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Inhibition of human cytochrome P450 enzymes by hops (*Humulus lupulus*) and hop prenylphenols

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Abstract

As hops (*Humulus lupulus* L.) are used in the brewing of beer and by menopausal women as estrogenic dietary supplements, the potential for hop extracts and hop constituents to cause drug-botanical interactions by inhibiting human cytochrome P450 enzymes was investigated. Inhibition of major human cytochrome P450 enzymes by a standardized hop extract and isolated hop prenylated phenols was evaluated using a fast and efficient assay based on ultrahigh pressure liquid chromatography-tandem mass spectrometry. The hop extract at 5 µg/mL inhibited CYP2C8 (93%), CYP2C9 (88%), CYP2C19 (70%), and CYP1A2 (27%) with IC₅₀ values of 0.8, 0.9, 3.3, and 9.4 µg/mL, respectively, but time-dependent inactivation was observed only for CYP1A2. Isoxanthohumol from hops was the most potent inhibitor of CYP2C8 with an IC₅₀ of 0.2 µM, whereas 8-prenylnaringenin was the most potent inhibitor of CYP1A2, CYP2C9 and CYP2C19 with IC₅₀ values of 1.1 µM, 1.1 µM and 0.4 µM, respectively. Extracts of hops contain prenylated compounds such as the flavanones isoxanthohumol and 8-prenylnaringenin and the chalcone xanthohumol that can inhibit CYP450s, especially the CYP2C family, which may affect the efficacy and safety of some CYP2C substrate drugs when co-administered.

Keywords

Cytochrome P450 inhibition; hops; isoxanthohumol; 8-prenylnaringenin; drug-botanical interactions

1. Introduction

The female flowers of hops (*Humulus lupulus* L.) are used in the brewing industry to add aroma and bitterness to beer. Although hop preparations are widely used as mild sedatives,

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most of the recent research has focused on their potential estrogenic and chemopreventive properties. Known active constituents of hops belong to the class of prenylphenols and may be divided into two groups: prenylated chalcones and prenylated flavanones. In hop cones, the most abundant prenylated chalcone is xanthohumol (XN; Fig. 1) which is contained in amounts up to 1% of the dry weight (Stevens et al., 1997; 2004). Studied primarily for its chemoprevention properties, XN has shown anti-proliferative activity against breast, colon and ovarian cancer cell lines (Colgate et al., 2007; Miranda et al., 1999) and is a potent inducer of quinone reductase (Dietz et al., 2005).

Among the prenylated flavanones, 8-prenylnaringenin (8-PN; Fig. 1) has been identified as one of the most potent of the known phytoestrogens (Milligan et al., 1999), and its estrogenic properties have been confirmed in numerous *in vitro* (Overk et al., 2005; Schaefer et al., 2003) and *in vivo* (Overk et al., 2008) assays. Isoxanthohumol (IX; Fig. 1), the 5-*O*-methyl derivative of 8-PN, has much weaker estrogenic activity (Overk et al., 2008). However, several *in vitro* and *in vivo* studies have shown that IX can be metabolically converted into 8-PN, either by the action of cytochrome P450 (CYP) enzymes (Nikolic et al., 2005) or by intestinal microflora (Possemiers et al., 2005; 2006). Therefore, IX may be considered a pro-phytoestrogen.

As women are using hop-based supplements as alternatives to estrogen replacement therapy (Zanolini and Zavatti, 2008), it is important to understand the potential of these supplements to interact with clinically used drugs. Drug-botanical interactions include inhibition and induction of drug metabolizing enzymes, especially the cytochrome P450 enzymes, and induction or inhibition of drug transporters. While studying cytochrome P450 enzymes involved in carcinogen activation, Henderson *et al.* (2000) reported that some hop prenylflavonoids can inhibit CYP1A1, CYP1B1 and CYP1A2. Foster *et al.* (2011) reported that hop-containing beers can inhibit CYP2C9, but no specific inhibitors were identified. Since hops have the potential to interfere with drug metabolism, more detailed studies of these drug-botanical interactions are needed.

