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Review article:

CYTOCHROME P450 ENZYME MEDIATED HERBAL DRUG INTERACTIONS (PART 1)

Sompon Wanwimolruk[1]*, Virapong Prachayasittikul[2]

[1] Center for Innovation Development and Technology Transfer, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
[2] Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

* Corresponding author: Dr. Sompon Wanwimolruk, E-mail: sompon.wan@mahidol.ac.th; Tel.: +66 2 441 4370, Fax: +66 2 441 4380

ABSTRACT

It is well recognized that herbal supplements or herbal medicines are now commonly used. As many patients taking prescription medications are concomitantly using herbal supplements, there is considerable risk for adverse herbal drug interactions. Such interactions can enhance the risk for an individual patient, especially with regard to drugs with a narrow therapeutic index such as warfarin, cyclosporine A and digoxin. Herbal drug interactions can alter pharmacokinetic or/and pharmacodynamic properties of administered drugs. The most common pharmacokinetic interactions usually involve either the inhibition or induction of the metabolism of drugs catalyzed by the important enzymes, cytochrome P450 (CYP). The aim of the present article is to provide an updated review of clinically relevant metabolic CYP-mediated drug interactions between selected herbal supplements and prescription drugs. The commonly used herbal supplements selected include Echinacea, Ginkgo biloba, garlic, St. John's wort, goldenseal, and milk thistle. To date, several significant herbal drug interactions have their origins in the alteration of CYP enzyme activity by various phytochemicals. Numerous herbal drug interactions have been reported. Although the significance of many interactions is uncertain but several interactions, especially those with St. John's wort, may have critical clinical consequences. St. John's wort is a source of hyperforin, an active ingredient that has a strong affinity for the pregnane xenobiotic receptor (PXR). As a PXR ligand, hyperforin promotes expression of CYP3A4 enzymes in the small intestine and liver. This in turn causes induction of CYP3A4 and can reduce the oral bioavailability of many drugs making them less effective. The available evidence indicates that, at commonly recommended doses, other selected herbs including Echinacea, Ginkgo biloba, garlic, goldenseal and milk thistle do not act as potent or moderate inhibitors or inducers of CYP enzymes. A good knowledge of the mechanisms of herbal drug interactions is necessary for assessing and minimizing clinical risks. These processes help prediction of interactions between herbal supplements and prescription drugs. Healthcare professionals should remain vigilant for potential interactions between herbal supplements/medicines and prescription drugs, especially for drugs with a narrow therapeutic index are used.

Keywords: Herbal drug interactions, CYP, dietary supplements, herbal medicines, botanical supplements, drug interactions

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INTRODUCTION

Products containing biologically active phytochemicals are often defined as "herbal" or "botanical" supplements. They are derived or extracted from a broad range of plant sources. Herbs and herbal supplements are used by people for purpose of treating medical or psychiatric disorders. Herbal supplements are now commonly used in both Eastern and Western countries. Herbal medicines or traditional medicines have a long history of use in the Eastern countries, such as China and India, and continue to be used today (Philp, 2004). The use of herbal supplements in the United States has grown at a rapid rate with a 380 % increase between 1990 and 1997 (Brevoort, 1998; Eisenberg et al., 1998). The Dietary Supplement Health and Education Act (DSHEA) of 1994 permits the direct marketing of herbal products and supplements without prior proof of safety or efficacy. Herbal supplements can be purchased in several forms. The most common formulation of herbal supplements are encapsulated extracts. Herbal extracts, because they are crude products, usually contain many natural phytochemicals. They are also accessible as tinctures and beverages (Markowitz et al., 2008). They can be ingredients in nutrition and sport drinks; powders, and "energy" bars. Patients may also consume food products containing certain phytochemical ingredients to treat common diseases. For instance, cranberry juice is widely used for urinary tract infections and garlic for hyperlipidemia (Markowitz et al., 2008).

The perception that herbal supplements and medicines, legally categorized as over-the-counter dietary supplements, may reduce or enhance the effects of prescription drugs has earned our attention slowly (Cott, 2008). Herbal supplements usually have many active phytochemical constituents. Therefore, the possibility of interactions is increased when compared with the likelihood of interactions between two prescription drugs. Presently, most herbal drug interactions are documented as case series or individual case reports (Sood et al., 2008). The common misconception is that herbal medicines or supplements are prepared from natural plants so therefore are safe to be taken concomitantly with prescription drugs. However, this does not guarantee that they are safe, free from adverse effects or toxicity, and devoid of any drug-drug interaction potential (Hermann and von Richter, 2012). The overwhelming majority of herbal use involves "self-medication" and these herbal products and supplements are commonly taken concomitantly with conventional medications with little or no information available on potential herbal drug interactions. Patients may feel embarrassed that they are self-medicating with complementary therapies and others may not consider that herbs are medicines in the conventional sense. A study in the United States revealed that only one-third of patients told their physician they were taking herbal remedies or supplements (Kennedy et al., 2008). In fact, there have been increasing reports on herbal drug interactions. Drug interactions can be caused by some natural products which are not be consumed as medicines. Grapefruit and grapefruit juice is a common example. The first major concern of herbal drug interactions was recognised in 1989 when grapefruit juice was reported to increase the blood concentrations of felodipine, an anti-hypertensive calcium channel antagonist (Bailey et al., 1998). Later in 1996, a similar grapefruit drug interaction was found with terfenadine, a non-sedative antihistamine (Bailey et al., 1998; Benton et al., 1996). Successive studies implied that these effects of grapefruit were caused by inhibition of intestinal CYP3A4 and a drug transporter protein, P-glycoprotein (P-gp) by furanocoumarins (e.g. 6', 7'-dihydroxybergamottin, bergamottin and bergapten), naturally-occurring ingredients in grapefruit (Bailey et al., 2000; Goosen et al., 2004; Ho et al., 2001; Lin et al., 2012; Paine et al., 2005). Serious adverse drug reactions arising from

grapefruit and terfenadine interaction was one of the main reasons for withdrawal of terfenadine from the market in 1998. These adverse drug reactions were due to inhibition of CYP3A4 by grapefruit active ingredients led to marked increase in blood terfenadine concentrations. This subsequently caused prolonged QT intervals and following cardiac arrhythmias. Grapefruits have also been shown to increase the plasma concentration and bioavailability of several drugs that are substrates for cytochrome P450 (CYP) 3A4 enzyme (Bailey et al., 2007; Greenblatt et al., 2003). This means that grapefruit interacts with most clinically used drugs as more than 50 % of prescription drugs are metabolized by CYP3A4 (Guengerich, 1999; Nebert and Russell, 2002). This made substantial concern regarding food or herbal drug interactions, and has also stimulated numerous research to fulfill the knowledge in this particular area. Cruciferous vegetables is an additional example, it has been involved in the interactions with a number of CYP1A2-substrate drugs (Ioannides, 1999; Zhou et al., 2004). An understanding of these herbal drug interactions has great benefit for drug therapy as it helps us to predict herbal drug interactions.

Herbal supplements and cancer chemoprevention

For many years it has been recognized that the use of herbs as medicines plays an important role in nearly every culture, including Asia, Africa, Europe and the Americas. Recent surveys suggest that one in three Americans use dietary supplements daily. It is interesting that the rate of herbal usage is much higher in cancer patients (Pierce et al., 2002; Richardson et al., 2000; Rock, 2003; Wargovich et al., 2001), in some cases, up to 50 % of patients treated in cancer centers. Many of these supplements are herbal in nature (Wargovich et al., 2001). Herbal supplements are generally taken for two main reasons. The first reason is that they are used to alleviate symptoms of illness. For example, the widespread use of St. John's wort for relief of depression, the use of Echinacea for relief of cold symptoms, and use of Ginkgo biloba for improvement in cognition (Wargovich, 2001). The second reason is that herbal supplements are used specifically with the hopes of preventing disease or reducing the risk for certain diseases. Examples of this include green tea, grape seed extract and other flavonoid-rich botanicals which are taken because of their natural antioxidants (Mandlekar et al., 2006). The use of garlic and its supplement preparation is another example, which has been demonstrated in animals, to prevent cancer (Wargovich, 1997; Wargovich et al., 2001).

With respect to cancer chemoprevention, herbal supplements can act through several mechanisms to provide a protective effect. Induction of phase I and phase II metabolic enzymes by herbal products is one of the common mechanisms (Wargovich et al., 2001). Garlic as well as many organosulfur compounds derived from garlic have been demonstrated to possess strong chemopreventive activity against experimentally induced cancers of the skin, esophagus, stomach, colon, liver, lung and mammary gland (Cerella et al., 2011; Chandra-Kuntal et al., 2013; Wargovich, 1997; Wargovich et al., 2001). One of the active constituents in garlic, diallyl sulfide, is a potent inhibitor of the phase I enzyme CYP2E1 (Brady et al., 1988). CYP2E1 is involved in the metabolic activation of several environmental and dietary carcinogens, e.g., nitrosamines (Yang et al., 2001; Zhou et al., 2003). In addition, diallyl sulfide significantly increases a number of phase II enzymes, including glutathione S-transferase, UDP-glucuronosyltransferase, and quinone reductase. These phase II enzymes are responsible for the detoxification of many carcinogens (Wargovich, 1997; Wargovich et al., 2001).

Cytochrome P450 (CYP)

CYP enzymes are responsible for detoxification of a wide range of xenobiotics including drugs, environmental pollutants, and cancer-causing agents (i.e., carcinogens). They are also involved in the biosynthesis of cholesterol and other important lipids such as prostacyclins and thromboxane A2 which are implicated in the causation of many cardiovascular diseases (Guengerich, 1999; Nebert and Russell, 2002). In fact, the role of CYP enzymes in protecting the body against foreign chemicals is as crucial as that of antibodies in dealing with invading organisms. Most chemical carcinogens require metabolic activation by CYP, to their genotoxic intermediates (Guengerich and Shimada, 1991; Badal et al., 2012). In some instances, these activated metabolites are subjected to detoxification by conjugation reactions. Thus, activities or levels of CYP enzymes may be one of the important host factors which determine whether exposure to the carcinogen results in cancer or not (Maliakal et al., 2011; Nebert, 1991). The CYP1A subfamily (CYP1A1 and CYP1A2) plays a vital role in the metabolism of two important classes of environmental carcinogens: polycyclic aromatic hydrocarbons and arylamines. Many clinically-important drugs are metabolized by this specific isoenzyme such as caffeine, theophylline, verapamil and clozapine. CYP2D6 is responsible for the metabolism of more than 30 clinically important drugs, such as metoprolol and several other β-blockers, antiarrhythmics, antidepressants, neuroleptics and morphine-related drugs (Gonzalez, 1992). More than 50 % of all prescription drugs are metabolized by CYP3A4 (Guengerich, 1999; Nebert and Russell, 2002). The activity of CYP can be influenced by many factors; including genetic composition of the host, certain medications, and exposure to certain dietary and environmental chemicals (Weisburger and Chung, 2002). It has been demonstrated that vegetables, particularly cruciferous ones (e.g., cabbage, brussel sprouts and cauliflower), induce CYP enzymes; namely CYP1A1 and CYP1A2 (Murray, 2006; Yoshida et al., 2004; Zhou et al., 2004). Cigarette smoking is known to induce CYP1A1 and CYP1A2; the high levels of these CYP enzymes have been linked to an increased risk of lung and colon cancer (Guengerich and Shimada, 1991; Smith et al., 2001; Zhou et al., 2010).

CYP3A4 is the most important human CYP isozyme as it is involved in the metabolism of most clinically used drugs (Guengerich, 1999; Nelson et al., 1996; Nebert and Russell, 2002). Human CYP3A4 is expressed in the prostate, breast, gut, colon and small intestine. However, its expression is most abundant in the liver which accounts for 30 % of the total CYP protein content (Guengerich, 1999; Lown et al., 1997; Shimada et al., 1994; Watkins et al., 1987). CYP3A4 plays an important role in the oxidation of both testosterone and estrogen. Activity of CYP3A4 can be inhibited or induced by drugs, herbs, pesticides, and carcinogens. Individual variation in CYP3A4 levels may play a role in breast and prostate carcinogenesis through modulation of sex hormone metabolite levels. Alternatively, CYP3A4 can be involved in bioactivation of exogenous carcinogens. It has been shown that CYP3A4 is involved in activation of many environmental carcinogens. These include polycyclic aromatic hydrocarbons, heterocyclic amines, aflatoxin B1, and nitrosamines (Guengerich and Shimada, 1991; Patten et al., 1997; Shimada et al., 1994). Furthermore, ingestion of polycyclic aromatic hydrocarbons and heterocyclic amines, and their metabolism mediated by CYP3A4 has been shown to result in the formation of carcinogen DNA adducts in mammary tissues (Lightfoot et al., 2000).

It has been hypothesized that alcoholic beverages, particularly red wine, offer extra cardiovascular protection due to their rich content of antioxidant flavonoid compounds. Many flavonoids, such as quercetin, resveratrol and naringenin, have been

shown to alter the activity of CYP enzymes (Chan and Delucchi, 2000; Niestroy et al., 2012). This effect could also be associated with a protective property against cancer. One target of the chemopreventive effect of naturally occurring flavonoids, such as grape constituents, could be inhibition of xenobiotic metabolizing phase I enzymes, i.e., CYP. Alternatively, it could be the induction of phase II conjugation enzymes, such as UDP-glucuronosyltransferase and glutathione S-transferase, that are responsible for the detoxification of carcinogens. Among the main human CYPs, CYP1A1 and CYP1A2 isoforms are involved in the activation of many procarcinogens; including polycylic aromatic hydrocarbons, polycyclic amines and aflatoxin B1 (Gonzalez and Gelboin, 1994). Resveratrol, a phytoalexin present in grapes, has been demonstrated to be an aryl hydrocarbon receptor antagonist which inhibits the induction of the CYP1A1 enzyme (Chan and Delucchi, 2000). CYP2E1 is responsible for the activation of volatile organic solvents and precarcinogenic N-nitrosamines (Gonzalez et al., 1992, Yang et al., 2001; Zhou et al., 2003). Human CYP3A4 activates aflatoxin B1 and the biotransformation of many clinically used drugs (Gonzalez, 1992). Moreover, it has been shown that resveratrol inactivated CYP3A4 in a time- and NADPH-dependent manner (Chan and Delucchi, 2000; Chan et al., 1998). The data suggest that resveratrol is an effective mechanism-based inactivator of CYP3A4.

From the above information, it clearly shows that developed countries recognize and value the importance of research on complementary alternative medicines, including herbal medicines. This is the case especially in the USA and UK where there are established research funding agencies, such as the Office of Dietary Supplements (ODS) under the National Institutes of Health (NIH) and the "Prince of Wales Foundation for Integrated Health", respectively (The Office of Dietary Supplements website, Ernst et al., 2006). These emphasize the relevance and clinical importance of pursuing research on herbal medicines.

Mechanisms of herbal drug interactions: general considerations

Like drug-drug interactions, herbal drug interactions result from the same principles. These principles are based on the same pharmacokinetic (i.e. changes of plasma drug concentration) and pharmacodynamic (i.e. drugs interacting at receptors) interactions. The currently recognized pharmacokinetic interactions between clinical drugs and herbs or herbal dietary supplements indicate that a number of herbs, most remarkably St. John's wort, can alter the plasma concentration of different conventional medicines metabolized by CYP, and/or are transported by P-gp (Izzo, 2012). P-gp is presented in the intestine, liver and kidney; it performs an important role in the absorption, distribution, or excretion of drugs. P-gp appears to limit the cellular transport from intestinal lumen into epithelial cells and also enhances the excretion of drugs via hepatocytes and renal tubules into the adjacent luminal space.

