort (three concentrations) and with rifampicin as positive inductive control (PIC, 50 μ M, 41.2 μ g/ml), $n = 4$. *Different from control ($P < 0.05$); †different from PIC ($P < 0.05$).

root) and Essiac[®] (Essiac Canada International, Calgary, Canada; contains burdock root, sheep sorrel, slippery elm and turkey rhubarb). However, each product may certainly contain several minor constituents, of which several have not yet been identified. Primary human hepatocytes were used for evaluating effects of these herbs on CYP1A2, CYP2D6 and CYP3A4 metabolism.

The results showed that, besides St. John's wort, common valerian was the strongest inducing herb. Out of the three CYPs, CYP3A4 was the one easiest to induce. St. John's wort and common valerian both induced statistically significant CYP3A4; common valerian also CYP2D6. *Ginkgo biloba* showed a complex induction/inhibition pattern of CYP1A2 and CYP2D6. We experienced statistically significant inhibition of all the investigated CYP enzymes by horse chestnut and *E. purpurea*.

Herbs are complex mixtures of different compounds, and we chose to use the whole herb in our study, and not just isolated ingredients. There are two main reasons for this: (i) people usually take the whole herb and not isolated ingredients; and (ii) although some of the pharmacologically active compounds have been identified, there might be other chemicals in the herb capable of modulating CYP enzymes. For making identity with the original products, constituents from each product were extracted into the same solvent as used by the manufacturer. Then we evaporated until dry, determined weight and added a limited amount of new solvent. In this way, we ended up with a concentrated product equal in composition to the one being traded. At the same time we obtained solutions with known concentrations (μ g/ml) for experimental use.

St. John's wort is a known inducer of CYP3A4, both *in vitro* and *in vivo* [13,14,30], and is capable of inhibiting CYP3A4 in isolated cDNA expressed human enzymes [12,31]. From the literature it seems that inhibition and induction depend on exposure time, as repeated exposures lead to induction, while a single dose may lead to inhibition [32]. However, our data indicate that the induction and inhibition of CYP3A4 by St. John's wort might also depend on dose, with induction at low concentrations and inhibition at high concentrations. This is also in line with what is reported for one of the main St. John's wort constituents, hyperforin, with a similar pattern [12,33].

In our study, an 8- μ g/ml solution of the St. John's wort preparation increased the formation of 6 β -OH-testosterone with 660% of control (1075% at 80 μ g/ml), supporting reports of decreased plasma concentrations of CYP3A4 substrates in patients taking St. John's wort as well as their medicines [34,35]. At 800 μ g/ml St. John's wort inhibited the enzymes investigated. This could be due to a cytotoxic effect, as previous experiments have shown that hepatocytes can take up no more than 2.5 μ M hyperforin in chronic exposure [14]. A concentration of 800 μ g/ml St. John's wort corresponds to approximately 3–5 μ M hyperforin, which supports such a statement. However, a possibility of an inhibitory effect of St. John's wort at very high concentrations, even when administered repeatedly, cannot be ruled out and should be further investigated.

Ginkgo biloba showed a moderate inductive effect on CYP1A2 (140% of control) at a concentration of 2.19 µg/ml, and inhibition for higher concentrations. The expression of CYP1A2 is controlled by the aryl hydrocarbon receptor, which is activated among others by some environmental toxins [36,37] and cigarette smoke [38]. If we compare the induction of CYP1A2 by *G. biloba* with the effect of omeprazole on CYP1A2 (300% of control) or the effect of St. John's wort on CYP3A4, we see that the obtained effects are quite modest. It is difficult to assess whether this interaction will be clinically significant. The magnitude of the induction is not very big, but as the effect takes place at a low concentration, further studies, with an extended range of low concentrations, should be initiated. Especially drugs that have a narrow therapeutic range (e.g. clozapine and theophylline, which are typical 1A2 substrates), could be candidates for clinically significant interactions with *G. biloba*. Research has suggested that omeprazole only induces CYP1A2 in poor metabolizers when given in clinical doses [39]; however, clinically significant interactions between omeprazole and clozapine have been reported [40] and give further indications of possible effects of *G. biloba*. Furthermore, Ryu and Chung [17] showed that a herbal dietary supplement containing *G. biloba* induced CYP1A2 *in vivo* (humans – up to 203%; rats – up to 440%) when taken in recommended daily doses and using caffeine as a substrate. However, Gurley et al. [41] did not find any effect of *G. biloba* when the herb was given to elderly patients. Caffeine was also used as a substrate in this study.

The *G. biloba* product demonstrates a complex induction/inhibition pattern as shown in figs 1 and 2. At low and clinically relevant systemic exposures, induction of CYP1A2 and an inhibition of CYP2D6 seem to occur. The opposite occurs at higher concentrations, the latter though outside the systemic *in vivo* range, and could be relevant for high intestinal concentrations. This observation may reflect the fact that herbs contain several constituents and that the effect of these different constituents dominates differently at different concentrations. This also shows the relevance in studying the herb as a whole, as we would probably miss these effects if using only isolated compounds.