To facilitate these studies, we developed a rapid, selective and sensitive assay using ultra high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS-MS) for the quantitative analysis of probe substrates of specific cytochrome P450 enzymes. This assay is similar to previous approaches that used a cocktail of probe substrates (Tolonen et al., 2007), but it is more than twice as fast per analysis as any previous approach. This assay was then used to measure inhibition of specific human cytochrome P450 enzymes by a standardized hop extract prepared for use as a dietary supplement by menopausal women. As a result, four hop prenylated phenols were identified that are inhibitors of members of the CYP2C family of drug metabolizing enzymes.

2. Materials and methods

2.1 Materials

All organic solvents were HPLC grade or better and were purchased from Thermo Fisher (Hanover Park, IL). Purified water was prepared by using a Millipore Milli-Q purification system (Millipore, Billerica, MA). Biochemicals, unlabeled internal standards, CYP probe

substrates, and CYP probe substrate metabolites were purchased from Sigma-Aldrich (St. Louis, MO). Biochemicals included reduced nicotinamide adenine dinucleotide phosphate (NADPH), magnesium chloride, ethylenediaminetetraacetic acid. CYP probe substrates included phenacetin, bupropion, amodiaquine, tolbutamide, S-(+)-mephenytoin, dextromethorphan, chlorzoxazone, and midazolam, and the reference standards of the metabolites of the CYP probe substrates included acetaminophen, hydroxylbupropion, desethylamodiaquine, hydroxytolbutamide, 4-hydroxy-mephenytoin, dextrorphan, 6-hydroxy-chlorzoxazone, and 1'-hydroxy-midazolam. Flurazepam was used as an unlabeled internal standard for the measurement of 1'-hydroxy-midazolam. Stable isotope-labeled internal standards were purchased from Cerilliant (Round Rock, TX) or BD Gentest (Woburn, MA) and included [d₄]-acetaminophen, [d₆]-hydroxy-bupropion, [d₃]-desethylamodiaquine, [d₉]-hydroxytolbutamide, [d₃]-4-hydroxy-mephenytoin, [d₃]-dextrorphan, [d₇]-6-hydroxy-chlorzoxazone. Mixed human liver microsomes pooled from 50 donors (protein concentration 20 mg/ml; CYP total activity 340 pmol/min/mg) and recombinant CYP2C8, CYP2C9, CYP2C19, and CYP1A2 were purchased from BD Biosciences (San Jose, CA).

The hop extract examined during this study was developed at the UIC/NIH Center for Botanical Dietary Supplements Research for use in clinical studies of safety and efficacy. The extract was prepared from botanically authenticated hops provided by Hopsteiner (New York, NY) that had been depleted of bitter acids and then standardized chemically to contain 33.84% XN, 0.35% 8-PN, 1.77% 6-prenylnaringenin (6-PN), and 1.07% IX (structures in Fig. 1). XN was isolated from hops and purified as previously described (Chadwick et al., 2004); the purity of XN was determined to be >99.5% by qHNMR. IX (> 99.0% pure by qHNMR) was prepared by cyclization of XN as described previously (Chadwick et al., 2004). 8-PN was synthesized chemically, and 6-PN was purified as previously reported (Stevens et al., 1999).

2.2 Methods

2.2.1 Screening for inhibitors of cytochrome P450 enzymes.—Incubation mixtures (100 μ L) contained 0.1 mg/mL human liver microsomes, 1 mM NADPH, hop extract (5 μ g/mL) or test compound (1 μ M or 10 μ M), and cytochrome P450 substrate (see substrates and their concentrations in Table I) in 100 mM potassium phosphate buffer (pH 7.4) containing 5 mM MgCl₂ and 1 mM EDTA. The hop extract and the substrates were dissolved in methanol (final methanol concentration < 1%). After pre-incubation at 37°C for 5 min, the reactions were initiated by addition of 1 mM NADPH. Enzymatic reactions (Table I) were carried out under conditions shown to be linear with respect to time, HLM protein concentration, and initial substrate concentration (equal to the apparent K_m). After incubation, the reaction was stopped by the addition of 400 μ L acetonitrile containing the internal standards. The samples were vortexed for 2 min and centrifuged at 13,000 $\times$ g at 4°C for 10 min. After centrifugation, 450 μ L supernatant was transferred to a micro-centrifuge tube and evaporated to dryness under a stream of nitrogen. Each residue was reconstituted in 50 μ L 20% aqueous acetonitrile, and 5 μ L was injected onto the column for analysis using UHPLC-MS-MS.