Many probe drugs have been used to assess potential effects of herbs on the activity of a specific CYP enzyme. These probe drugs include midazolam, alprazolam, nifedipine (CYP3A4); debrisoquine, dextromethorphan (CYP2D6); chlorzoxazone (CYP2E1); tolbutamide, diclofenac and flurbiprofen (CYP2C9); caffeine, tizanidine (CYP1A2); and omeprazole (CYP2C19). Fexofenadine, digoxin and talinolol have been extensively used in pharmacokinetic studies as P-gp substrates (Izzo, 2012). Polymorphisms in the genes for CYP enzymes and P-gp may influence the interactions mediated through these pathways (Lei et al., 2009a). Pharmacodynamic-based interactions between herbs and drugs have been less investigated. However, the outcomes of pharmacodynamic interactions may be either additive or synergetic (i.e. the herbal medicines potentiate the pharmacological/toxicological action of conventional

drugs) or antagonistic (i.e. the herbal medicines reduce the efficacy of the drugs). Warfarin and herbal interactions are a classical example of pharmacodynamic interactions. Theoretically, augmented anticoagulant effects could be likely when warfarin is administered concomitantly with coumarin-containing herbs (some plant coumarins exert anticoagulant effects) or with antiplatelet herbs (Izzo, 2012). On the other hand, vitamin K-containing herbs can antagonize the effect of warfarin (the action of warfarin is due to its ability to antagonize the cofactor function of vitamin K).

A number of recently published review articles have comprehensively written and exclusively drawn attention to the mechanisms of herbal drug interactions (Colalto, 2010; Izzo, 2012; Tomlinson et al., 2008; Zhou et al., 2004, 2010; Zhang et al., 2009). They have provided evidence of herbs that can modify CYP or P-glycoprotein.

CYP mediated herbal drug interactions: evidence in humans

Evidence is emerging that particular herbs and herbal medicines may contain specific phytochemicals which can modulate the activity of drug metabolizing enzymes CYP and drug transporting protein, P-glycoprotein (P-gp). These modulations may lead to potential interaction between herbal medicines and prescription drugs. This review will focus on important herbal drug interactions having clinical relevance and discuss the mechanism of drug interactions which is mediated by CYP enzymes. Some interactions mainly involved with function of P-gp are omitted, but may be mentioned. Studies in animals are generally excluded because of the marked species, which makes extrapolation of such results to humans difficult (European medicines Agency, 2013; Hermann and von Richter, 2012). Herbal drug interactions caused by pharmacodynamic mechanisms including partly cover toxicities, while not less important, are outside the scope of this review article. Nevertheless, these have been reviewed elsewhere (Izzo and Ernst, 2009; Johne and Roots, 2005).

Echinacea

Echinacea is a genus of herbaceous flowering plants (purple coneflowers), and belongs to Asteraceae family. It comprises of nine species, which are native and endemic to eastern and central North America. Marketed preparations of Echinacea arise from underground as well as aerial parts of three different species (*E. purpurea*, *E. angustifolia*, *E. pallida*), whereby the majority (i.e., about 80 %) of the products are based on *E. purpurea*. Due to its immunostimulant properties, echinacea is commonly used for the prevention and treatment of acute virus infections of the upper respiratory tract, such as the common cold and influenza (Capasso et al., 2008; Ernst et al., 2006, 2008). Recommended daily doses of echinacea products vary broadly depending on the formulation and content of the product. Usually daily doses of echinacea for adults range between 900 to 1000 mg, to be taken in 3 to 4 divided doses.

Echinacea is considered to be one of the safest herbal medicines, with few reported adverse effects and very few reports of *in vivo* drug interactions. Echinacea seems to cause no serious risk for drug interactions in humans (Izzo, 2012). No verifiable case reports of herbal drug interactions with any echinacea product have been published to date. The effect of echinacea (*E. purpurea* root) on specific CYP activities in humans was evaluated by using a single dose administration of the CYP probe drugs in 12 healthy subjects (Gorski et al., 2004). These CYP probes included caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6), and midazolam (hepatic and intestinal CYP3A4). The probe drugs were administered before and after a short course of Echinacea [*E. purpurea* root extract 400 mg, 4 times daily (= 1600 mg/day) for 8 days], and respective plasma concentration-time profiles of the probe drugs were

determined. In this study, it was found that echinacea dosing significantly decreased the oral clearance of the CYP1A2 probe caffeine by 27 % (Gorski et al., 2004), indicating inhibition of *in vivo* CYP1A2 catalytic activity. Theophylline is a widely prescribed CYP1A2 drug that has a narrow therapeutic window. Therefore modest changes of 20 % in clearance of theophylline are considered to be clinically significant (Gorski et al., 2004). There was considerable inter-individual variability in this interaction, with 2 individuals (out of 12 subjects) presenting a greater than 50 % reduction in caffeine oral clearance (Gorski et al., 2004). The co-administration of echinacea and theophylline may give rise to an increased incidence of toxicity developing from increased plasma theophylline concentrations. Other CYP1A2-metabolized drugs which have adverse effect concerns, such as clozapine, tacrine, and cyclobenzaprine, may be influenced by taking echinacea concomitantly (Gorski et al., 2004).

Echinacea dosing also significantly reduced (average by 11 %) the oral clearance and enhanced the systemic exposure of tolbutamide (Gorski et al., 2004). This indicates that the *in vivo* hepatic CYP2C9 activity was slightly inhibited by echinacea. However, the maximum plasma tolbutamide concentration was not significantly modified by echinacea. These results showed a minor change in the pharmacokinetics of tolbutamide which is mainly metabolized by CYP2C9. The observed magnitude of changes in tolbutamide pharmacokinetic parameters, though statistically significant, is most likely lack clinical importance with this echinacea product. Thus it suggests that co-administration of echinacea has no considerable effect on the activity of CYP2C9 enzyme (Gorski et al., 2004). However, the co-administration of echinacea with warfarin, a substrate known to be metabolized by multiple CYP enzymes including CYP2C9 and CYP3A4 (Abernethy et al., 1991; Rettie et al., 1992), may result in variable responses from a decrease in efficacy due to CYP3A4 induction to toxicity as a result of CYP2C9 inhibition to no effect (offsetting changes). Gorski and coworkers (Gorski et al., 2004) commented that the co-administration of other echinacea products that differ in phytochemical content to the brand they studied (*E. purpurea* root, Nature's Bounty) with CYP2C9 substrates having a narrow therapeutic index such as phenytoin and warfarin may require to be cautiously monitored.

In the same study, it was also shown that echinacea co-administration did not significantly affect the pharmacokinetic parameters (i.e., AUC0-∞, oral clearance, $t_{1/2}$) of the CYP2D6 probe dextromethorphan (Gorski et al., 2004). This suggested that co-administration of echinacea does not alter the metabolism and pharmacokinetics of drugs metabolized by CYP2D6.

The systemic clearance of intravenously administered CYP3A4 probe midazolam was significantly enhanced by 42 %, and there was a corresponding significant decrease in mean AUC0-∞ of 23 % (Gorski et al., 2004). Collectively it indicates that hepatic CYP3A4 activity was increased. On the other hand, the oral clearance and the AUC0-∞ after oral midazolam dosing were not significantly changed by echinacea co-administration. Nevertheless, the mean absolute oral bioavailability of midazolam was increased from 24 % to 36 % (i.e., by 50 %). This is consistent with the inhibition of intestinal CYP3A4 by echinacea. The distinction effects of echinacea on intestinal and hepatic CYP3A4 activity may possibly be due to a diversity of mechanisms. These include, for example, locally acting CYP3A4 inhibiting constituents of echinacea that do not become systemically available, or fast absorption of CYP3A4 inducing constituents of echinacea, thus limiting intestinal exposure and intestinal CYP3A4 induction (Gorski et al., 2004). Alternatively, it may be due to a systemically formed metabolite of an echinacea's constituent that is capable of inducing hepatic CYP3A4

but not intestinal CYP3A4. In spite of this, these mechanistic considerations remain hypothetical and warrant further research.

A further study by Gurley and colleagues (Gurley et al., 2004) employed single-time point phenotypic metabolic ratios to determine whether long-term supplementation of *Echinacea purpurea* affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4 activity. This clinical study was performed in 12 healthy volunteers. They were randomly assigned to receive *E. purpurea* (800 mg twice a day, or 1600 mg daily) for 28 days. Probe drug cocktails of midazolam (CYP3A4) and caffeine (CYP1A2), followed 24 hours later by chlorzoxazone (CYP2E1) and debrisoquine (CYP2D6) were administered before (baseline) and at the end of echinacea supplementation. Activities of CYP3A4, CYP1A2, CYP2E1, and CYP2D6 enzymes during pre-supplementation and post-supplementation were assessed by use of 1-hydroxymidazolam/midazolam serum ratios (1-hour sample), paraxanthine/caffeine serum ratios (6-hour sample), 6-hydroxychlorzoxazone/chlorzoxazone serum ratios (2-hour sample), and debrisoquine urinary recovery ratios (8-hour collection), respectively. In this study, high-dose *E. purpurea* given over 28 days did not significantly change the activities of CYP3A4, CYP2E1, and CYP2D6 as estimated by single timepoint metabolic ratios. The only appreciable alteration in mean phenotypic ratios appeared with 6-hour paraxanthine/caffeine values. Co-administration of *Echinacea purpurea* caused an approximately 13 % decrease in the ratio of paraxanthine/caffeine, this suggested that there was a possible inhibitory effect of *E. purpurea* on CYP1A2 enzyme. This minor difference, however, was not statistically significant ($P = 0.07$), nor was it thought clinically relevant. This finding suggests that prolonged use of *E. purpurea* poses a minimal risk of producing CYP1A2-, CYP2D6-, CYP2E1-, or CYP-3A4-mediated herbal drug interactions in humans. In respect to the effects of *E. purpurea* extract on CYP3A4 and CYP2D6, the findings of Gurley et al. (2004) are consistent with those of Gorski et al. (2004). This consistency happened regardless of differences in the type of *E. purpurea* products used (root extract versus whole plant extract), duration of supplementation (8 days versus 28 days), and CYP phenotype assessment methodologies (AUC versus single-time point phenotypic ratio). The lack of or little effect of *E. purpurea* supplementation on human CYP2D6 was supported by a clinical study by Gurley et al. (2008b). The evidence has shown that supplementation of a standardized *E. purpurea* extract (267 mg three times a day = 801 mg/day, for 14 days) caused no significant inhibition of CYP2D6 in 16 healthy subjects using debrisoquine as the CYP2D6 probe.

Taken together, the study showed that an 8-day treatment with relatively high doses of *E. purpurea* root extract (i.e., 400 mg, four times daily = 1600 mg/day) displayed differential effects on the activity of various CYP enzymes. There were no alterations of CYP2D6 noted, negligible to modest inhibition of CYP2C9, modest inhibition of CYP1A2, and differential (i.e., inductive/ inhibitory) effects on hepatic and intestinal CYP3A4.

Although the observed mean effects on CYP1A2 and CYP2C9 activities were generally moderate, they displayed some more pronounced effect sizes in individual subjects. Considering CYP-based Echinacea-drug interactions of potential clinical significance, the study suggests that the bioavailability of orally administered CYP3A4 substrates with low oral bioavailability may be significantly increased by Echinacea co-administration. Also the exposure to CYP1A2 and CYP2C9 substrates (e.g., theophylline, clozapine) may be modestly increased, at least in some individual subjects. The observed effects of high doses of *E. purpurea* root extract on CYP1A2 and CYP2C9, however, are overall small. Therefore, this is unlikely to be of clinical

significance in clinical practice, unless drugs with a narrow therapeutic index are involved. These examples are theophylline in case of CYP1A2 and (S)-warfarin in case of CYP2C9.

A clinical study was conducted in 16 healthy volunteers received lopinavir-ritonavir (400/100 mg) twice daily for 30 days (Penzak et al., 2010) to determine the effect of *Echinacea purpurea* on the pharmacokinetics of lopinavir-ritonavir, and on CYP3A4 and P-glycoprotein (P-gp) activity using the probe substrates midazolam, and fexofenadine, respectively. *Echinacea purpurea* extract was given in a dosage of 500 mg three times daily, which they continued for four weeks, the first two weeks in combination with lopinavir-ritonavir. The HIV protease inhibitor lopinavir is extensive metabolized by CYP3A4, whereas ritonavir is a potent inhibitor of CYP3A4 and P-gp. Ritonavir is combined with lopinavir to boost (i.e., increase) its systemic exposure. The results have shown that neither lopinavir nor ritonavir pharmacokinetics were significantly altered by 2 weeks of Echinacea co-administration (Penzak et al., 2010). However, with respect to activity of CYP3A4, Echinacea co-administration considerably reduced AUC0–12h and increased oral clearance of midazolam, a CYP3A4 probe. These suggested that the Echinacea co-administration induced CYP3A4 enzyme activity and this in turn increased the clearance of midazolam (Penzak et al., 2010). Thus co-administration of *Echinacea purpurea* may cause moderate decrease in plasma concentrations of other CYP3A4 substrates.

The above finding was also supported by a study conducted by Moltó et al. (2011) in which they determined the potential of *Echinacea purpurea* to interact with the boosted protease inhibitor darunavir-ritonavir in 15 HIV-infected patients. Patients were receiving antiretroviral therapy including darunavir-ritonavir (600/100 mg twice a day) for at least 4 weeks. *E. purpurea* root extract capsules (500 mg every 6 h, for 14 days) were concomitantly given on top of their antiretroviral treatment. In general, supplementation of *Echinacea purpurea* did not affect the overall pharmacokinetics of darunavir and ritonavir, although individual patients did show a decrease in darunavir concentrations (Moltó et al., 2011). While no dose adjustment is required, monitoring darunavir concentrations on an individual basis may give reassurance in this setting. Their findings with darunavir exposure was reduced in some individual patients by 30 to 40 % after *E. purpurea* supplementation, would be harmonious with the finding of a modest induction of hepatic CYP3A4 by echinacea administration previously reported by Gorski et al. (2004). This evidence would be further consistent with the assumption that low-dose ritonavir (100 mg twice daily) might not efficiently inhibit hepatic CYP3A4 activity in individual patients (Hermann and von Richter, 2012).

Overall, the outcomes from both studies (Moltó et al., 2011; Penzak et al., 2010) regarding the noticed changes in CYP3A4 activity is consistent for the effects of *E. purpurea* supplementation on a darunavir-ritonavir combination. These studies reliably suggest that *E. purpurea* co-administration is unlikely to considerably alter the pharmacokinetics of ritonavir-boosted protease inhibitors. This is most likely due to the presence of the potent CYP3A inhibitor ritonavir, but may be capable to moderately induce hepatic CYP3A activity.