These concentration-dependent biphasic effects of *G. biloba* on CYP1A2 and CYP2D6 are in line with that earlier shown for hyperforin [12,33] and for St. John's wort in this study, towards CYP3A4. This may indicate more concentration-dependent biphasic effects of herbal products and xenobiotics towards several CYP isoforms than earlier anticipated.

Common valerian and *G. biloba* both increased CYP2D6 activity, 141% and 143% of control, respectively, at the highest herbal concentration (1875 µg/ml and 219 µg/ml). To our knowledge, there are no reports on induction of CYP2D6, and it has even been claimed to be non-inducible [42,43]. The observed 'induction' can therefore be attributed to an allosteric activation directly on the CYP2D6 enzyme itself by one or more of the compounds in these herbs [44–46]. The modest increase in CYP2D6 activity by rifampicin

has been noted by many investigators [47–49], but the underlying mechanism has not yet been described.

Common valerian also induced CYP3A4, 200% relative to control. However, the concentration at which the induction becomes statistically significant is too high for systemic effects. However, there might be a potential for an inductive effect on intestinal CYP3A4 metabolism and, hence, on a decreased drug bioavailability. To our knowledge, this is the first study to assess the inductive properties of common valerian on CYP enzymes *in vitro*. Two recent reports have investigated the effects of common valerian in humans and found no effect on either CYP3A4 or CYP2D6. Donovan et al. [50] say that common valerian is unlikely to produce interactions with CYP2D6, but they draw no categorical conclusion. The study focused on inhibition, but should also observe induction, if any. CYP3A4 activity is also unaffected in the study, using alprazolam as substrate. Gurley et al. [21] seem to have used a product lacking the marker compound valerenic acid in the study and suggest that their result might not be representative for other valerian products. Anyhow, our study demonstrates a dose-dependent increase of both CYP2D6 and 3A4 with increasing exposure to common valerian. The above results are interesting and further experiments should be initiated, perhaps also in liver microsomes, in order to elucidate further the findings in this study.

The steady-state plasma and intestinal concentrations in man of all herbal constituents should ideally be known as a basis for the selection of *in vivo*-relevant herbal concentrations. Adequate concentrations in *in vitro* experiments are important for *in vivo* evaluations; bioavailability has been mentioned as an important link between basic pharmacological research and clinical situations [51]. However, few data are available for herbal constituents. Some reports though are available on the plasma uptake of the herbal constituents hypericin and hyperforin in St. John's wort [52–54]. After daily repeated dosages to humans in the range of 300 mg to 900 mg, hypericin plasma C_{max} concentrations were measured to 1–22 ng/ml. Ginkgolides and bilobalides (terpenoids) in *G. biloba* [55,56] produced terpenoids plasma C_{max} concentrations of 58.9–181.1 ng/ml after daily repeated dosages of 120–160 mg. There might be other constituents in the herbs that are more or less systemically available. In our approach for systemic herbal uptake (Materials and Methods), we used the daily administered dose of total herb diluted in the total body water (53 l) as a common guide for estimation of relevant systemic plasma concentrations. Furthermore, 5.3 l and 0.53 l were used as common guides for a relevant range of gastrointestinal concentrations. From our dosages of total herbs, a systemic (plasma) hypericin concentration of 16 ng/ml and terpenoid concentration of 110 ng/ml can be estimated.

Although our estimates of *in vivo* systemic (plasma) concentrations of total herb (µg/ml) from daily dosages are very rough, fairly good approximates were achieved for constituents from St. John's wort and *G. biloba*, where adequate literature values are available. Objections that no herbal constituents have 100% bioavailability and no protein binding

are true; however, this may be balanced by the fact that no accumulation factors are included. As a first step approach to an estimate of systemic (plasma) concentrations of total herb from a given daily dose, the presented approach could be useful and well fitted for the selection of adequate *in vitro* test concentrations.

The use of human hepatocytes closely resembles an *in vivo* situation with all co-factors and other substances present that the P450 enzymes normally encounter in the liver. Also, the CYP enzymes can be induced *in vitro* in a way that correlates to an *in vivo* situation [57], given that the experiments are conducted at clinically and pharmacologically relevant concentrations. Another great advantage with the human hepatocytes is that you avoid the problem with species difference; for example is rifampicin, an effective inducer of human CYP3A, but not an inducer of the rat enzyme CYP3A [58,59]. The metabolic profiles of drugs also differ from species to species. However, one problem in this study is that only hepatocytes from one donor were used. In the CYP superfamily, there are many polymorphisms [60–63], and this study does not cover variation, neither was this our intention with the study. As the basic activities of the investigated CYP enzymes were all inside the accepted normal variation of the laboratory (data not shown), no or minor polymorphism is indicated. The fact that the liver donor was a Mongolian does not exclude that these data on CYP activity can be extrapolated to Caucasians, as there are no major differences in CYP metabolism between ethnic groups [64].

Acknowledgements

This study was supported by the Norwegian Research Council and the Norwegian University of Science and Technology.

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