To test for time-dependent inactivation of the cytochrome P450 enzymes that were determined to be inhibited by hops, recombinant enzymes (300 pmol/mL) were pre-incubated with hop extract (50 µg/mL) or test compound (10 µM) for 30 min at 37 °C in 100 mM potassium phosphate buffer (pH 7.4) with or without (control) NADPH (1 mM). The concentrations of hop extract and test compound were selected to be 10-fold higher than the corresponding IC₂₅ value or 10 µM if no IC₂₅ was available (Grimm et al., 2009). At 0 min and 30 min after incubation, 5 µL aliquots of each mixture were transferred to secondary incubation mixtures containing a probe substrate (85 µM phenacetin, 3.5 µM amodiaquine, 400 µM tolbutamide, or 85 µM S-(+)-mephenytoin for CYP1A2, CYP2C8, CYP2C9, or CYP2C19, respectively) and 1 mM NADPH in a total volume of 100 µL. The secondary incubation was carried out as described in Table I. Note that aliquots of the pre-incubation mixtures were diluted 20-fold with probe substrate at a saturating concentration (4-fold K_m) to measure residual enzyme activities. After incubation, each reaction was stopped by the addition of 20 µL water/acetonitrile/formic acid (92:5:3, v/v) containing the internal standards. The samples were vortexed for 30 s and centrifuged at 13,000 × g at 4°C for 10 min. After centrifugation, 5 µL aliquots of the supernatants were analyzed using UHPLC-MS-MS.

2.2.2 UHPLC-MS-MS—Formation of metabolites from probe substrates was measured using UHPLC-MS-MS on a Shimadzu (Kyoto, Japan) Nexera UHPLC system interfaced with a Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Analytes were separated on a Shimadzu Shim-pack XR-ODS III UHPLC column (2.0 × 50 mm, 1.6 µm) using a 2 min linear gradient from 5-100% acetonitrile in 0.1% aqueous formic acid. The UHPLC column was re-equilibrated at 5% acetonitrile for 1 min before the next injection. The total run time including equilibration was 3 min. The flow rate was 0.5 mL/min. As some metabolites required positive ion electrospray and others required negative ion electrospray, polarity switching was used so that all species could be measured during a single UHPLC-MS-MS analysis. The selected reaction monitoring (SRM) transitions that were used for the analyses are summarized in Table II. The dwell time was 20 ms/ion, and the polarity switching time was 15 ms. The mass spectrometer source parameters were as follows: DL temperature 300°C, spray voltage 3500 V, nebulizing gas flow 3 L/min, and drying gas flow 20 L/min.

The simultaneous analysis of the metabolites of all 8 probe substrates and their corresponding 8 internal standards was carried out within 2 min using UHPLC-MS-MS (Fig. 2). Polarity switching was used during electrospray tandem mass spectrometry for optimum sensitivity, since acetaminophen, dextorphan, desethylamodiaquine, hydroxybupropion, 4-hydroxy-mephenytoin, and 1'-hydroxy-midazolam ionized more efficiently using positive ion electrospray, while 6-hydroxy-chlorzoxazone and hydroxytolbutamide ionized most efficiently in negative ion mode (Table II). Stable isotope labeled analogs of the metabolites were added to each sample as internal standards after incubation with human liver microsomes to control for sample losses during handling, instrument variations between analyses, and matrix effects that might cause ion suppression or enhancement. These internal standards co-eluted or nearly co-eluted with their respective metabolites during UHPLC-MS-MS (Fig. 2). As a structurally similar analog of 1'-hydroxy-midazolam was

already in use in our laboratory (flurazepam), this compound was used as an internal standard instead of a stable isotope labeled analog of 1'-hydroxy-midazolam (Table II and Fig. 2G).