Abdul and colleagues (Abdul et al., 2010) studied the pharmacokinetic and pharmacodynamic interactions of Echinacea with warfarin in 12 healthy subjects who received a single oral 25 mg dose of warfarin alone and after 2 weeks of pretreatment with high-dose Echinacea (1275 mg four times daily = 5100 mg/day). Pharmacodynamic parameters including the international normalized ratio (INR), platelet activity) and pharmacokinetic (warfarin enantiomer concentrations) end points were evaluated. The apparent oral clearance of

(S)-warfarin was found to be significantly higher during concomitant treatment with echinacea but this did not lead to a clinically significant change in INR. Because (S)-warfarin is metabolized by CYP2C9 and CYP3A4, whereas (R)-warfarin is metabolized by CYP3A4 and CYP1A2 (Wittkowsky, 2003), it was proposed that a 2-week treatment with high doses of echinacea (i.e., 5100 mg/day) does not appreciably alter the activities of these CYP enzymes. Generally, the small extents of the effect observed are doubtful to be of clinical relevance.

In summary, the existing *in vivo* evidence reveals that echinacea products at recommended doses have little potential to produce clinically relevant or worrying metabolism-based pharmacokinetic interactions involving CYP1A2, CYP2C9, CYP2D6 and CYP2E1. Sequentially, there is reasonable clinical evidence suggesting that Echinacea products in fact have potential to moderately induce hepatic CYP3A4 activity. However, at the same time Echinacea products also may inhibit the presystemic (intestinal) metabolism of CYP3A4 drugs. As both mechanisms work against each other in terms of the net effects on the systemic exposure of CYP3A4 drugs, the onset of each mechanism probably occurs at different times (i.e., onset of inhibition occurs faster than onset of induction), and also will vary in their extent of effect on specific substrate characteristics (e.g., oral bioavailability of CYP3A4 drugs). General predictions on the clinical relevance of Echinacea and CYP3A4 drug interactions are complicated to generate. In spite of this, warning is advised when CYP3A4 drugs with low oral bioavailability due to pronounced intestinal CYP3A4-mediated metabolism (e.g. verapamil, cyclosporine A and tacrolimus) or CYP3A4 drugs with narrow therapeutic index, are co-administered with Echinacea supplementation. There are no clinical studies addressing the potential impact of Echinacea products on other important metabolic pathways such as CYP2C19 and phase II metabolism (e.g. glucuronidation).

Ginkgo biloba

Leaves from the tree Ginkgo (*Ginkgo biloba*; family Ginkgoaceae) have been used for 4000 years to improve mentation and respiratory function (Philp, 2004). The Ginkgo tree, also known as maidenhair tree, is the surviving member of ancient family Ginkgoaceae, with no close living relatives. Ginkgo biloba is claimed to improve cerebral and peripheral blood flow. Currently available pharmaceutical products of Ginkgo biloba represent leave extracts. Ginkgo biloba extract contains two constituents: flavonoids and terpenoids, which have antioxidant properties (Kennedy et al., 2011; Pierre et al., 2008). Oral standardized dry extracts of Ginkgo biloba generally contain between 22-27 % flavones glycosides; 5-7 % terpene lactones, and should contain not more than 5 ppm of ginkgolic acids, constituents with known allergic potency (Kressmann et al., 2002). Many studies have been conducted using EGb 761, a well-defined extract of Ginkgo biloba. Ginkgo biloba extract is widely used in some countries by patients with cognitive disorders such as memory decline with ageing and Alzheimer's disease. Early studies showed that the extract was superior to placebo in improving symptoms of dementia, and this has been confirmed by more recent research (Andrieu et al., 2003; Ihl, 2012). Standardized ginkgo biloba extract has a good safety profile, although some case reports have suggested an increased risk of bleeding.

Ginkgo biloba preparations may convene appreciable anti-platelet effects that are evidently caused by various ginkgolides. Consequently, information from case reports or controlled trials revealed that Ginkgo biloba extract potentiates the effects of anti-coagulant or anti-platelet drugs such as warfarin (Bone, 2008; Vaes and Chyka, 2000). However, these interactions are related to the pharmacodynamic

interactions. While they are clinically important, the pharmacodynamic interactions are outside the scope of the current review on CYP mediated herbal drug interactions.

There were various studies showing the *in vitro* effects of Ginkgo biloba extracts or specific constituents to alter activities of CYP enzymes. However, these results were generally inconsistent. Overall, the obtained findings indicated no effect and either inhibition or induction of various human CYP enzymes. Some reports found the effects of Ginkgo biloba extracts on CYP are concentration-dependently (i.e. inhibition at low concentrations, induction at high concentrations). In some studies, a substrate-dependent fashion effects have been described. Of note, the results derived from *in vitro* studies have been obtained in part at very high concentrations of the herb (Ginkgo biloba). Such concentrations are unlikely to be achieved in humans (*in vivo*) after recommended doses of Ginkgo biloba extracts. This particular aspect is considered to be a major disadvantage of *in vitro* studies. Together, the buildup of evidence from *in vitro* studies offers little guidance in the reliable prediction of relevant metabolic-based Ginkgo biloba mediated drug interactions *in vivo* (Hermann and von Richter, 2012).

Luckily, there are a number of clinical studies that have been carried out to determine the effects of Ginkgo biloba on many CYP isoforms and other drug metabolizing enzymes including phase II conjugations. These investigations mainly employed specific probe drugs as substrates for CYP enzymes in which it allows the identification and quantification of changes of specific CYP enzyme activity. Gurley et al. (2002) employed single-time point phenotypic metabolic ratios to determine whether long-term supplementation of St. John's wort, garlic oil, Panax ginseng, and Ginkgo biloba affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4 activity in healthy young volunteers. Dosages of 60 mg Ginkgo biloba dietary supplements were given (four times daily) to 12 healthy subjects over 28 days. In this particular study, probe drug cocktails of caffeine, debrisoquine, chlorzoxazone, and midazolam were administered before and at the end of Ginkgo biloba supplementation. Activities of CYP1A2, 2D6, 2E1, and 3A4 were assessed by the use of paraxanthine/caffeine serum ratios (6-hour sample), debrisoquine urinary recovery ratios (8-hour collection), 6-hydroxychlorzoxazone/chlorzoxazone se-rum ratios (2-hour sample), and 1-hydroxymidazolam/midazolam serum ratios (1-hour sample), respectively. Comparisons of pre-treatment and post-treatment ratios revealed that Ginkgo biloba did not cause any appreciable change of CYP1A2, 2D6 and 3A4 activity. In contrast, Ginkgo biloba appeared to moderately increase the activity of CYP2E1 by 23 %, even though this effect was not statistically significant (Gurley et al., 2002). This suggests a trend of CYP2E1 is being induced by Ginkgo biloba. A similar study was conducted by the same group of researchers (Gurley et al., 2005a) but in 12 elderly subjects, using the same Ginkgo biloba product, dose and treatment duration, as well as the same CYP probe drugs. Surprisingly, Ginkgo biloba had no significant effect on the activity of CYP1A2, 2D6, 2E1, and 3A4 in this elderly subjects. Thus these results do not confirm the earlier observed trend regarding a moderate CYP2E1 induction (Gurley et al., 2005a).

Lack of effect of Ginkgo biloba on the activity of CYP2D6 and CYP3A4 was supported by the evidence derived from a clinical study conducted by Markowitz and colleagues (Markowitz et al., 2003a). This study was similar in nature to those previously conducted by Gurley et al. (2005a). However, they assessed the influence of standardized Ginkgo biloba extract on the activity of CYP2D6 and CYP3A4 in healthy young adults, instead of elderly volunteers as used in Gurley and coworkers' study (Gurley et al., 2005a). In the trial by Markowitz et al. (2003a), dextromethorphan (CYP2D6 activity) and alprazo-

lam (CYP3A4 activity) were employed as specific substrates for the CYPs of interest. These probe drugs were co-administered orally at baseline, and following treatment with Ginkgo biloba extract (120 mg twice a day) for 14 days. There were no statistically significant differences between baseline and post-Ginkgo biloba treatment on dextromethorphan metabolic ratios. This indicated a lack of Ginkgo biloba effect on CYP2D6 activity. Regarding the CYP3A4 activity, a statistically significant decrease in the AUC of alprazolam was detected after treatment with Ginkgo biloba. The AUCs were decreased by 17 %, an amount that could be clinically significant for drugs with narrow therapeutic indices. However, when the effects of Ginkgo biloba are compared with synthetic medications known to induce alprazolam metabolism such as carbamazepine and rifampin, the magnitude of Ginkgo biloba effects were quite small (Markowitz et al., 2003a). Therefore, the authors concluded that standardized extracts of Ginkgo biloba, when taken in normally recommended doses, do not significantly alter the activity of human CYP2D6 and CYP3A4 enzymes. Thus, there appears to be little likelihood of significant herbal drug interactions between Ginkgo biloba and drugs predominantly metabolized by CYP2D6 or CYP3A4 isoforms (Markowitz et al., 2003a).

In addition, the effects of Ginkgo biloba supplementation (90 mg/day for 30 days) on the steady-state plasma concentration of donepezil were examined (Yasui-Furukori et al., 2004) in elderly patients with Alzheimer's disease; the patients received donepezil 5 mg/day. Donepezil, the 'first-line' cholinesterase inhibitor, in the treatment of Alzheimer's disease, is metabolized by CYP2D6 and CYP3A4 (Jann et al., 2002). The results from this study demonstrated that taking relatively low doses of Ginkgo biloba (90 mg/day) in elderly Alzheimer's Japanese patients did not alter the steady-state plasma concentrations of donepezil. This implies that daily doses of 90 mg of Ginkgo biloba do not have significant inhibitory or inducing effects on the CYP2D6- and CYP3A4-mediated metabolism of donepezil in the target population. This finding supports previous studies by Gurley et al. (2005a) and Markowitz et al. (2003a) in regard to the lack of interaction between Ginkgo biloba and drugs that are metabolized by CYP2D6 or CYP3A4.

Robertson and coworkers (Robertson et al., 2008) also used a similar methodology to determine whether Ginkgo biloba extract causes any alterations in the activity of human CYP3A4. Midazolam was employed as a probe drug for human CYP3A4. Besides midazolam, the investigators also used fexofenadine as a marker drug to monitor the influence of Ginkgo biloba on the activity of drug transporter protein, P-glycoprotein (P-gp). Effect of Ginkgo biloba extract on pharmacokinetics of midazolam and fexofenadine (after a single dose), and lopinavir's pharmacokinetics at steady-state, in healthy subjects was assessed before and after the subjects received Ginkgo biloba at a dose of 120 mg, two times daily (for 4 weeks). Lopinavir, ritonavir, and fexofenadine exposures were not significantly affected by Ginkgo biloba co-administration, while total AUC and maximum plasma drug concentration (Cmax) of the CYP3A4 substrate midazolam were both significantly lower post Ginkgo biloba supplementation, i.e. by 34 % and 31 %, respectively, as compared to baseline. The apparent oral clearance of midazolam increased in 10 of 13 subjects studied post Ginkgo biloba supplementation. These results suggest that Ginkgo biloba moderately induces CYP3A4 metabolism. The authors commended that as the reductions in midazolam AUC and Cmax were similar, with no change in half-life of midazolam, it appears likely that the interaction occurred secondary to CYP3A4 induction at the intestinal level (Robertson et al., 2008). However, it cannot be ruled out that hepatic induction of CYP3A4 may also have contributed. These findings obtained

from the study of Robertson et al. (2008) are in contrast with those previously reported by Gurley et al. (2005a) who found that 28 days of Ginkgo biloba extract (60 mg, four times daily) had no apparent effect on midazolam metabolism in 12 healthy subjects. Robertson and co-authors (Robertson et al., 2008) thought that it is unlikely that the disparity in their findings is due to differences in Ginkgo biloba extract formulation, as the total daily dose of Ginkgo biloba (240 mg/day) was the same in both studies and both products were standardized to the same flavonol glycoside and terpene lactone contents. To our knowledge, as herbal dietary supplements generally have problems with quality control aspects, including Ginkgo biloba (Draves and Walker, 2003; Foster et al., 2005; Haller et al., 2004), thus the differences in the content of active ingredients, dissolution and absorption characteristics of the formulation used cannot be excluded.

However, the two studies were different in their approach to assessing CYP3A4 phenotype. Robertson and colleagues (Robertson et al., 2008) assessed CYP3A4 activity by determining midazolam AUC before and after 4 weeks of Ginkgo biloba extract administration. Whereas, in Gurley et al. (2005a) investigation, a 1-h post-dose plasma concentration ratio of 1-hydroxymidazolam/midazolam was used to assess human CYP3A4 activity. Use of this ratio to determine human CYP3A4 activity has produced inconsistent results in previous studies, perhaps caused by significant interpatient heterogeneity in the glucuronidation of 1-hydroxymidazolam. This can alter the 1-hydroxymidazolam/midazolam ratio independent of CYP3A4-mediated metabolism (Eap et al., 2004; Rogers et al., 2002; Streetman et al., 2000). Therefore, it has been suggested that using midazolam concentrations alone, as opposed to 1-hydroxymidazolam ratios, offers a more accurate assessment of CYP3A4 activity (Nafziger and Bertino, 2007; Penzak et al., 2008).

Ginkgo biloba extract is also widely used as herbal dietary supplement in Japan. The effects of Ginkgo biloba extract on the pharmacokinetics and pharmacodynamics of nifedipine, a calcium-channel blocker, were studied using 8 healthy volunteers. Concurrent oral ingestion of Ginkgo biloba extract (240 mg/day) did not significantly affect any of the mean pharmacokinetic parameters of either nifedipine or its major metabolite dihydronifedipine (Yoshioka et al., 2004). However, unexpected observation was found in which the maximal plasma nifedipine concentrations in 2 subjects were approximately doubled during these subjects were taking Ginkgo biloba extract concomitantly with nifedipine. The authors also observed that these 2 subjects had experienced more severe and longer-lasting headaches during Ginkgo biloba extract phase than the control phase (Yoshioka et al., 2004). The mean heart rate after oral administration of nifedipine with Ginkgo biloba extract had a tendency to be faster than that without Ginkgo biloba extract at every time point. The adverse drug reactions observed were coincident with the abnormally high maximum plasma nifedipine concentrations in these particular two subjects. From this it was concluded that Ginkgo biloba extract and nifedipine should not be simultaneously administered, and careful monitoring is necessary when nifedipine and Ginkgo biloba extract need to be taken together in humans (Yoshioka et al., 2004). Although the authors emphasized the finding of abnormally increased maximal plasma nifedipine concentrations in two subjects upon co-administration of Ginkgo biloba extract, they also discussed possible underlying mechanisms. Nifedipine has a well recognized pharmacokinetic variability this together with the overall study results implies the absence of any Ginkgo biloba effects in respect of the total nifedipine exposure (i.e., AUC). This supports the hypothesis that there is no systematic effect due to Ginkgo biloba on the bioavailability of nifedipine. As nifedipine is an antihyper-

tensive drug known to be primarily metabolized by CYP3A4 (Bailey et al., 2013; Nebert and Russell, 2002), this interpretation would be in agreement with previous reports from other investigators showing lack of effect of Ginkgo biloba on metabolism and pharmacokinetics of CYP3A4 metabolized drugs.

