Effects of Herbal Supplements on Drug Glucuronidation. Review of Clinical, Animal, and *In Vitro* Studies

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Abstract

The use of herbal supplements has increased steadily over the last decade. Recent surveys show that many people who take herbal supplements also take prescription and nonprescription drugs, increasing the risk for potential herb-drug interactions. While cytochrome P450-mediated herb-drug interactions have been extensively characterized, the effects of herbal extracts and constituents on UDP-glucuronosyl transferase (UGT) enzymes have not been adequately studied. Thus, the purpose of this review is to evaluate current evidence on the glucuronidation of phytochemicals and the potential for UGT-mediated herb-drug interactions with the top-selling herbal supplements in the United States and Europe. *In vitro* and animal studies indicate that cranberry, *Ginkgo biloba*, grape seed, green tea, hawthorn, milk thistle, noni, soy, St. John's wort, and valerian are rich in phytochemicals that can modulate UGT enzymes. However, the *in vivo* consequences of these interactions are not well understood. Only three clinical studies have investigated the effects of herbal supplements on drugs cleared primarily through UGT enzymes. Evidence on the potential for commonly used herbal supplements to modulate UGT-mediated drug metabolism is summarized. Moreover, the need for further research to determine the clinical consequences of the described interactions is highlighted.

Introduction

Herbal supplements are commonly used in many countries around the world. Sales of herbal supplements in Europe and the US combined exceed $10 billion annually [1,2]. Survey studies estimate that more than half the German, Danish, and Northeast Brazilian populations and nearly 40% of Australians use herbal supplements [3–5]. Moreover, approximately 80% of German physicians regularly prescribe herbal products [3]. In the US, the herbal supplement market has grown steadily in the last decade. In 2006, Americans spent $4.6 billion dollars on herbal supplements, representing a 4% growth in sales from 2005 [2]. Surveys indicate that about 20% of Americans use at least one herbal supplement. Meanwhile, one in four herbal supplement users takes one or more prescription drugs, raising the potential for herb-drug interactions [6,7]. Additionally, patients with chronic diseases, which are likely to be treated by multiple drugs, use herbal supplements more frequently than the general population, thereby increasing the risk for interactions [8,9]. The top selling herbal supplements in the US and Europe are listed in **Table 1**.

In the last decade, interest in studying the pharmacologic effects of herbal supplements, including their potential to interact with drug metabolizing enzymes, has grown. The number of publications citing herbal supplements has increased by nearly eightfold over the last twenty years, from about 200 to nearly 1600 annual citations in PubMed (www.ncbi.nlm.nih.gov). This upsurge coincided with an escalation in the use of herbal supplements, which has also raised concern by health professionals regarding the potential for herbs to adversely affect drugs pharmacokinetics and pharmacodynamics [10].

Several milestone events have contributed to the increased interest in studying herb-drug interactions as summarized in **Fig. 1**. These events shaped the current widespread use of herbal supplements and highlighted the knowledge gap regarding their safety. In 1994, the United States Congress passed the Dietary Supplement Health and Education Act (DSHEA). Under the provisions of this law, dietary supplements, including herb-

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Table 1 Top selling herbal supplements in the United States and Europe. Source: NBJ's Supplement Business Report, October 2007 [2] and IMS Health 2009 (http://www.imshealth.com).

	US		Europe	
	Top herbs	Sales ($ millions)	Top herbs	Sales ($ millions)
1	Noni juice	257	*Ginkgo biloba*	300
2	Garlic	155	Saw palmetto	114
3	Mangosteen juice	147	Valerian	61
4	Green tea	144	English ivy	54
5	Saw palmetto	134	*Pelargonium sidoides*	52
6	Echinacea	129	Psyllium	50
7	*Ginkgo biloba*	106	Diosmin	45
8	Ginseng	98	Grape seed	43
9	Milk thistle	93	Myrtol	43
10	Psyllium	85	Echinacea	40
11	Soy	69	Milk thistle	40
12	Cranberry	68	St. John's wort	40
13	Maca	66	Sennosides A & B	34
14	Goji	65	Hawthorn	34
15	Green foods	64	Cranberry	33
16	St. John's wort	60		
17	Aloe	60		
18	Stevia	58		
19	Black cohosh	57		
20	Valerian	55		