2.2.3 IC₅₀ determination—When cytochrome P450 enzyme inhibition by a single compound at 10 μ M exceeded 50%, follow-up experiments were carried out to determine the IC₅₀ value. The IC₅₀ value was determined by measuring the cytochrome P450 enzyme activities at 8 or more inhibitor concentrations spanning 4 orders of magnitude. The initial substrate concentrations were identical to their respective K_m values. Incubations with human liver microsomes were carried out using individual substrates as described above. Comparison was made with negative control incubations containing no inhibitor, and activity was expressed as the percentage of control activity remaining.

2.2.4 Statistical analysis—All experiments were carried out in triplicate, and all data are expressed as the mean $\pm$ SD. The statistical analysis of these results consisted of a student t-test and ANOVA using GraphPad Software (La Jolla, CA) Prism 5.0. Peak areas were determined and evaluated using Shimadzu quantitation software. Inhibition curves were constructed consisting of percent control activity versus the logarithm of the test compound concentration, and IC₅₀ values were calculated via exponential decay with single, four-parameter curve fitting analysis using Sigma Plot 8.0 software (Systat Software, Chicago, IL).

3. Results

3.1 UHPLC-MS-MS

Eight cytochrome P450 enzymes, which are responsible for the metabolism of the vast majority of clinically useful drugs, and the corresponding probe substrates selected for these studies of enzyme inhibition assays are shown in Table I. These enzymes and probe substrates were recommended by the U.S. Food and Drug Administration (2012) for properties including robust turnover, high selectivity for one CYP enzyme, and good aqueous solubility. Phenacetin was selected as a substrate for CYP1A2 (Table I), and its metabolite acetaminophen was measured using UHPLC-MS-MS (Fig. 2 and Table II). Bupropion and amodiaquine were used as substrates for CYP2B6 and CYP2C8, respectively, and their metabolites, hydroxybupropion and desethylamodiaquine were measured. The formation of hydroxytolbutamide from tolbutamide was measured as an indication of CYP2C9 activity, and the metabolite of mephenytoin, 4-hydroxy-mephenytoin, was measured to indicate CYP2C9 activity. Dextromethorphan was used as a probe substrate for CYP2D6 by measuring the formation of dextorphan using UHPLC-MS-MS (Fig. 2). Finally, 6-hydroxy-chlorzoxazone and 1'-hydroxy-midazolam, which are metabolites of chlorzoxazone and midazolam, were measured as indicators of CYP2E1 and CYP3A4 activity, respectively (Table I and Table II).

3.2 Inhibition of CYP isoforms

The hop extract and isolated prenylated phenols from hops were assayed for inhibition of the eight cytochrome P450 enzymes which are responsible for the metabolism of the vast

majority of clinically useful drugs. The four major prenylated compounds in hops (XN, IX, 6-PN, and 8-PN) were tested individually at 1 μ M and 10 μ M for inhibition of CYP enzymes. There was no significant inhibition of CYP3A4, CYP2D6 or CYP2E1 by the hop extract or any of the prenylated compounds isolated from hops (Table III). However, at 10 μ M, all 4 prenylated compounds showed >60% inhibition of CYP2C8 and CYP2C9. The prenylated phenols IX and 8-PN, but not XN or 6-PN, were potent inhibitors (>90%) of CYP2C19. 8-PN was also a potent (>88%) inhibitor of CYP1A2 (Table III).

The hop extract at 5 μ g/mL showed significant (70%) inhibition of CYP2C8, CYP2C9 and CYP2C19 but only weak inhibition of CYP2B6 (36%) and CYP1A2 (27%) (Table III). Inhibition of the CYP2C family was probably due to the actions of multiple prenylated compounds present at relatively high levels in the hop extract. The weak inhibition of CYP1A2 can be ascribed to 8-PN, which represents a potent inhibitor of CYP1A2 but was present at only 0.35% of the hop extract. A mixture of XN, 8-PN, 6-PN, and IX at the same concentrations as in the standardized hop extract (33.84% XN, 0.35% 8-PN, 1.77% 6-PN, and 1.07% IX) did not show any significant inhibition of CYP2B6. Therefore, the hop extract might contain as yet unknown constituents which are responsible for this effect.