Effects of a higher daily dose (360 mg/day, for 28 days) of Ginkgo biloba extract on CYP3A4 and CYP2C9, were evaluated (Uchida et al., 2006). CYP3A4 probe (midazolam) and CYP2C9 probe (tolbutamide) were orally administered as a single dose to 10 healthy volunteers before and after intake of Ginkgo biloba extract. AUC(0-infinity) for midazolam was significantly increased (25 %) by Ginkgo biloba extract intake and oral clearance was significantly decreased (26 %). These results suggested that Ginkgo biloba extract may inhibit the activity of CYP3A4, therefore it increased exposure to drugs cleared by CYP3A4 (Uchida et al., 2006). Furthermore, these researchers examined the potential interaction between Ginkgo biloba extract and CYP2C9-metabolized drugs using tolbutamide as a CYP2C9 probe. Their results have shown that the AUC(0-infinity) for tolbutamide after Ginkgo biloba extract intake was slightly but significantly (16 %) lower than that before Ginkgo biloba extract intake. Co-administration of Ginkgo biloba extract with tolbutamide tended to reduce AUC(0-2h) of blood glucose-lowering effect of tolbutamide. Therefore, the authors suggested that the combination of Ginkgo biloba extract and drugs should be cautiously in terms of the potential interactions, especially in elderly patients or patients treated with drugs exerting relatively narrow therapeutic windows (Uchida et al., 2006). When considering the magnitude of changes in pharmacokinetic parameters of CYP3A4 and CYP2C9-metabolized drugs caused by taking Ginkgo biloba extract concomitantly with the drugs, it revealed a minor alteration in the activities of these CYP enzymes of interest. This minor CYP2C9 induction and some inhibition of intestinal CYP3A4 were achieved with supra-therapeutic doses (360 mg/day) of Ginkgo biloba extract. The study results of drug interactions with Ginkgo biloba extract on CYP3A4- and CYP2C9 metabolically cleared drugs show minimal effect due to Ginkgo biloba extract (Hermann and von Richter, 2012). Thus, this is in agreement with reports from other studies that suggested there was no effect by Ginkgo biloba extract on the activity of these enzymes at recommended doses.

Zuo et al. (2010) employed diazepam as a probe substrate of CYP2C19 and CYP3A4 to determine the effects of Ginkgo biloba extract on the pharmacokinetics of diazepam. A single oral dose (10 mg) of diazepam was given either alone or concomitantly with oral Ginkgo biloba extract (120 mg twice daily) for 28 days to 12 healthy volunteers. They found that total AUC for diazepam and the main metabolite N-desmethyldiazepam were essentially unaltered. This data indicate that the disposition of CYP2C19 and CYP3A4 substrates is unlikely to be markedly modified by recommended doses of Ginkgo biloba products (Zuo et al., 2010).

The effect of Ginkgo biloba extract on the pharmacokinetics and pharmacodynamics of warfarin was investigated in 12 healthy subjects (Jiang et al., 2005). A single 25-mg dose of warfarin (Coumadin™) was given to each subject with and without pretreatment with multiple doses of Ginkgo biloba for 1 week (Tavonin™; 40 mg three times a day = 120 mg/day). Dosing of Ginkgo biloba was continued for a further week after warfarin administration. S-warfarin is mainly metabolized to S-7-hydroxywarfarin by CYP2C9, and R-warfarin is metabolized by CYP1A2 and CYP3A4. Using warfarin as a probe substrate, thereby allows for a separate mechanistic assessment of any potential change of these metabolic pathways by concomitant Ginkgo biloba treatment. Co-administration of recommended doses of a commonly used

herbal supplement, Ginkgo biloba did not affect the pharmacokinetics and the pharmacodynamics of warfarin enantiomers after a single dose of warfarin in healthy male subjects (Jiang et al., 2005). Also, Ginkgo biloba did not affect blood clotting status or platelet aggregation.

In addition, the effect of Ginkgo biloba on the activity of CYP2C9, the isoform responsible for S-warfarin clearance, was assessed in 11 healthy volunteers who received single 100-mg doses of the nonsteroidal anti-inflammatory drug (NSAID) flurbiprofen, a probe substrate for CYP2C9 (Greenblatt et al., 2006a). Subjects also received either a standardized Ginkgo biloba leaf preparation (Ginkgold, 3 doses of 120 mg) or matching placebo in a randomized crossover study. The study showed that pretreatment of healthy subjects with usual clinical doses of Ginkgo biloba has no detectable effect on the pharmacokinetics of a single dose of flurbiprofen or on the apparent extent of formation of the principal hydroxylated metabolite. The findings suggest that short-term exposure to Ginkgo biloba does not inhibit CYP2C9 activity *in vivo* (Greenblatt et al., 2006a). As the study involved only short-term exposure to Ginkgo biloba, it was not intended to capture possible CYP2C9 induction that could occur with long-term treatment. The results confirm previous controlled clinical studies showing no effect of ginkgo on the pharmacokinetics or pharmacodynamics of warfarin which is metabolized by CYP2C9. This finding is also supported by an *in vivo* study conducted by Mohutsky et al. (2006). They carried out two pharmacokinetic studies in healthy subjects using tolbutamide and diclofenac as probe substrates of CYP2C9. They found there were no interactions between Ginkgo biloba extract and these CYP2C9 probe substrates *in vivo* as demonstrated by the lack of effect on the steady-state pharmacokinetics of diclofenac and the urinary metabolic ratio of tolbutamide (Mohutsky et al., 2006). This evidence seems to be contradictory to a number of case reports having documented possible interactions between Ginkgo biloba and warfarin (Fugh-Berman and Ernst, 2001; Izzo and Ernst, 2001). Such an interaction is mostly relevant to elderly patients on anticoagulant therapy. However, this interaction appears to be attributable to the inhibition of platelet activating factor by various ginkgolides (Zhu et al., 1999). That is mediated by a pharmacodynamic mechanism. Explanation by a CYP-mediated metabolism for the Ginkgo biloba and warfarin interaction looks less possible based on the clinical data described above (Gurley et al., 2002; Jiang et al., 2005).

Yin and coworkers (Yin et al., 2004) investigated the potential herbal drug interaction between Ginkgo biloba and omeprazole, a widely used CYP2C19 substrate, in subjects with different CYP2C19 genotypes. Eighteen healthy Chinese subjects previously genotyped for CYP2C19 were studied. All subjects received a single omeprazole 40 mg at baseline and then at the end of a 12-day treatment period with Ginkgo biloba (140 mg, twice a day). Multiple blood samples were collected over 12 h, and 24 h urine was collected post omeprazole dosing. Plasma concentrations of omeprazole and its metabolite omeprazole sulfone were significantly decreased, and 5-hydroxyomeprazole significantly increased following Ginkgo biloba administration in comparison to baseline. A significant decrease in the ratio of AUC of omeprazole to 5-hydroxyomeprazole was observed in the homozygous extensive metabolizers, heterozygous extensive metabolizers, and poor metabolizers, respectively. The decrease was greater in poor metabolizers than extensive metabolizers. No significant changes in the AUC ratios of omeprazole to omeprazole sulfone were observed. Renal clearance of 5-hydroxyomeprazole was significantly decreased after Ginkgo biloba, but the change was not significantly different among the three genotype groups. Their results show that Ginkgo biloba can induce omeprazole hydro-

xylation in a CYP2C19 genotype-dependent manner and concurrently reduce the renal clearance of 5-hydroxyomeprazole. Co-administration of Ginkgo biloba with omeprazole or other CYP2C19 substrates may significantly reduce their effects, but further studies are warranted (Yin et al., 2004).

Possible effects of Ginkgo biloba as an inducer of CYP2C19 on single-dose pharmacokinetics of voriconazole were examined in 14 Chinese volunteers genotyped as either CYP2C19 extensive or poor metabolizers (Lei et al., 2009b). Pharmacokinetics of oral voriconazole 200 mg after administration of Ginkgo biloba 120 mg twice daily for 12 days were determined for up to 24 hours in a 2-phase randomized crossover study. For extensive metabolizers, the median value for voriconazole's AUC(0 to infinity) after administration of voriconazole alone was not significantly different from that after voriconazole with Ginkgo biloba ($P > 0.05$). The other pharmacokinetic parameters of voriconazole such as time to reach maximum concentration, half-life, and apparent clearance also did not change significantly for extensive metabolizers in the presence of Ginkgo biloba. Pharmacokinetic parameters followed a similar pattern for poor metabolizers. The authors concluded that 12 days of treatment with Ginkgo biloba did not significantly alter the pharmacokinetics of voriconazole in either CYP2C19 extensive or poor metabolizers. Thus, the pharmacokinetic interactions between voriconazole and Ginkgo biloba may have limited clinical significance. Based on these results, it can be assumed that - even at the highest recommended doses - Ginkgo biloba-mediated CYP2C19-based pharmacokinetic drug interactions appear to be light and may have restricted clinical significance.

In recent study, cocktail phenotyping design was used to evaluate the metabolic drug interaction profile of Ginkgo biloba extract EGb 761® with respect to the activities of major CYP enzymes (Zadoyan et al., 2012). These included CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. In random order, the following pretreatments were administered to 18 healthy men and women for 8 days each: placebo twice daily, EGb 761® 120 mg twice daily, and EGb 761® 240 mg in the morning and placebo in the evening. The phenotyping cocktail was orally administered before and after the EGb 761®/placebo pretreatment periods. CYP probe drugs and metrics used were: tolbutamide (CYP2C9, plasma concentration 24-h postdose), omeprazole (CYP2C19, omeprazole/5-hydroxy omeprazole plasma ratio 3-h postdose), dextromethorphan (CYP2D6, dextromethorphan/ dextrorphan plasma ratio 3-h postdose), and midazolam (CYP3A4, plasma concentration 6-h postdose). EGb 761®/placebo ratios for phenotyping metrics were close to unity for all CYP enzymes studied, except for CYP2C9, which may suggest a weak trend towards induction of these CYP2C family enzymes (Zadoyan et al., 2012). Their data obviously show that even a relatively high dose of 240 mg daily doses of EGb 761® extract, it has no inhibitory effect towards CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, irrespective whether the product is given once or twice daily (Zadoyan et al., 2012). The study also demonstrates that EGb 761® extract does not induce the activity of human CYP1A2, CYP2D6, and CYP3A4 enzymes. Supplementation of Ginkgo biloba (EGb 761® extract) may present a weak induction of CYP2C9 and CYP2C19 enzymes, which, however, seems very small to be of clinical relevance (Hermann and von Richter, 2012; Zadoyan et al., 2012).

Together, the existing evidence reveals that taking Ginkgo biloba extract at recommended doses up to daily doses of 240 mg do not have substantial or clinically meaningful effects on the activity of human CYP enzymes including CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Apparently, CYP2C19 enzyme was shown to be moderately inducible by Ginkgo biloba extract products. This effect

appears to occur at the highest recommended dose of level of 240 mg/day (Lei et al., 2009a). Also the induction effect on CYP2C19 may become somewhat more prominent with increasing doses of Ginkgo biloba extract (Yin et al., 2004). Furthermore the extent of this induction effect might depend on the individual CYP2C19 genotype of subjects (Yin et al., 2004) and possibly on characteristics of that particular CYP substrate. The existing evidence on this issue appears not to be consistent and further investigation is warranted.

Findings on pharmacokinetic alterations of CYP3A4 due to taking Ginkgo biloba concomitantly are not entirely consistent. Some studies suggested the possibility of presystemic induction of CYP3A4 with a high-dose treatment of Ginkgo biloba, whereas some data suggest CYP3A4 inhibition. For example, a study by Robertson et al. (2008) showed a significant induction effect of Ginkgo biloba extract on CYP3A4 enzyme, but the extent of enzyme induction (approximately 30 % reduction in AUC of midazolam) was relatively small as compared to a large inter-individual variation in CYP3A4 activity observed in humans (Watkins, 1994; Wrighton et al., 1993; Zhang et al., 1997). Thus the overall extent of CYP3A4-related effects produced by Ginkgo biloba are usually weak and doubtful to be of clinical relevance, unless for CYP3A4 drugs with a narrow therapeutic index are involved.

The evidence described previously also suggests that standard daily dose of Ginkgo biloba extract has no appreciable effect on the activity of two important drug metabolizing enzymes, human CYP3A4 and CYP2D6. Human CYP2D6 enzyme is involved in the metabolism of approximately 25 % of drugs on the market (Zhou, 2009). It metabolizes several clinically used drugs such as antidepressants (imipramine, desipramine and fluoxetine), cardiovascular drugs especially antiarrhythmic drugs (encainide, flecainide), opioid analgesics (morphine, codeine and tramadol) in which some have a narrow therapeutic index. Also approximately 50 % of clinically used drugs are metabolized by human CYP3A4. Therefore, lack of drug interaction (via CYP metabolism) between majority of clinically used drugs metabolized by CYP2D6 and CYP3A4, and Ginkgo biloba provides a safety for patients who currently take this herbal supplement.

Ginkgo biloba happen to be at this time, the second best studied natural health product, in regards to the clinical investigation of its metabolic and transporter-based pharmacokinetic drug interaction potential (Hermann and von Richter, 2012). The available array of published mechanistic herbal drug interaction studies permits reasonable interpretations on the particular drug metabolizing enzyme and transporter effects of Ginkgo biloba products *in vivo*. The information derived from these studies is largely consistent, with some minor disagreements that may be accounted for differences in study design, population studied, dose size, treatment durations, and perhaps differences in the composition of products investigated (Hermann and von Richter, 2012).

St. John's wort

St. John's wort (*Hypericum perforatum* L.; family Clusiaceae) is a popular medicinal herb used for the treatment of depression. *Hypericum perforatum* is a yellow-flowering, perennial herb native to Europe which has been introduced to many temperate areas of the world and grows wild in many meadows (Gurley et al., 2012). The common name comes from its traditional flowering and harvesting on 24th June, the birthday of John the Baptist (St. John's Day). St. John's wort is the most studied botanical dietary supplement in the world. With more than 2000 peer-reviewed articles published on its safety and efficacy (Gurley et al., 2012). Many clinical trials have shown antidepressive efficacy of St. John's wort superior to placebo and comparable to standard antidepressants but with fewer side

effects than conventional antidepressive agents (Linde et al., 2008). When used as a single agent, a risk/benefit ratio has made St. John's wort one of the most readily consumed dietary supplements in the world.

St. John's wort affects the pharmacokinetics of several drugs by inducing CYP isozymes, such as CYP3A4, CYP2C19, CYP2C9, and the drug transporter protein P-gp. This causes St. John's wort to be a highly problematic botanical with regard to CYP-mediated herbal drug interactions (Ang-Lee et al., 2001; Brazier and Levine, 2003; Gurley and Hagan, 2003; Izzo and Ernst, 2001). St. John's wort contains numerous pharmacologically active compounds, including hyperforin, hypericin, pseudohypericin, and flavonoids (e.g., quercetin, quercitrin and I3,II8-biapigenin). Hyperforin, the principal mediator of St. John's wort antidepressive action, and is the main reason for St. John's wort herbal drug interaction potential. Hyperforin is a good ligand for the pregnane xenobiotic receptor (PXR) and thus acts as a potent inducer of CYP3A4 and P-gp, the gene product of MDR1 (Moore et al., 2000; Watkins et al., 2003). According to one estimate, hyperforin is the most potent PXR activator discovered to date. The clinical severity of St. John's wort-drug interactions was first documented in 1999-2000 (Gurley et al., 2005a). At that time, numerous clinics around the world reported that concomitant use of St. John's wort and cyclosporine A produced remarkable reductions in blood levels of the immunosuppressant among organ transplant recipients resulting in graft rejection. Since that time, an overabundance of clinical studies has investigated the effect of St. John's wort on the pharmacokinetics of several medications (Gurley et al., 2005a).