als, are exempt from regulations applied to drugs, including premarketing safety and efficacy studies [11]. Concurrently, the Internet became widely accessible and was commonly used to market herbal products, which led to an increase in the use of herbal supplements in the mid to late 1990s [12]. In 1998, the Congress established the National Center for Complementary and Alternative Medicine (NCCAM) with the goal of funding research on the safety and efficacy of complementary and alternative medicine, including herbal supplements (nccam.nih.gov). Two years later, a milestone case report published in *The Lancet* described an interaction between St. John's wort, an herbal supplement commonly used for depression, with the immunosuppressant drug cyclosporine [13]. This case report sparked a wave of clinical, *in vitro*, and animal studies addressing St. John's wort interactions with drug metabolizing enzymes and transporters [14]. Meanwhile, reports emerged associating ephedra use with heart attacks; these reports eventually led to a ban in sales of over the counter ephedra-containing products in several countries including the US, Canada, Australia, and Germany (http://www.erowid.org; accessed May 2010). These events set off an alarm that research was needed to characterize the safety of herbal supplements as well as their potential to interact with conventional drugs.

In general, regulation of herbal products is greater in the European Union (EU) than in the US. A 2004 EU directive mandates manufacturers of herbal products to register and license their products by the European Agency prior to marketing. In addition, it mandates premarketing safety evaluations as well as postmarketing surveillance for serious adverse events [1]. In the US, the scientific community has recently requested that the FDA play a more rigorous role in evaluating safety and efficacy of herbal supplements with calls for premarketing safety data and studies on interactions with drug metabolizing enzymes [15].

Several case studies, reports, and review articles have described the potential of herbal supplements and phytochemicals to modulate cytochrome P450 (CYP) enzymes. Conversely, the effect of herbal extracts on glucuronidation, a major conjugative metabolism pathway, has not been sufficiently studied. The aim of this review is to summarize evidence regarding the potential of the top-selling herbal supplements in the US and Europe to interact with UGT enzymes.

Potential for Herb-Drug Interactions through Drug Metabolizing Enzymes

Enzymatic biotransformation (i.e., metabolism) plays a major role in the disposition of endogenous and exogenous compounds including both drugs and herbal constituents. Biotransformation reactions are generally divided into phase I and phase II reactions, each of them encompassing a wide range of enzymes and catalytic activities [16]. Phase I reactions involve hydrolysis, reduction, and oxidation and usually result in only a small increase in hydrophilicity [17]. In phase I, CYP enzymes rank first in terms of clinical importance and number of substrates. On the other hand, phase II reactions include conjugation of compounds with