After these initial screening experiments, IC_{50} values were determined for the hop extract and for those individual hop prenylated phenols that showed >50% inhibition of cytochrome P450 enzymes at 10 μ M (Table III). As expected, the hop extract showed strong inhibition of CYP2C isoforms with IC_{50} values of 0.8 ± 0.1 μ g/mL and 0.9 ± 0.1 μ g/mL for inhibition of CYP2C8 and CYP2C9, respectively (Table IV). The hop extract also inhibited CYP2C19, showing an IC_{50} value of 3.3 ± 0.7 μ g/mL. In addition to the pronounced inhibition of the CYP2C family, the hop extract only moderately inhibited CYP1A2 with an IC_{50} value of 9.4 ± 0.6 μ g/mL.

Time-dependent enzyme inactivation experiments showed that the hop extract produced no irreversible inhibition of CYP2C8, CYP2C9 or CYP2C19 (data not shown). However, preincubation of the hop extract with CYP1A2 did show partial inactivation (Fig. 3). Although 8-PN was found to be an inhibitor of CYP1A2 (Tables III and IV), preincubation with the enzyme showed no time-dependent inactivation. Instead, XN was found to produce time-dependent inactivation of CYP1A2 (Fig. 3).

The isolated hop prenylated compounds showed potent inhibition against CYP2C8 and CYP2C19 with some compounds such as IX and 8-PN showing inhibition in the upper nanomolar range (Table IV). Compared with its isomer 6-PN, 8-PN was a more potent inhibitor of CYP1A2, CYP2C8, CYP2C9, and CYP2C19. The prenylated flavanol IX was a more potent inhibitor of CYP2C8, CYP2C9 and CYP2C19 than was its isomer, the chalcone XN (Table IV).

4. Discussion

The majority of drug-drug interactions result from inhibition of cytochrome P450 enzymes, and inhibition of the metabolism of drugs by cytochrome P450 enzymes is also responsible for most clinical problems associated with drug-botanical interactions. The enzyme

CYP3A4 accounts for ~30% of the cytochrome P450 enzymes in human liver and is responsible for the metabolism of ~70% of all drugs (Wienkers and Heath, 2005). Our data did not indicate any significant inhibition of CYP3A4 by hops or prenylated hop phenols, and these results are consistent with those of Henderson *et al.* (2000). Although hops and prenylated hop compounds did not inhibit CYP3A4, they were significant inhibitors of enzymes of the CYP2C family, including CYP2C8, CYP2C9 and CYP2C19. Among the hop compounds tested, IX was the most potent inhibitor of CYP2C8, and 8-PN was the most potent inhibitor of CYP2C9 and CYP2C19 (Table IV). Enzymes of the 2C subfamily account for approximately 25% of total human liver cytochrome P450 (Ioannides, 1996) and metabolize a large number of drugs. For example, CYP2C8 is responsible for the metabolism of statins that are widely prescribed to combat hypercholesterolemia (Rokitta and Fuhr, 2010), and CYP2C9 is responsible for the metabolism of many clinically important drugs such as the anticoagulant drug warfarin and many nonsteroidal anti-inflammatory drugs (Goldstein and De Morais, 1994). Therefore, inhibition of CYP2C enzymes by hop dietary supplements might be worth further investigation in clinical trials as a potential source of drug-botanical interactions.

A mixture of XN, IX, 8-PN, and 6-PN, prepared at concentrations identical to their levels in the standardized hop extract, was tested for inhibition of cytochrome P450 enzymes. This mixture inhibited CYP2C enzymes in a manner similar to that of the hop extract (Table 3) and accounted for all of the CYP2C8 and CYP2C19 inhibition and the majority of the CYP2C9 inhibition. These results indicate that the contributions of the prenylated compound to the inhibition of the CYP2C enzymes were additive rather than synergistic.