Clinical data revealed that St. John's wort may cause both pharmacokinetic and pharmacodynamic drug interactions. Employing well-established probe drugs, a large number of clinical studies have consistently demonstrated that St. John's wort induced P-gp, a drug transporter protein. Co-administration of St. John's wort also markedly induced human CYP3A4, CYP2E1 and CYP2C19 whereas it had no appreciable effect on CYP1A2, CYP2C9 and CYP2D6 (Arold et al., 2005; Dresser et al., 2003; Hafner et al., 2010; Markowitz et al., 2003b; Roby et al., 2001; Wang et al., 2001, 2004, 2009; Wenk et al., 2004; Xie et al., 2005).

Induction of activity of CYP enzymes and P-gp is produced by hyperforin through activation of the PXR (Hall et al., 2003; Mai et al., 2004; Mueller et al., 2006). Interestingly, it has been reported that the CYP induction effect of St. John's wort was dependent upon the active hyperforin content (Mai et al., 2004; Mueller et al., 2006, 2009). Mai et al. (2004) found that co-administration of cyclosporine A with the conventional St. John's wort preparation contained high-hyperforin content (7 mg) to renal transplant patients led to the expected 40 % to 60 % decrease in AUC, Ctrough, and Cmax values of cyclosporine A. This significant decrease in cyclosporine A's AUC values caused by the interaction with St. John's wort necessitated a substantial increase in cyclosporine A dose to maintain sufficient immunosuppression. In contrast, the St. John's wort preparation contained with low-hyperforin content (0.1 mg) did not significantly affect cyclosporine A pharmacokinetics and did not require cyclosporine A dose adjustments compared with baseline (Mai et al., 2004). The magnitude of CYP3A4 induction mediated by St. John's wort correlated significantly with the content of hyperforin in the St. John's wort extracts but not with the content of hypericin (Gödtel-Armbrust et al., 2007; Komoroski et al., 2004; Mai et al., 2004).

Interactions of St. John's wort with another immunosuppressant, tacrolimus (FK506), in renal transplant patients were also tested (Bolley et al., 2002). During long-term treatment with 2 mg/day of tacrolimus, the blood trough levels of tacrolimus in the renal transplant patient had been sta-

ble in a range from 6-10 mcg/L. The patient started a self-medication with St. John's wort extract at a dosage of 600 mg per day because of a depressive mood disorder. Approximately a month later, tacrolimus trough levels decreased sharply to a minimum level of 1.6 mcg/L during St. John's wort co-administration (Bolley et al., 2002). A re-evaluation of the patient's drug history revealed the above-mentioned antidepressive self-medication, which was consequently stopped. Tacrolimus trough levels returned to the previous range of 6–10 mcg/L without change of dosage. Since tacrolimus is a substrate for CYP3A4 and P-gp (Zhou, 2008), the decrease in the blood trough concentrations of tacrolimus is likely to be due to the induction of CYP3A4 and P-gp by St. John's wort. Low tacrolimus blood levels may present with the risk of under-immunosuppression and result in rejection episodes with the risk of graft loss.

Another clinical relevance example is the interaction of St. John's wort with the oral contraceptive pill. Several cases of women becoming pregnant unintentionly while taking oral contraceptives and St. John's wort have been reported by authorities in the UK (7 cases), Germany (4 cases) and Sweden (2 cases) (Murphy, 2002). An interesting case report described a 36-year-old woman became pregnant unexpectedly while using a combined oral contraceptive, ethinylestradiol/dienogest (Schwarz et al., 2003). An evaluation revealed that she began self-medicated with the over-the-counter St. John's wort extract Helarium®425 (Bionorica) with daily doses up to 1700 mg approximately 3 months prior to conception. Prior to conception no other medication was taken except the hormonal contraceptive pills. The clearance of hormones such as ethinylestradiol, norethindrone and ketodesogestrel has previously been shown to be increased by St. John's wort (Hall et al., 2003; Murphy et al., 2005; Pfrunder et al., 2003). Even though, co-administration with a low hyperforin content St. John's wort extract does not change the plasma concentrations of oral contraceptives (Will-Shahab et al., 2009). Concomitant use of St. John's Wort (at a daily consumption of 2.7 mg of hyperforin) was associated with a significant increase in the oral clearance of norethindrone and a significant reduction in the half-life of ethinylestradiol (Hall et al., 2003). Serum concentrations of follicle-stimulating hormone, luteinizing hormone, and progesterone were not changed by St. John's wort. Breakthrough bleeding happened in 7 of 12 women consuming St. John's wort compared with only 2 of 12 women in the control group (Hall et al., 2003). Changes in the plasma concentrations of ethinylestradiol might also explain several reports of breakthrough bleeding (Hall et al., 2003; Murphy et al., 2005; Pfrunder et al., 2003). Induction of ethinylestradiol and norethindrone metabolism caused by St. John's wort is in agreement with increased CYP3A4 enzyme activity. The authors also suggested that women taking oral contraceptive pills should be advised to expect breakthrough bleeding and should consider adding a barrier method of contraception when using St. John's wort.

With regard to the herbal drug interactions via CYP caused by St. John's wort co-administration, there were many case reports in humans. Some of these are that are worth mentioning are as follows. Concomitant administration, St. John's wort causes a significant decrease in the blood concentrations of cyclosporine A (Dresser et al., 2003) as previously mentioned. It was suggested that this was due to St. John's wort inducing human CYP3A4 and also the drug transporter protein P-gp. This in turn increased the elimination of cyclosporine A. Long term co-administration of St. John's wort with amitriptyline, reduced the plasma concentrations of amitriptyline (Venkatakrishnan et al., 1999). Similarly the mechanism was proposed to be attributed to the induction of CYP3A4. In addition, consistent observations were found with other

CYP3A4-metabolized drugs. These included the anti-HIV agents indinavir, nevirapine and ritonavir, in which co-administration of St. John's wort produced a considerably lower blood concentration of these anti-HIV agents (Piscitelli et al., 2000). The suggested mechanism was attributed to the induction of CYP3A4 by St. John's wort.

Furthermore, short-term (1-3 days) administration of St. John's wort did not induce CYP3A4 or P-gp, and long term (14 days) treatment is generally required to show the inductive effect (Markowitz et al., 2000; Markowitz et al., 2003b). Wang et al. (2001) used midazolam as a specific CYP3A4 probe and found that long-term (300 mg, three times a day for 14 days) St. John's wort administration caused a significant increase in oral clearance of midazolam. While short-term administration of St. John's wort (900 mg single dose) did not have significant effect on CYP activities. This significant increase in oral clearance of midazolam was due to the induction of CYP3A4 mediated by St. John's wort co-administration (Wang et al., 2001). The authors commented that reduction in therapeutic efficacy of drugs metabolized by CYP3A4 should be anticipated during long-term administration of St. John's wort. The induction of CYP3A4 initiated by St. John's wort was demonstrated to be time-dependent. This was illustrated by evidence showing that oral clearance of midazolam was significantly increased after administration of St. John's wort (300 mg three times daily for 14 days), and CYP3A4-induced activity progressively returned to basal levels approximately 1 week after completion of St. John's wort treatment (Imai et al., 2008).

In addition, a case report of drug interaction between St. John's wort and methadone was documented (Eich-Höchli et al., 2003). In this particular report it was found that prescription of St. John's wort decreased methadone blood levels and induced withdrawal symptoms which, if not correctly identified and handled, might cause unnecessary discomfort to the patient, leading to resumption of illicit drug use. Methadone is mainly metabolized by human CYP3A4 enzyme. Thus, co-administration with St. John's wort, as expected, caused induction of CYP3A4 and increased the metabolism and clearance of methadone. Another CYP3A4-metabolized drug that was reported to significantly affected by St. John's wort administration, was simvastatin. Taken with St. John's wort concomitantly, St. John's wort caused a marked decrease in the blood concentrations of simvastatin (Sugimoto et al., 2001). The induction of CYP3A and P-gp by St. John's wort was attributed to an increase in the elimination of simvastatin, and this caused a decrease in the blood concentrations of this drug during St. John's wort co-administration. Besides causing induction of CYP3A4, St. John's wort may also induce two other human CYP enzymes, CYP1A2 and CYP2C9. A case report described a 42-year old female started self-medicating with the over-the-counter St. John's wort extract supplement. This caused a significant decrease in the plasma concentrations of theophylline possibly due to the induction of CYP1A2, the major enzyme responsible for the metabolism of theophylline (Nebel et al., 1999). However, this observation was not confirmed by a well defined pharmacokinetic study conducted by Morimoto et al. (2004). They found that a 15-day treatment with St. John's wort did not cause any significant change in the pharmacokinetics of theophylline (Morimoto et al., 2004). Thus, indicating that the effect of St. John's wort on the activity of human CYP1A2 needs further investigation.

Evidence suggesting a possible induction of CYP2C9 was derived from a clinical study carried out by Jiang et al. (2004). Their results have shown that St. John's wort significantly induced the clearance of both S-warfarin and R-warfarin, which in turn caused a significant reduction in the pharmacological effect of racemic warfarin.

On the other hand, St. John's wort does not have a significant influence on the pharmacokinetics of drugs such as ibuprofen (Bell et al., 2007) and carbamazepine (Burstein et al., 2000). Even though CYP2C9 is primarily responsible for the clearance of ibuprofen and carbamazepine.

A case report described a psychiatric patient who experienced adverse reactions from clozapine and St. John's wort interaction (van Strater and Bogers, 2012). This patient was stable on a fixed dose with stable plasma level of clozapine. Her psychiatric condition deteriorated after she started self-medicating with St. John's wort. This was consistent with a decreased plasma clozapine concentration during the St. John's wort's co-administration. After the withdrawal of St. John's wort, the reduced plasma clozapine level and the psychiatric condition normalized. It is likely that St. John's wort induced CYP3A4, leading to a decrease in plasma clozapine levels in this patient. However, some studies have shown that plasma clozapine levels would decrease in interaction with St. John's wort as a result of induction of CYP1A2 enzyme (Nebel et al., 1999; Miller, 2001). Physicians should be alert to patients self-medicating with over-the-counter medicines, especially St. John's wort which can lower clozapine concentrations below the therapeutic range (van Strater and Bogers, 2012).

In summary, a great number of studies have shown that St. John's wort can produce both pharmacokinetic and pharmacodynamic interactions. The clinical consequences of such St. John's wort-drug interactions are dependent upon a range of factors such as the dosage, duration and therapeutic range of the treatment. Hyperforin appears to play an important role in the induction effect on CYP and P-gp caused by St. John's wort. As the potential for St. John's wort-drug interactions is very significant therefore, patients taking prescription drugs should be strongly advised not to take herbal products containing St. John's wort. Well-documented and clinically relevant interactions between St. John's wort and drugs include: (1) reduced blood cyclosporine A concentration associated in some cases to rejection episodes; (2) reduced efficacy of the oral contraceptive pill, resulting in unwanted pregnancy, and (3) reduced plasma concentration of antiretroviral, e.g. indinavir, nevirapine, and anticancer drugs, e.g. imatinib, irinotecan (Izzo, 2012). Furthermore the use of St. John's wort preparations is not recommended in patients taking immunosuppressants or cardiovascular drugs. When St. John's wort needs to be taken with other medications, it is recommended that practitioners should only use St. John's wort preparations with a low hyperforin content and ensure a careful monitoring scheme. It is also suggested that because of the reduction in the bioavailability of oral contraceptives administered concurrently with St. John's wort, women taking St. John's wort preparations should utilize additional preventive methods to avoid unintended pregnancy. When compared with earlier studies that employed young subjects, the data suggest that some age-related changes in CYP responsivity to herbal supplementation may be present (Gurley et al., 2005a). Therefore, concomitant ingestion of herbal supplements with prescription medications should be strongly discouraged in the elderly.

Garlic

Garlic (*Allium sativum*; family Amaryllidaceae) is the third best-selling herbal supplement in the United States. This is mainly due to the number of its clinically established pharmacological properties – antihypertensive, hypolipidemic, antiatherogenic, antitumorigenic, fibrinolytic, anticarcinogenic, immunomodulatory, antimicrobial and hypoglycemic activities (Foster et al., 2007). Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. It is also taken in the form of an extract in dietary supplements. Fresh garlic and garlic in dietary supplement form may have different physiological

effects and properties, with both forms purported to have antibacterial and cholesterol-lowering properties. Daily dosages generally recommended in the literature for adults are 4 g (1-2 cloves) of raw garlic, one 300 mg dried garlic powder tablet (standardized to 1.3 % allicin or 0.6 % allicin yield) two to three times per day, or 7.2 g of aged garlic extract per day (Tattelman, 2005). Oil-soluble compounds also present in garlic, mainly allicin, sulfides, ajoenes, and vinyldithiins, are quickly metabolized in the body after consumption and are not found in the urine and blood.

Organosulfur compounds, flavonoids, sapogenins and saponins, selenium compounds and fructosamines have been recognized as the main bioactive principles in raw garlic and different garlic supplements (Berginc and Kristl, 2012). Polyphenolic compounds (i.e., flavonoids) apigenin, quercetin, nobiletin, tangeretin, rutin, allixin, myricetin and bergamottin from garlic are good antioxidants with potential cardiopreventive and anticancer activities (Berginc and Kristl, 2012). Flavonoids have also been shown to modulate the activities of intestinal/hepatic transporters (P-glycoprotein – P-gp, multidrug resistance-associated protein 2 - MRP-2, breast cancer resistance protein - BCRP, organic anion-transporting polypeptide - OATP) and enzymes (mainly CYP) *in vitro*. Therefore, these increase the probability of garlic-drug interactions.

Pharmaceutical products developed from garlic bulbs/cloves are made available in the forms of powders, oily preparations, or aqueous-alcoholic extracts of fresh or aged garlic. Due to the complex chemistry, different manufacturing processes result in preparations of quite different chemical composition and pharmaceutical properties. This in turn may yield the products with different pharmacological, safety, and bioavailability characteristics (Greenblatt et al., 2006b; Johne and Roots, 2005).

Up to now, the identified active constituents of garlic include alliin, allinase, diallyldisulfide, ajoens, and others. Alliin is enzymatically converted to allicin, the major garlic component, which displays considerable chemical instability and is quickly degraded to an array of organosulfur compounds (e.g., diallyl sulfide, diallyl disulfide, diallyl trisulfide) that are supposed to ultimately present the main pharmacological effects (Lam and Ernst, 2006).

Even though the use of garlic preparations is extremely popular, preclinical evidence and clinical case reports on the potential garlic drug interactions have been documented. There were also some suspected pharmacodynamic-based drug interactions associated with garlic use. Well-designed clinical studies assessing drug interactions with garlic preparations are remarkably very limited (Hermann and von Ritcher, 2012). Fukao et al. (2004) investigated the *in vivo* effect of garlic allyl sulfides on the phase I and phase II drug-metabolizing enzymes in rats. A highly purified form of each sulfide was administered i.p. as a bolus to rats at a concentration of 10 or 100 micromol/kg body weight for 14 consecutive days. As to the phase I enzymes, diallyl sulfide (100 micromol/kg) slightly but significantly increased CYP2E1 activity (1.6-fold vs. control), whereas diallyl disulfide and diallyl trisulfide did not affect it or the hepatic total CYP level or CYP1A1/2 activity. With regard to the phase II enzymes, diallyl trisulfide (10 micromol/kg) and diallyl disulfide at a 10-fold higher dose (100 micromol/kg) significantly increased the activities of glutathione S-transferase, quinone reductase, and antioxidative enzyme glutathione peroxidase; whereas diallyl sulfide did not. The authors suggested that the upregulation of glutathione S-transferase enzyme by garlic sulfide constituents may have been implicated in the detoxification of carcinogens (Fukao et al., 2004). On the other hand, conflicting results have been reported in which garlic was shown to inhibit the activities of CYP2E1, CYP2C9, and CYP2C19 (An and Morris, 2010).