In contrast, the prenylated hop phenol mixture contributed only partially to the inhibition of CYP1A2 and CYP2B6 that was observed for the hop extract (Table III). This suggests that other compounds in hops are responsible for much of the observed inhibition of CYP1A2 and CYP2B6. However, due to the relatively low inhibitory activity of the hop extract toward CYP1A2 and CYP2B6 (< 40% at 5 µg/mL), identification of additional inhibitors of these enzymes in the extract was not pursued. CYP1A2 showed time-dependent inactivation by hops, and XN was found to be responsible, at least in part, for this effect. However, the magnitude of CYP1A2 inactivation by the hop extract relative to that of XN alone (Fig. 3) suggests that other compounds in hops might also cause time-dependent inactivation of this enzyme.

Polymorphisms of drug metabolizing enzymes influence the efficacy and safety of some drugs, and drug-botanical interactions can potentiate these effects. Approximately 5-10% of the population has defects in CYP2D6, which result in exaggerated responses to certain drugs such as the chemoprevention agent tamoxifen and the antitussive agent dextromethorphan (Puga et al., 1997). A genetic polymorphism in the metabolism of mephenytoin has been traced to defects in CYP2C19 which are rare in Caucasians (2-5%) but are more common (12-23%) in Orientals subjects (Ioannides, 1996). Although the hop extract and hop compounds did not inhibit CYP2D6, the hop extract and the prenylated flavanols 8-PN and IX showed significant inhibition of CYP2C19 (Table III and Table IV). Interestingly, the prenylated chalcone, XN, did not significantly inhibit CYP2C19.

Human liver cytochrome P450 is composed of 15-20% CYP1A2, and substrates for CYP1A2 include acetaminophen, phenacetin, imipramine, theophylline, caffeine, and warfarin (Ioannides, 1996). CYP1A2 is also involved in the metabolic activation of many environmental carcinogens. The hop extract showed weak inhibition of CYP1A2, and 8-PN, which was present at only 0.35% in the hop extract, was a significant inhibitor of this enzyme (Table III). Therefore, while hop dietary supplements have the potential to interact with drugs that are metabolized extensively by CYP1A2, hops can also protect against the formation of carcinogenic metabolites that are products of CYP1A2 metabolism. These results are consistent with those of Miranda *et al.* (2000), who reported >90% inhibition of CYP1A2 by 8-PN at 10 μ M. Although Miranda also reported >90% inhibition of CYP1A2 by IX at 10 μ M, we found only 48.2% inhibition of CYP1A2 at the same concentration of IX. This minor discrepancy is probably the result of using different enzyme inhibition assays; we used human liver microsomes instead of recombinant CYP1A2 and phenacetin as the probe substrate instead of acetanilide.

If serum levels of XN, IX, 6-PN, and 8-PN can reach high nM concentrations, then these compounds would have the potential to inhibit several human cytochrome P450 enzymes *in vivo*. In a recent human pharmacokinetics study of xanthohumol, Legette, *et al.* (2013) reported that after single doses of xanthohumol of 20, 60 or 180 mg each to 24 women and 24 men, the maximum serum levels (measured as aglycons after enzymatic deconjugation) were 33 mg/L (93 μ M), 48 mg/L (135 μ M) or 120 mg/L (340 μ M), respectively. Furthermore, the mean half-life values of XN were 20 h and 18 h for the 60 mg and 180 mg doses, respectively. The serum concentrations of XN in this pharmacokinetics study exceeded by over 100-fold the IC₅₀ values we determined for XN inhibition of CYP2C8 (1.1 ± 0.1 μ M) and CYP2C9 (3.3 ± 0.4 μ M). In another human study, Bolca, *et al.* (2010) administered to women daily for 5 days a hop dietary supplement containing 2.04 mg XN, 1.20 mg IX and 0.1 mg 8-PN per dose. Serum levels of XN and IX were measured after deconjugation of the Phase II metabolites and were as high as 0.0176 μ M and 0.0315 μ M, respectively (approximately 100-fold and 10-fold lower, respectively, than our measured IC₅₀ values for the inhibition of cytochrome P450 enzymes by these compounds). Both of these human studies suggest that hop prenylated phenols such as XN circulate at high levels but primarily as conjugates such as glucuronides. The half-lives of these prenylated hop phenols are as long as 20 h due to enterohepatic recirculation of their metabolites, but the effect of these conjugates on cytochrome P450 enzymes is unknown.