Two *in vitro* studies on cell-free enzymes demonstrated that garlic constituents competitively inhibit several recombinant CYP enzymes, namely CYP2C9, CYP2C19 and CYP3A4 (Foster et al., 2001; Zou et al., 2002). Aqueous extracts from odorless garlic products inhibited CYP3A4 to a slightly greater extent than CYP2C9. Steam-distilled garlic oil inhibited CYP2C9 and CYP3A4 to a comparable extent, while freeze-dried garlic tablets did not inhibit CYP2C9 activity (Zou et al., 2002). Allicin, a major organosulfur constituent of some garlic supplements, was a more potent inhibitor of cDNA-expressed CYP2C9 than CYP3A4 (Zou et al., 2002). On the other hand, a study using human liver microsomes found that only two garlic constituents, *S*-allyl- or *S*-methyl-L-cysteine at 0.1 mg/mL significantly inhibited CYP3A4, with no appreciable effect on CYP2C9, CYP2C19 or CYP1A2 (Foster et al., 2001). Another *in vitro* study investigated the effects of garlic phytochemicals and aged garlic extract on the hepatic pharmacokinetics of two antiretroviral drugs (saquinavir and darunavir) which are primarily metabolized by human CYP3A4 (Berginc et al., 2010). The results have shown that the garlic phytochemicals including diallyl sulfide and S-methyl-L-cysteine, and aged garlic extract inhibited CYP3A4-mediated metabolism of both anti-retroviral drugs, saquinavir and darunavir.

In addition, Ho and co-workers (Ho et al., 2010) used an immortalized human hepatocyte, Fa2N-4 cell line, to evaluate the effects of garlic extract on the activity of CYP2C9 and CYP3A4 in the cells. They found that garlic extract suppresses CYP2C9 activity without affecting CYP3A4. These results suggest that the concomitant administration of garlic supplements with drugs metabolized by CYP2C9 may lower metabolic clearance of the CYP2C9 drugs and potentially cause adverse drug reactions.

A number of clinical studies were conducted to determine the effects of garlic supplements or garlic dietary constituents on the activities of human CYP enzymes. The effects of acute administration of dietary supplement garlic oil extract, diallyl sulphide on cytochrome CYP2E1 activity in healthy volunteers were evaluated using the selective CYP2E1 probe substrate, chlorzoxazone (Loizou and Cocker, 2001). These studies have demonstrated that garlic dietary constituent diallyl sulfide, administered at dietary concentration can inhibit human CYP2E1 activity. The inhibitory effect of diallyl sulfide may be additive with daily consumption of allium vegetables in particular. This may explain the lower CYP2E1 phenotypic metabolic ratios measured in various European and Mexican cohorts and is consistent with the lower incidence of stomach, liver and colon cancers observed in southern Europeans (Loizou and Cocker, 2001). These results are consistent with an earlier study on effects of garlic oil extract on CYP2E1 activity, which also employed chlorzoxazone as a selective CYP2E1 probe (Gurley et al., 2002, 2005b). Repeat administration of garlic oil over 28 days was shown to moderately reduce CYP2E1 activity by 39 % in healthy adult subjects (Gurley et al., 2002) and by approximately 22 % in elderly individuals (Gurley et al., 2005a). Of note, these data derived from human study are in contrast with those results obtained by Fukao et al. (2004) in which they investigated the *in vivo* effect of garlic allyl sulfides on the CYP2E1 enzyme in rats. Fukao et al. (2004) reported that diallyl sulfide (100 micromol/kg) significantly increased CYP2E1 activity (1.6-fold vs. control) in rats suggesting garlic diallyl sulfide induced rat CYP2E activity. This difference in their results could simply be due to species difference in CYP activity and specificity.

The benzodiazepines alprazolam and midazolam, as well as the anticancer drug docetaxel are extensively used as probes for CYP3A4 activity because they are exclusively metabolized by intestinal and hepatic CYP3A4. Markowitz and co-workers ex-

amined the influence of a garlic extract on the activity of CYP2D6 and CYP3A4 in 14 healthy volunteers (Markowitz et al., 2003c). Probe substrates dextromethorphan (CYP2D6) and alprazolam (CYP3A4) were administered orally at baseline and again after treatment with garlic extract (3 x 600 mg twice daily) for 14 days. They found no significant differences in the activity of CYP2D6 between the baseline and garlic phases. For CYP3A4 as assessed by using alprazolam, there were no significant differences in pharmacokinetic parameters of alprazolam at baseline and after garlic extract treatment. These results indicate that garlic extracts are unlikely to alter the disposition of co-administered medications primarily metabolized by CYP2D6 or CYP3A4. Latter study also showed that a 28-day treatment with garlic oil did not have a significant effect on the pharmacokinetics of midazolam in the elderly (Gurley et al., 2005a). Moreover, Cox et al. (2006) have demonstrated that a 12-day treatment with garlic did not affect the pharmacokinetics of a CYP3A4 substrate, docetaxil in 10 women with metastatic breast cancer. The lack of garlic treatment on the activity of human CYP3A4 in healthy subjects was further confirmed by a clinical study using another CYP3A4 substrate drug, saquinavir. In this study, the results have shown that garlic extract had no significant effect on saquinavir pharmacokinetics and hepatic CYP3A4 function measured by the erythromycin breath test (Jacek et al., 2004). Collectively, these trials signify that garlic has no effect on the CYP3A4 enzyme in humans. These findings are consistent with an *in vitro* observation in which a number of water-soluble garlic components of aged garlic have been shown to not affect the activity of human CYPs *in vitro* (Greenblatt et al., 2006b). These suggest that drug interactions involving inhibition of CYP3A4 enzyme by aged garlic extract are very unlikely.

Conflicting results were reported in a clinical study showing a garlic supplement containing alliin and allicin significantly decreased the AUC and Cmax of the protease inhibitor saquinavir (Piscitelli et al., 2002a). It suggested that garlic supplement caused an induction of human CYP3A4. This was not consistent with the findings described previously (Jacek et al., 2004; Markowitz et al., 2003c). The induction effect of garlic supplement could be a result of the use of higher doses, at least with respect to allicin content, or possibly the longer dosing period (20 days versus 14 days in Markowitz et al.'s study). Another study with supplements devoid of allicin, for a period of 4 days, also indicated changes in pharmacokinetic parameters of ritonavir, although the results were not statistically significant (Gallicano et al., 2003). The authors also commented that as the effects of both ritonavir and garlic on enzyme inhibition and enzyme induction are complicated, the results of their study should not be extrapolated to steady state conditions, where the possibility of an interaction still needs to be evaluated (Gallicano et al., 2003). Both saquinavir and ritonavir not only are metabolized by CYP3A4 but also are substrates for P-glycoprotein (Lee et al., 1998). It was proposed that the pharmacokinetic changes observed for saquinavir and ritonavir after garlic treatment may not be due to induction of CYP3A4 but rather the influence of garlic constituents on another enzyme or transporter such as P-glycoprotein (Markowitz et al., 2003c). However, any conclusions on the metabolic effects of garlic based on inter-study comparisons are somewhat restricted. This is mainly because all published interaction studies have used different garlic formulations, treatment durations, and probe substrates (Markowitz et al., 2003c).

The well-known adverse interaction between garlic and warfarin is probably due to the antiplatelet effects of garlic. A number of case reports have proposed that garlic may influence platelet function and blood coagulation which can lead to a risk of bleeding (Borrelli et al., 2007). Two pa-

tients having the international normalized ratio (INR) previously stabilized on warfarin were found to have changed INR values after garlic intake (Sunter, 1991). There is also evidence that some of the antiplatelet activity might be irreversible and thus it has been suggested that patients stop ingestion at least seven days prior to surgery (Ang-Lee et al., 2001). Few case reports have highlighted the possibility that garlic may increase the risk of bleeding, particularly in patients undergoing surgery. In contrast, the results obtained from two clinical studies have suggested that garlic (enteric-coated tablets or aged garlic extract) did not alter the pharmacokinetics or pharmacodynamics of warfarin and that garlic had no serious haemorrhagic risk for adequately monitored patients receiving warfarin (Macan et al., 2006; Mohammed Abdul et al., 2008). Astonishingly, garlic has been suggested to decrease the INR of the anticoagulant fluindione in an 82-year-old man (Pathak et al., 2003). A rational explanation is not known for such an interaction.

The above evidence indicates reliably that neither single nor repeat dose administrations of several garlic formulations up to 28 days might have the ability to exert important alterations (i.e., inhibition or induction) of the activities of CYP1A2, CYP2D6, or CYP3A4 in humans. These CYP isozymes play an important role in the metabolic pathways of many drugs that are likely to be co-administered with garlic supplements. In clinical expressions, these findings reveal that garlic preparations are unlikely to modify the disposition of co-administered drugs which are metabolized by these CYP enzymes. In contrast, repeat administration of garlic oil over 28 days appeared to moderately decrease CYP2E1 activity in healthy and elderly individuals (Gurley et al., 2002, 2005a). However, as the overall extent of CYP2E1 inhibition by garlic is moderate, and scarcely any clinically used drugs are dependant on CYP2E1 as an exclusive or predominant metabolic pathway (except chlorzoxazone), the detected effects of garlic on CYP2E1 do not emerge to have crucial clinical implications (Hermann and von Ritcher, 2012; Yuan et al., 2004). This viewpoint is further supported by a study in healthy adult subjects, in which a 3-month administration of aged garlic extract (10 mL once daily) did not have any significant effect on oxidative metabolism of acetaminophen, to which CYP2E1 is known to be an important contributor, besides CYP3A4 and CYP1A2 (Gwilt et al., 1994).

Collectively, there is sound consistent evidence that recommended doses of many varieties of garlic preparations do not have any significant effect on CYP3A4 activity *in vivo*. To our knowledge, the question of whether garlic preparations might be engaged in transporter-based drug interactions has not yet been dealt with in human pharmacokinetic studies. Additionally the preclinical evidence regarding this question is still not well recognized (An and Morris, 2010). With the limited existing evidence from human *in vivo* drug interaction studies, there is no indication that garlic may present any remarkable or clinically important metabolism-based pharmacokinetic interactions with drugs, mainly cleared by CYP1A2, CYP2D6, CYP2E1, or CYP3A4 enzymes. Therefore, many of the findings from *in vitro* studies still need to be confirmed by results from clinical interaction studies before we can implement recommendations for herbal drug interactions.

Goldenseal

Goldenseal (*Hydrastis canadensis*, orangeroot) is a perennial herb in the buttercup family Ranunculaceae, indigenous to southeastern Canada and the northeastern United States. Botanical goldenseal supplement is used for the common cold and upper respiratory tract infections, as well as stuffy noses and hay fever. Some people use goldenseal for digestive disorders including stomach pain and swelling (gastritis), peptic ulcers, colitis, diarrhea, constipation, and hemorrhoids, as well as men-

strual disorders. In addition, goldenseal is being used as a topical antimicrobial remedy to disinfect cuts and scrapes (Weber et al., 2003). Goldenseal contains a number of isoquinoline alkaloids such as hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, and canalidine. Hydrastine and berberine are the main active constituents (Weber et al., 2003). Commercial dietary supplement preparations of goldenseal standardized extracts are available in liquid, tablet, and capsule forms. Recommended doses of the powdered root vary widely and range from 750 mg to 6 grams in tablet or capsule form per day, to be given 2 to 3 times daily. Goldenseal preparations possess widespread popularity and are among the top 20 bestselling botanical products in the United States (Ettefagh et al., 2011).

Evidence obtained from *in vitro* studies have suggested that goldenseal root was the most potent inhibitor of CYP3A4 among 21 popular herbal products tested for the inhibitory potential. A number of clinical studies on goldenseal and drug interactions have been reported. Sandhu et al. (2003) investigated the influence of goldenseal root on the pharmacokinetics of the CYP3A4 substrate indinavir in 10 healthy volunteers. The pharmacokinetics of a single oral dose (800 mg) indinavir were characterized in subjects before and after 14 days of treatment with goldenseal root (1140 mg twice daily). No statistically significant differences in peak concentration (11.6 vs. 11.9 mg/L) or oral clearance (26.8 vs. 23.9 mg*h/L) of indinavir were detected following treatment with goldenseal root. These results suggest that patients being treated with indinavir can safely take goldenseal root and that interactions with other drugs metabolized by hepatic CYP3A4 are unlikely (Sandhu et al., 2003).

Single-time point phenotypic metabolic ratios were used to determine if long-term supplementation of goldenseal (*Hydrastis canadensis*) affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4 activity (Gurley et al., 2005b) in 12 healthy volunteers. They were given a pretreatment of goldenseal dietary supplement for 28 days. Probe drug cocktails of midazolam (CYP3A4) and caffeine (CYP1A2), followed 24 hours later by chlorzoxazone (CYP2E1) and debrisoquine (CYP2D6), were administered before (baseline) and at the end of supplementation. Presupplementation and postsupplementation phenotypic trait measurements were determined for activity of CYPs of interest as previously described (Gurley et al., 2005b). Comparisons of pre-supplementation and post-supplementation phenotypic ratio means revealed significant inhibition (approximately 40 %) of CYP2D6 and CYP3A4 activity for goldenseal. Whereas, there was no considerable effect of goldenseal on CYP1A2 and CYP2E1 activities. Thus, this was the first clinical study convincingly indicating that goldenseal root extract, given at recommended doses, showed to remarkably inhibit CYP2D6 and CYP3A4 *in vivo*. Accordingly, serious adverse interactions may rise from the concomitant intake of goldenseal supplements and drugs that are metabolized by CYP2D6 and CYP3A4 (Gurley et al., 2005b).

Researchers from the same group have continued studying the effects of goldenseal supplementation on human CYP3A4 activity (Gurley et al., 2008a). This was evaluated by using midazolam as a phenotypic probe for human CYP3A4 and studied in 16 healthy volunteers. The subjects were randomly assigned to receive either goldenseal or placebo for 14 days. Midazolam (8 mg) was administered before and after goldenseal supplementation, and pharmacokinetic parameters were assessed. The results have shown that means of midazolam's AUC0-∞ and Cmax were increased by approximately 63 % and 40 %, respectively, upon goldenseal supplementation. These indicated that goldenseal supplementation caused significant inhibition of CYP3A4 (Gurley et al., 2008a). In contrast, the potent CYP3A4 inhibitor clarithromycin produced about 5.5-fold (i.e., 548 %) and about 2-fold (217 %)

increases in midazolam's AUC0-∞ and Cmax values, respectively (Gurley et al., 2008a). Therefore, according to current FDA drug interaction guideline recommendations (US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research 2012), these data classified goldenseal root extract, at the given recommended dose, as a weak inhibitor of CYP3A4 (i.e., < 2-fold increase in AUC of the sensitive CYP3A4 probe midazolam).

Gurley et al. (2008b) also conducted a similar study to determine the effects of goldenseal supplementation on *in vivo* activity of another human CYP enzyme, CYP2D6. Healthy subjects (N = 16) were randomized to receive a standardized goldenseal extract for 14 days on separate occasions. The CYP2D6 probe substrate, debrisoquine (5 mg), was administered before and at the end of supplementation. Pre- and post-supplementation phenotypic trait measurements were assessed for CYP2D6 activity (Gurley et al., 2008b). Comparisons of pre- and post-supplementation, results revealed significant inhibition (approximately 50 %) of CYP2D6 activity caused by goldenseal supplementation. Consequently, adverse herbal drug interactions may result with concomitant ingestion of goldenseal supplements and drugs that are CYP2D6 substrates (Gurley et al., 2008b).

In addition, there were other two clinical studies evaluating the effect of berberine, a major alkaloid constituent in goldenseal extract, on the pharmacokinetics of cyclosporine A. An immonosupressive agent, cyclosporine A is a drug with narrow therapeutic index and primarily metabolized by CYP3A4. These studies were conducted in Chinese renal transplant patients (Wu et al., 2005) and in healthy Chinese subjects (Xin et al., 2006). The first study was a randomized, controlled clinical trial in 104 renal transplant recipients, in which Wu and colleagues (Wu et al., 2005) examined the effects of oral berberine supplementation (tablets 200 mg, three times a day, for 3 months) on the pharmacokinetics of cyclosporine A. Blood trough concentration of cyclosporine A and biochemistry indexes for hepatic and renal functions were determined. For the comprehensive pharmacokinetic study, 6 renal-transplant recipients were treated with a 3-mg/kg dose of cyclosporine A, twice a day, before and after oral co-administration of 200 mg berberine three times a day, for 12 days. The trough blood concentrations and the ratios of concentration/dose of cyclosporine A in the berberine-treated group increased by 88.9 % and 98.4 %, respectively, compared with those at baseline ($P < 0.05$). For the berberine-free group, these parameters rose by 64.5 % and 69.4 %, respectively, when compared to those at baseline ($P < 0.01$). However, the final blood concentrations and the ratios of concentration/dose of cyclosporine A in berberine-treated patients were still 29.3 % and 27.8 %, respectively, higher than those in berberine-free patients ($P < 0.05$). No significant effects on liver or renal functions were detected under co-administration of berberine. For the comprehensive pharmacokinetic study, the results showed that following co-administration of berberine in six patients for 12 days, the mean AUC of cyclosporine A was increased by 34.5 % ($P < 0.05$). The average percentage increase in the steady-state drug concentration (Css) was 34.5 % ($P < 0.05$). The mean half-life ($t_{1/2}$) of cyclosporine A was increased by 2.7 h ($P < 0.05$). In addition, the average percentage decrease in apparent oral clearance (CL/F) of cyclosporine A was 40.4 % ($P < 0.05$). This study has demonstrated that berberine can markedly elevate the blood concentration of cyclosporine A in renal-transplant recipients in both clinical and pharmacokinetic trials. This combination may allow a reduction of the cyclosporine A dosage. The mechanism for this interaction is most likely explained by inhibition of CYP3A4 by berberine in the liver and/or small intestine (Wu et al., 2005).

The second study on effects of berberine on the pharmacokinetics of cyclosporine

A was carried out in 12 healthy Chinese volunteers (Xin et al., 2006). Six volunteers were orally treated with 0.3 g berberine, twice daily for 10 days. Pharmacokinetic studies of cyclosporine A at 6 mg/kg dosage were performed before and at the end of the berberine treatment period. Another six healthy volunteers participated in the pharmacokinetic study with 3 mg/kg cyclosporine A, in which the second single dose of 3 mg/kg cyclosporine A was given concomitantly with a single oral dose of 0.3 g berberine. The blood cyclosporine A concentrations were monitored. In the pharmacokinetic study with 6 mg/kg cyclosporine A and time-separated intake of the last berberine dose, berberine did not cause any significant changes in the pharmacokinetic parameters of cyclosporine A. However, in the trial with 3 mg/kg cyclosporine A and the concomitant administration of cyclosporine A and berberine, the average percentage increase in AUC of cyclosporine A was 19.2 % ($P < 0.05$), without altering elimination half-life, and apparent oral clearance (CL/F). The present results suggest that berberine can increase the oral bioavailability of cyclosporine A at the dosage of 3 mg/kg (Xin et al., 2006). The berberine-mediated increase in cyclosporine A bioavailability may be partly attributed to a decrease in liver and/or intestinal metabolism through the inhibition of CYP3A4 in the liver and/or gut wall.

The outcomes derived from these two clinical studies even though are not totally consistently, but the evidence pointed to a moderest inhibition of human CYP3A4 isozyme by berberine. However, with even small changes in pharmacokinetic parameters including AUC, Css and apparent oral clearance, caused by co-administration with berberine, it may be important to consider in case of concomitant treatment with narrow therapeutic index drugs that are substrates of CYP3A4, such as cyclosporine A.

With regard to the effects of goldenseal root extract constituent, berberine on other human CYP enzymes, a recent study has described findings on these enzymes. This particular study was conducted in 18 Chinese healthy subjects who received orally either placebo or berberine capsules at a dose of 300 mg three times a day (= 900 mg/day) for 14 days (Guo et al., 2012). Phenotypic effects of berberine supplementation on the activities of various CYP isozymes were evaluated by the administration of the CYP-specific probe substances namely caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4). A remarkable decrease in CYP2D6 activity was observed followed berberine supplementation, as the CYP2D6 index, 0-8 h urinary dextromethorphan/ dextrorphan ratio increased about 9-fold ($P < 0.01$). In addition, the losartan/E-3174 ratio doubled ($P < 0.01$) after berberine administration, indicating a notable decrease in CYP2C9 activity. In contrast, CYP3A4 activity was only modestly inhibited, as the Cmax, AUC0-∞, and AUC0-12h of midazolam were significantly increased after berberine treatment. Accordingly, the oral midazolam clearance was also modestly decreased by 27 % ($P < 0.05$). There were no significant changes of the other probe substances caffeine (CYP1A2) and omeprazole (CYP2C19). These results suggested that repeated administration of berberine (900 mg/day) markedly decreased the activity of CYP2D6, and appears to modestly inhibit CYP2C9 and CYP3A4 activities. On the other hand, berberine administration does not change CYP1A2 and CYP2C19 function. Herbal drug interactions should be considered when berberine is concomitantly administered with drugs that are metabolized by CYP2D6, CYP2C9 and CYP3A4 (Guo et al., 2012).

In summary, a number of pharmacokinetic interaction studies with different goldenseal root extract products at daily doses up to 4 g revealed overall consistent results and categorized goldenseal root extract as a weak inhibitor of CYP3A4 and CYP2D6 enzymes, according to FDA drug interac-

tion guideline criteria. Of note, the existing evidence for this assessment is most vigorous for CYP3A4, and based on phenotypic serum ratios or metabolite urinary recovery data only, for CYP2D6 enzyme. Moreover, results from single studies suggest that goldenseal root extract does not alter the activities of CYP1A2 and CYP2E1 enzymes. To our knowledge, there is no information available in respect to potential *in vivo* effects of goldenseal on CYP2C9 and CYP2C19 enzymes. In addition, the effects of goldenseal root extract constituent, berberine were similar to those of goldenseal root extract. The evidence suggests that berberine products have weak potential to inhibit CYP3A4 and CYP2D6, but is unlikely to alter CYP1A2 and CYP2C19 enzymes. However, these findings and their clinical implication have to be confirmed by future studies. To be safe, care should be applied when goldenseal root extract or berberine products are co-administered with narrow therapeutic index drugs, which its major metabolic pathway is catalyzed by CYP3A4, CYP2D6 or CYP2C9 enzymes.

Milk thistle

Milk thistle (*Silybum marianum*) is a thistle of the genus Silybum, a flowering plant of the daisy family (Asteraceae). The plant is native to the Mediterranean regions of Europe, North Africa and the Middle East. The name "milk thistle" originates from a feature of the leaves, which are notably banded with splashes of white. Historically, these milky bands were said to be Mother Mary's milk, and this is the origin of another common name, St. Mary's thistle. For many centuries extracts of milk thistle have been recognized as “liver tonics”. Milk thistle has been reported to have protective effects on the liver and to greatly improve its function. It is typically used to treat liver cirrhosis, chronic hepatitis, toxin-induced liver damage including the prevention of severe liver damage from Amanita phalloides ('death cap' mushroom poisoning), and gallbladder disorders (Pradhan and Girish, 2006). Milkthistle, along with dandelion and other extracts are often referred to as hangover cures as the bitter tincture helps organs clear toxins after heavy drinking.

Milk thistle is one of the oldest and most extensively studied plants in the treatment of alcoholic, toxic, and viral liver diseases. Many years of research disclose the active flavanoid-lignan (flavanolignan) group of constituents, called silymarin has liver-protective and regenerative properties, as well as antioxidant effects. Silymarin represents a mixture of the flavonolignan constituents’ sylibin (silibinin), isosilybin, silidianin, and silichristin. Silymarin is extracted from dried milk thistle seeds, in which it is contained at higher contents than in other parts of the plant. Silymarin has structural similarities to steroid hormones, which may be linked to its pharmacological actions. Silybin is the predominant and pharmacologically most active component, constituting approximately 60-70 % of the isomers, followed by silychristin (20 %), and silydianin (10 %) (Pradhan and Girish, 2006). The silymarin content in milk thistle extracts may range from 40-80 %. Recommended daily doses of milk thistle extract range between 210 to 800 mg, depending on patient characteristics and therapeutic objectives (Pradhan and Girish, 2006). The terms milk thistle, silymarin, and silybin are generally used interchangeably.

In the United States and Europe, about 65 % of patients with liver disease take herbal preparations. The cost of the use of silymarin reaches $ 180 million in Germany alone. In spite of the wealth of literature, there is no firm clinical evidence to endorse the use of these substances in clinical practice (Loguercio and Festi, 2011). This discrepancy is attributed to various factors, such as quality of clinical trials, heterogeneity of diagnoses, lack of standardized preparations, and frequently inconsistent dosing and outcome parameters.

A number of *in vitro* studies revealed that silymarin and silybin can exhibit inhi-

bition of various CYP enzymes such as CYP3A4, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 (Budzinski et al., 2007; Etheridge et al., 2007; Sridar et al., 2004; Venkataramanan et al., 2000). The existing data suggest that the clearance of a variety of drugs may be reduced by concomitant use of milk thistle via inhibition of CYP enzymes. In contrast, there is no conclusive *in vitro* evidence that milk thistle would have considerable effects on the activity of the drug transporter protein P-gp (Budzinski et al., 2007; Etheridge et al., 2007).

A number of clinical studies have been performed to assess the *in vivo* effects of milk thistle products on various CYP enzymes. Most of these were done by using specific probe drugs, so this allows us to identify and characterize the effects of milk thistle on specific metabolic pathways. In the very early work, silymarin at the lower end of usually recommended daily doses (Legalon®, 70 mg three times a day = 210 mg/day) was given orally to 16 healthy subjects for 28 days. Their results have shown that co-administration of silymarin had no effect on the pharmacokinetics of the two non-specific CYP probes, aminopyrine or phenylbutazone (Leber and Knauff, 1976).

Later on, a defined pharmacokinetic drug interaction was conducted (Gurley et al., 2004) to determine whether long-term supplementation of milk thistle (*Silybum marianum*) extract affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4 enzyme activity. The study was performed in 12 healthy subjects who were randomly assigned to receive milk thistle (175 mg twice a day = 350 mg/day, standardized to 80 % silymarins) for 28 days. Probe drug cocktails of midazolam (CYP3A4) and caffeine (CYP1A2), followed 24 hours later by chlorzoxazone (CYP2E1) and debrisoquine (CYP2D6), were administered before (baseline) and at the end of supplementation. Pre-supplementation and post-supplementation phenotypic trait assessments were determined for activity of CYP3A4, CYP1A2, CYP2E1, and CYP2D6. The results from this study showed that daily doses of 350 mg of a standardized milk thistle extract did not affect human CYP1A2, CYP2D6, CYP2E1, and CYP3A4 enzyme activities *in vivo*. Therefore, the long term use of usual recommended dose of milk thistle does not appear to cause a significant interaction potential for drugs which are metabolized by these enzymes (Gurley et al., 2004).

The same group of researchers conducted another similar pharmacokinetic study in 19 healthy young subjects (Gurley et al., 2006). The difference was the subjects were dosed with substantially higher daily doses of a standardized milk thistle supplement (900 mg/day) for 14 days. The subjects also received rifampin (300 mg twice a day) and clarithromycin (500 mg twice a day) for 7 days as positive controls for CYP3A4 induction and inhibition, respectively. Identical oral dose of midazolam (a CYP3A4 probe substrate) was administered before and after milk thistle supplementation and control periods (Gurley et al., 2006). Unlike those observed for rifampin and clarithromycin, midazolam pharmacokinetics were unaffected by milk thistle. Milk thistle supplementation appears to have no clinically relevant effect on CYP3A activity *in vivo*. Thus, these results confirm the previous finding that milk thistle extract is not a clinically relevant modulator of human CYP3A4 enzyme activity *in vivo*. However, given the inter-product variability in phytochemical content, potency, and formulation among herbal supplements, these results may not extend to regimens utilizing higher dosages, longer supplementation periods, or brands with improved dissolution and/or bioavailability characteristics (Gurley et al., 2006).

Furthermore, a similar observation regarding the effect of milk thistle extract was found with another CYP3A4 probe drug, nifedipine (Fuhr et al., 2007). This pharmacokinetic study was conducted to determine

whether inhibition of CYP3A4 by silymarin is present *in vivo*. Immediate release nifedipine (10 mg) was administered as a CYP3A4 probe drug either alone or with co-administration of silymarin (560 mg) to 16 healthy volunteers. It was found that nifedipine AUC was 13 % higher in the silymarin period, and Cmax values were 30 % lower than those of the baseline period. There was a trend to delayed absorption of nifedipine in the silymarin period. Intra-individual variability especially for Cmax (intra-subject CV 120 %) was surprisingly high. Overall, this study confirmed that silymarin is not a potent CYP3A4 inhibitor *in vivo* (Fuhr et al., 2007). Their data also suggest that co-administration of silymarin does not considerably alter the extent of absorption or metabolism of nifedipine but may delay the absorption rate (Fuhr et al., 2007). This potential effect of silymarin co-medication very much resembles the effect of food on pharmacokinetics of immediately release nifedipine, which probably is caused by delayed and more irregular gastric emptying in the presence of food (Fuhr et al., 2007).

There were two other pharmacokinetic studies on milk thistle and drug interactions. These studies determined whether milk thistle extract may have the potential to alter the pharmacokinetics of the HIV protease inhibitor indinavir. Indinavir is a known CYP3A4 and P-gp substrate. In the first study (Piscitelli et al., 2002b), pharmacokinetics of indinavir was investigated in 10 healthy volunteers. Blood samples were collected over 8 hours after the volunteers took four doses of indinavir 800 mg every 8 hours for baseline pharmacokinetics. This dosing and sampling were repeated after the subjects took milk thistle 175 mg (contained silymarin 153 mg as the active ingredient) three times a day for 3 weeks. After an 11-day washout, indinavir dosing and blood sampling were repeated to evaluate the offset of any potential interaction. The results have demonstrated that milk thistle did not alter significantly the overall exposure of indinavir, as evidenced by only 9 % reduction in the indinavir AUC after 3 weeks of dosing with milk thistle (Piscitelli et al., 2002b). This suggests that milk thistle in commonly recommended dosages should not interfere with indinavir therapy in patients infected with the human immunodeficiency virus.