We believe that human liver microsomes represent perhaps a more appropriate experimental system than recombinant enzymes for CYP inhibition studies. The Food and Drug Administration (2012) also recommends the use of tissues such as liver microsomes instead of recombinant enzymes for drug interaction studies. Human liver microsomes provide a complete set of human cytochrome P450 enzymes as well as other catalytic factors such as NADPH/P450 reductase and cytochrome *b*₅ at their natural relative levels. Interactions between substrates, metabolites and inhibitors may be detected when using human liver microsomes but may be missed when using just one recombinant CYP enzyme at a time. However, we recognize that inhibition assays using human liver microsomes might suffer from non-CYP inhibition activities. For example, non-specific binding to proteins and lipids

of human liver microsomes can lower the free concentration of a probe substrate and alter the apparent IC₅₀ value (Tucker et al., 2001). Therefore, we used human liver microsomes with a protein concentration of only 0.1 mg/ml to minimize such non-specific binding.

5. Conclusion

Hop extracts containing prenylated flavanols such as 8-PN and chalcones such as xanthohumol have the potential to inhibit some cytochrome P450 enzymes, especially members of the CYP2C family and CYP1A2. If hop dietary supplements are co-administered with therapeutic agents that are substrates for these enzymes, there is the potential for drug-botanical interactions which might include prolonged drug half-life and elevated serum concentrations. Clinical evaluation would be required to determine the clinical relevance of these *in vitro* findings.

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Figure 1.
Structures of XN, IX, 8-PN and 6-PN

Figure 2. Positive/negative ion electrospray UHPLC-MS-MS SRM chromatograms of cytochrome P450 probe substrate metabolites (solid lines) and internal standards (dashed lines). The separation was carried out within 2 min. (A) CYP1A2; (B) CYP2B6; (C) CYP2C8; (D) CYP2C9; (E) CYP2C19; (F) CYP2D6; (G) CYP2E1; and (H) CYP3A4.

Figure 3.

Time-dependent inactivation of CYP1A2 by the standardized hop extract and prenylated compounds from hops. After a 30 min preincubation of recombinant CYP1A2 with the extract or individual hop compounds and NADPH (1 mM) or without NADPH (control), the residual activities of the enzyme were determined at 0 min and 30 min. (Each data point represents the mean of two determinations.)

Table I

Experimental conditions for assays of specific isoforms of cytochrome P450 enzymes in human liver microsomes

CYP	Substrate	Concentration (μ M)	Incubation time (min)
1A2	Phenacetin	80	15
2B6	Bupropion	20	15
2C8	Amodiaquine	2	15
2C9	Tolbutamide	100	12
2C19	S-(+)-Mephenytoin	30	30
2D6	Dextromethorphan	3	12
2E1	Chlorzoxazone	40	15
3A4	Midazolam	2	5

Table II

Tandem mass spectrometer parameters for the selection of precursor and product ions of probe substrate metabolites and internal standards

CYP enzyme	Compound	SRM ^a transition	Electrospray polarity	Q1	CE	Q3
<i>Metabolites</i>						
1A2	Acetaminophen	152.0>110.0	+	-17	-20	-20
2B6	Hydroxybupropion	256.0>238.0	+	-19	-10	-26
2C8	Desethylamodiaquine	328.0>283.0	+	-17	-15	-19
2C9	Hydroxytolbutamide	285.0>186.1	-	20	20	21
2C19	4-Hydroxy-mephenytoin	235.0>149.9	+	-12	-20	-14
2D6	Dextorphan	258.1>133.1	+	-19	-45	-18
2E1	6-Hydroxy-chlorzoxazone	183.9>120.1	-	19	20	22
3A4	1'-hydroxy-midazolam	342.0>203.2	+	-17	-25	-21
<i>Internal standards</i>						
1A2	[d ₄]-Acetaminophen	156.0>114.0	+	-17	-15	-20
2B6	[d ₆]-Hydroxybupropion	262.1>244.3	+	-19	-10	-26
2C8	[d ₅]-Desethylamodiaquine	333.3>283.3	+	-17	-20	-19
2C9	[d ₉]-Hydroxy-tolbutamide	294.1>186.1	-	14	20	22
2C19	[d ₃]-4-Hydroxy-mephenytoin	238.0>150.0	+	-12	-25	-17
2D6	[d ₃]-Dextorphan	261.1>133.1	+	-13	-45	-24
2E1	[d ₂]-6-Hydroxy-chlorzoxazone	185.9>122.1	-	20	20	24
3A4	Flurazepam	388.1>315.1	+	-20	-30	-30

^aSRM, selected reaction monitoring

Table III

Screening of a hop extract (5 µg/ml) and prenylated phenols from hops (1 µM and 10 µM) for inhibition of cytochrome P450 enzymes.

	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
	% Inhibition (1 µM)							
6-PN	< 10 ^a	< 10	< 10	< 10	< 10	< 10	< 10	< 10
8-PN	55.8 ± 0.7 ^b	< 10	32.2 ± 1.2	ND	43.8 ± 0.2	< 10	< 10	< 10
IX	< 10	< 10	87.7 ± 1.1	31.6 ± 8.0	51.0 ± 8.6	< 10	< 10	< 10
XN	< 10	< 10	47.8 ± 5.1	< 10	< 10	< 10	< 10	< 10
	% Inhibition (10 µM)							
6-PN	21.4 ± 0.8	35.7 ± 1.9	85.5 ± 1.2	63.8 ± 2.0	14.3 ± 0.1	< 10	< 10	< 10
8-PN	88.4 ± 1.6	34.7 ± 1.7	97.6 ± 0.1	93.0 ± 0.6	92.8 ± 5.5	< 10	< 10	31.4 ± 2.8
IX	48.2 ± 4.6	< 10	98.9 ± 0.1	82.1 ± 1.7	96.3 ± 1.5	< 10	< 10	< 10
XN	11.8 ± 1.1	43.9 ± 1.3	92.9 ± 0.4	69.7 ± 3.0	15.0 ± 1.9	< 10	< 10	< 10
Hops (5 µg/ml)	26.7 ± 8.1	36.4 ± 5.1	92.7 ± 0.4	88.1 ± 2.5	69.8 ± 10.8	19.5 ± 2.5	13.7 ± 12.6	19.2 ± 2.9
Compound mix ^c	16.4 ± 0.7	14.9 ± 1.4	92.7 ± 0.6	75.9 ± 2.1	65.5 ± 6.8	20.8 ± 4.9	< 10	29.2 ± 2.3

^a Insignificant inhibition (<10%)

^b Data expressed as mean ± SD

^c Mixture of 6-PN, 8-PN, IX, and XN at concentrations identical to those in the standardized hop extract

Table IV

IC₅₀ values (μM) for the inhibition of specific cytochrome P450 enzymes by the standardized hop extract and individual hop prenylated phenols.

<i>Compound</i>	CYP1A2	CYP2C8	CYP2C9	CYP2C19
<i>Hops (μg/ml)</i>	9.4 ± 0.6 ^a	0.8 ± 0.1	0.9 ± 0.1	3.3 ± 0.7
<i>IX</i>	ND ^b	0.2 ± 0.1	2.1 ± 0.3	0.5 ± 0.1
<i>8-PN</i>	1.1 ± 0.2	0.6 ± 0.1	1.1 ± 0.2	0.4 ± 0.1
<i>XN</i>	ND	1.1 ± 0.1	3.3 ± 0.4	ND
<i>6-PN</i>	ND	1.9 ± 0.1	5.9 ± 0.7	ND

^aData expressed as mean ± SD.

^bND: Not determined since 50% inhibition at 10 μM.