In the second study (DiCenzo et al., 2003), pharmacokinetic drug interactions were evaluated in 10 healthy volunteers. Indinavir (800 mg, three times a day) was given for four doses on days 1 and 2. Silymarin (160 mg, three times a day = 480 mg/day) was given on days 3-15. On day 16 and for one dose on day 17, both indinavir and silymarin were given at the same dosages. Pharmacokinetic parameters of indinavir were assessed at steady state both before and after co-administration of silymarin for 14 days. The results showed that pretreatment with silymarin for 14 days had no considerable effect on the AUC at steady state and the trough plasma concentration of indinavir. So these indicate that silymarin has no apparent effect on the pharmacokinetics of indinavir. Taken together, both indinavir pharmacokinetic drug interaction studies generated consistent results, thus denoting that milk thistle extract supplementation at usually recommended dose does not modify the activity of CYP3A4 *in vivo* to a clinically relevant extent (DiCenzo et al., 2003).

Another pharmacokinetic drug interaction study was carried out to evaluate the effect of milk thistle root extract on the activity of CYP3A4 (Rao et al., 2007). This study used the H_2-receptor antagonist ranitidine as a model CYP3A4 substrate and ranitidine is also a putative P-gp substrate. They found that there was no notable influence of silymarin on the pharmacokinetics of ranitidine. This was revealed by evidence showing that concomitant administration of silymarin (140 mg, three times a day for 7 days) did not alter ranitidine Cmax and AUC(0-infinity). So this finding of lack of effect of milk thistle on CYP3A4

activity, is consistent with those reported previously (Gurley et al., 2004, 2006).

Van Erp and co-workers (van Erp et al., 2005) conducted a study to examine whether milk thistle affects the pharmacokinetics of the anticancer drug irinotecan, a substrate for CYP3A4 and UDP glucuronosyltransferase isoform 1A1 (UGT1A1) enzymes, in humans. The study involved 6 cancer patients who were treated with irinotecan (125 mg/m^2) given as a 90-minute infusion once weekly. Four days before the second irinotecan dose, patients received 200 mg milk thistle capsules, three times a day (= 600 mg/day), for 14 consecutive days. Each milk thistle capsule contained 200 mg milk thistle seed extract consisting of 80 % silymarin. Short-term (4 days) or more prolonged intake of milk thistle (12 days) had no significant effect on clearance of irinotecan. Whereas the extent of glucuronidation of the irinotecan's metabolite SN-38 was relatively similar between pre-supplementation and post-supplementation with milk thistle. These results suggested that the concentrations silybin achieved after intake of milk thistle at this usual recommended are too low to produce any significant effect on the activities of CYP3A4 and UGT1A1 *in vivo* (van Erp et al., 2005). Thus, herbal drug interaction with milk thistle is unlikely in regard to anticancer drugs metabolized by these two enzymes. Moreover, Flaig et al. (2007) provided the best evidence that silybin can be administered to humans at doses producing anticancer-relevant concentrations, with minimal or no side effects.

With respect to CYP2C9, an early clinical study (Rajnarayana et al., 2004) was undertaken in 12 healthy volunteers to determine the effects of milk thistle extract silymarin on the pharmacokinetics of metronidazole (a substrate for CYP3A4 and CYP2C9). At first, subjects received metronidazole alone at a dose of 400 mg every 8 h for 3 days. On day 4, blood and urine were collected at different time points and metronidazole concentrations were measured. After a washout period of one week silymarin was given at a daily dose of 140 mg for 9 days. From day 7 both silymarin (140 mg/day) and metronidazole (3 x 400 mg/day) were given till the 9th day. On day 10, blood and urine were collected as above and the concentrations of metronidazole and its metabolite were determined. The results have shown that co-administration of silymarin increased the clearance of metronidazole and its major metabolite, hydroxymetronidazole both by approximately 30 %, with a concomitant decrease in half-life, Cmax and AUC. These findings indicate that silymarin might induce both intestinal P-glycoprotein and CYP3A4 upon multiple dose administration (Rajnarayana et al., 2004). However, this finding is in contrast with all other previous reports, and reserved further investigations to clarify the issue and give insight to its underlying mechanism(s). In addition, a recent study has reported that milk thistle extract pro-ducts significantly inhibited PXR-mediated CYP3A4 induction by rifampicin, erlotinib and paclitaxel at the transcriptional level (Mooiman et al., 2013). Their results demonstrate that milk thistle is able to prevent CYP3A4 induction and that silybin and isosilybin are responsible for this effect.

Han et al. (2009) examined the effects of silymarin on the pharmacokinetics of the angiotensin II receptor antagonist losartan and its active metabolite E-3174 and its relationship with CYP2C9 genotypes in 12 healthy Chinese subjects. These subjects were of known CYP2C9 genotype (six CYP2C9*1/*1 and six CYP2C9*1/*3). The pharmacokinetics of losartan and E-3174 were studied before and after a 14-day treatment with 140 mg of silymarin three times daily. The AUC of losartan increased (by approximately 2-fold) significantly following a 14-day silymarin treatment in subjects with the CYP2C9*1/*1 genotype, but not in those with the CYP2C9*1/*3 genotype. Hence, the oral apparent clearance (CL/F) of losartan was also significantly

decreased by more than 2-fold after a 14-day silymarin treatment in these CYP2C9*1/*1 genotype subjects. On the other hand, in carriers of CYP2C9*1/*3, there were no significant differences in any pharmacokinetic parameters of losartan between the placebo and silymarin treatment groups. The AUC of E-3174 decreased significantly with a silymarin pretreatment in both CYP2C9*1/*1 and the CYP2C9*1/*3 subjects. The authors concluded that silymarin inhibits the metabolism of losartan to E-3174, however the magnitude of the inhibition on CYP2C9 is different dependent upon CYP2C9 genotypes (Han et al., 2009). The findings from Han et al. (2009) were followed up by Brantley and co-workers (Brantley et al., 2010) who did study *in vitro* and found that silybin A and silybin B were the most potent CYP2C9 inhibiting constituents of silymarin extract. In systematic expressions, the available evidence on CYP2C9-related issue suggests that milk thistle co-administration might have a potential to inhibit the clearance and to increase the exposure of other sensitive CYP2C9 substrates such as warfarin *in vivo*. The consequence of this particular herbal supplement is remaining to be confirmed by clinical investigations.

Gurley and co-workers (Gurley et al., 2008b) evaluated the effect of milk thistle extract on the activity of human CYP2D6 in 16 healthy volunteers. Subjects were randomized to receive a standardized milk thistle extract for 14 days on separate occasions. Debrisoquine (a CYP2D6 probe) was administered before (baseline) and at the end of milk thistle supplementation. Pre- and post-supplementation phenotypic trait measurements were determined for CYP-2D6 activity. Comparisons of pre- and post-supplementation results revealed no significant effect of milk thistle supplementation on human CYP2D6 activity. Accordingly, adverse herbal drug interactions are unlikely to occur when the patients taking milk thistle concomitantly with prescription drugs which are metabolized by CYP2D6 enzyme.

Overall, the existing evidence from clinical interaction studies is reasonably consistent and suggests that milk thistle or silymarin products consumed at recommended doses; do not change the activities of CYP3A4, CYP1A2, CYP2D6, and CYP2E1 enzymes. The evidence supporting a lack of interaction potential is most strong for CYP3A4. Also the findings from a single study in irinotecan-treated cancer patients suggest that co-administration of milk thistle or silymarin products is unlikely to markedly modify the activity of UGT1A1 and carboxylesterase 2 enzymes. On the other hand, the available evidence regarding CYP2C9 from a well-designed study in Chinese subjects (Han et al., 2009) convincingly proposes the possibility of significant inhibition of CYP2C9 by milk thistle or silymarin products. These findings invite for further studies to confirm the clinical implication of this interaction, especially with narrow therapeutic index CYP2C9 drugs such as warfarin.

Special consideration for elderly patients

Herbal medicines and dietary supplement use is common in the elderly (Olesen et al., 2013). The concomitant use of prescription medications and herbal products by the elderly is also a common situation (Izzo, 2012). Many older patients do not disclose their use of the herbal dietary supplements to their physicians. In addition, because older adults have multiple health problems, they are particular at risk for herbal drug interactions. Special concerns have been raised for elderly patients because of well-documented polypharmacy interactions, increased sensitivity to certain drugs, and diminished metabolism of many drugs. These can potentially increase the risk of adverse events from herbal drug interactions. In spite of this, clinical studies targeted at exploring the herbal drug interactions in elderly patients are limited. Gurley et al. (2005a) found that elderly sub-

jects, like their younger counterparts, are susceptible to herb-mediated changes in CYP activity and that some age-related changes in CYP responsivity to herbal products may exist. Gurley et al. (2005a) reported that ginseng marginally inhibited CYP2D6 in elderly subjects. On the other hand, no such inhibition was observed in young subjects (Gurley et al., 2002). It is recommended that concomitant ingestion of herbal supplements with prescription medications should be strongly discouraged in the elderly.

CONCLUSIONS

Abundant research data have shown that herbal supplements or herbal medicines can modify drug metabolism mediated by human CYP enzymes. This causes herbal drug interaction which has the clinical consequence of adversely affecting the pharmacokinetics of several drugs. Thus, when dietary supplements are taken concomitantly with prescription drugs, this can result in serious herbal drug interactions. This may be likely to happen when multiple drugs, and multiple herbal supplements are taken concurrently. However, as noted in this article, the drug interactions do not appear to happen with every single herbal supplement and with every drug. This is somewhat similar to grapefruit drug interactions in which grapefruit or grapefruit juice does not interact with every drug. As we have discovered the mechanism of the grapefruit drug interactions, it was revealed that there are certain characteristics of the drug required for the interactions to happen. These are that the drug has to be mainly metabolized by CYP3A4 and possess low oral bioavailability (Bailey et al., 2013; Ho et al., 2000; Shimomura et al., 2003). Regarding herbal drug interactions, the clinical implications of the interaction is dependent on a variety of factors, such as characteristics of co-administered drugs, the involved CYP enzymes, active constituents of the herb, the applied dosage regimens, and health status of the patients. The drug interactions with St. John's wort represent the best studied drug interactions among the other botanical supplements. The numerous data available revealed that the active ingredient hyperforin has a strong affinity for the pregnane xenobiotic receptor (PXR) and thus acts as a potent inducer of CYP3A4. Consequently, CYP3A4 induction caused by St. John's wort can reduce the oral bioavailability of many drugs making the drug less effective. If a drug with a narrow therapeutic index (e.g. warfarin or cyclosporine A) is involved, the interactions may cause serious or occasionally life-threatening adverse effects. The experience with the St. John's wort case shows that there was so much useful information that we know about this interesting interaction. These include the mechanism of the interaction, the active herbal interaction involved, the particular CYP enzymes induced or inhibited by St. John's wort, etc. This information is very useful and can help us predict herbal drug interactions, thus the adverse drug interactions can be avoided and helps make the use of herbal supplements safe for patients. With respect to other herbal supplements, this comprehensive data have not been completed. For example identification of the major active constituents in herbal products that are responsible for drug interactions with specific herbal supplement is required to be undertaken. Also characterization of the interaction by individual active constituent and mechanism of the interaction need to be studied in detail.

With regard to future research, it is worth to note that there are numerous research on herbal drug interactions that have been conducted *in vitro* and in animals. *In vitro* studies are useful for providing mechanistic information and assessing various components in herbal medicines. In general, characterization of these processes assists prediction of interactions between herbal supplements and conventional drugs that may put the patient's health at risk. Although these experiments are relatively easy to carry out but they suffer from limitations

in extrapolating the results obtained from these studies to clinical situations (Goey et al., 2013; Hermann and von Richter, 2012). It is not uncommon to find that the findings observed from *in vitro* studies were inconsistent with those found in clinical studies. Clinical studies are needed for confirmation and evaluation of the clinical relevance of herbal drug interaction results obtained from *in vitro* studies. Despite the progress having been attained over the previous years, the overall number of clinical studies on herbal drug interactions still seems inadequate. A need for additional well-designed clinical studies is desirable as these will assist in confirming the *in vitro* studies and help assess the clinical significance of these potential interactions. We agree with Hermann and von Richter (2012) that there are numerous interactions studies with warfarin, a narrow therapeutic index drug and a CYP2C9 substrate. Whereas studies with other narrow therapeutic index drugs are scarce. The exceptions to this were for cyclosporine A and antiretroviral drugs. Thus, clinical studies to verify whether herbal drug interactions exist with respect to other narrow therapeutic index drugs are warranted.

There is also limited information on the pharmacokinetics and pharmacodynamics of herbal supplements or herbal medicines per se. Without this comprehensive data, it is difficult to characterize and predict the interactions that may occur. It is also necessary to understand the mechanisms of herbal drug interactions to assist this process. The study of herbal drug interactions is further complicated by the nature of the herbal supplements. The manufacture of herbal supplements or medicines is not subject to the same regulations as prescription drugs. Thus the content of the active ingredients may vary among manufacturers, potentially causing a large variation in efficacy and safety. The ingredients specified on the labels of herbal products may be incomplete or incorrect. The herbal ingredients could also vary from batch to batch due lack of quality control. Even though sources and specification of herbs have been cited in many studies of herbal drug interactions, generally it is very difficult to generalize and compare the results derived from different studies because of this. Often we see contradictory findings obtained from separate investigations. Occasionally, it was suspected that the differences in their results were related to different methology approaches used. However, as the herbal supplements are not precisely regulated, the difference in the active ingredients of the particular herbal supplement used cannot be ruled out. Products from different manufacturers of the same herb may have different chemical compositions and hence different biological actions. It has been suggested that the same rigorous regulations that apply to conventional drugs with regard to quality, safety and efficacy should be required for herbal supplements. There is a distinct necessity for well designed clinical trials, pre-marketing approvals regarding labelling and safety, and comprehensive post-marketing surveillance systems for monitoring the adverse effects of herbal drug interactions.

An important herbal drug interaction is St. John's wort drug interactions. St. John's wort is remarkably problematic in relation to its ability to induce CYP3A4, an enzyme that metabolizes more than 50 % of all prescription medications. The extraordinary effect of St. John's wort clearly shows the caution is needed when this herb is used by patients who are taking other drugs that are metabolized by CYP3A4. Garlic and milk thistle extracts do not cause severe drug interactions in humans. There have been inconsistent results reported on the effects of sylibin, which is one of the active components of milk thistle. Currently, we recognize that many herbal drug interactions are possible; some of these are a serious risk to the health of our patients. Interactions between herbal supplements and drugs often lead to toxicity and loss of therapeutic efficacy. Therefore it is essential that health-

care professionals are well informed about this fast growing field. Consequently healthcare professionals should check if their patients are using herbal supplements. Patients should be encouraged to inform their healthcare providers which herbal supplements or herbal medicines they are taking so that the risk of herbal drug interactions can be prevented or minimized with appropriate management and to ensure that consuming herbal medicines is safe as possible.

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