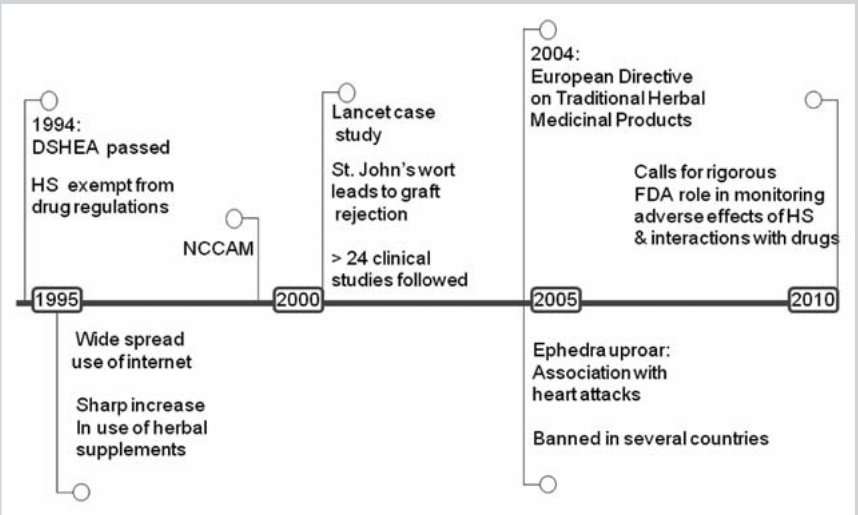

Fig. 1 Timeline for milestone events that have increased interest in studying herb-drug interactions. Abbreviations: DSHEA, Dietary Supplement Health and Education Act; HS, Herbal Supplements; NCCAM, the National Center for Complementary and Alternative Medicine; FDA, the Food and Drug Administration.

a hydrophilic group producing a more hydrophilic and easily excreted product (except for acetylation and methylation). Phase II reactions may or may not be preceded by phase I reactions. For some substrates, such as morphine and mycophenolic acid, phase II conjugation with glucuronic acid represents the primary metabolic pathway [17].

Herbal supplements contain a myriad of natural chemicals that share the same metabolic pathways with prescription drugs [18]. This may result in activation or inhibition of the metabolism of concomitantly taken drugs, under- or overexposure to drugs, and consequently, treatment failure or toxicity. At least 30 clinically proven herb-drug interactions mediated through CYP enzymes have been described [19–21]. Induction of CYP2C19, for example, by *Ginkgo biloba* resulted in subtherapeutic levels of anticonvulsant drugs, which precipitated fatal seizures [22]. St. John's wort has the most documented evidence of pharmacokinetic drug interactions with more than 100 publications in the last 10 years on its interactions with prescription drugs [20]. For example, induction of CYP3A4 and P-glycoprotein by St. John's wort resulted in decreased exposure to midazolam (↓ 44%), tacrolimus (↓ 59%), alprazolam (↓ 52%), verapamil (↓ 80%), and cyclosporine A (↓ 52%) [23]. In contrast, interactions through glucuronidation have not been adequately characterized.

Glucuronidation enzymes

Conjugation with glucuronic acid (glucuronidation) represents the main phase II reaction and one of the most essential detoxification pathways in humans [24]. The UDP-glucuronosyl transferases (UGT) are a superfamily of 18 different enzymes divided into two families, UGT1 and UGT2, and three subfamilies, UGT1A, 2A, and 2B based on sequence homology (**Fig. 2**) [25]. UGT enzymes are widely and differentially expressed throughout the human body [26]. Although the majority of UGT enzymes are expressed in the liver, UGT1A7, 1A8, and 1A10 are expressed exclusively extrahepatically, mainly in the intestine [27,28]; UGT1A9, 2B7, and 2B11 are expressed at relatively high amounts in the kidney. **Fig. 2** depicts the difference in UGT expression between the liver and intestine, which are the main sites for xenobiotic glucuronidation.

Glucuronidation as a pathway for drug interactions

Several reports document the clinical significance of interactions through UGT enzymes. The glucuronidation pathway has been frequently described as a low affinity pathway, with a relatively small impact on substrate exposure *in vivo* as a result of inhibition [29,30]. This has been observed for substrates that have alternative metabolic pathways and relatively low affinity for UGT enzymes. However, if the substrate is metabolized mainly through glucuronidation, inhibition can result in a significant increase in exposure. For example, exposure to zidovudine, a substrate for UGT2B7, increased by 31% and 74% due to inhibition of glucuronidation by atovaquone and fluconazole, respectively [31,32]. Moreover, rash, which could be life-threatening, resulted from inhibition of lamotrigine N-glucuronidation by valproic acid [33]. In addition to inhibition, interactions with glucuronidation can occur through induction of UGT enzymes. Studies have reported that rifampicin and lopinavir/ritonavir induced lamotrigine glucuronidation, which required a doubling of the dose to maintain a therapeutic plasma concentration [34,35]. These examples show that drug-drug interactions through modulation of glucuronidation can be clinically significant. Similarly, since many phytochemicals are substrates for UGT enzymes, herb-drug interactions may occur through this pathway.

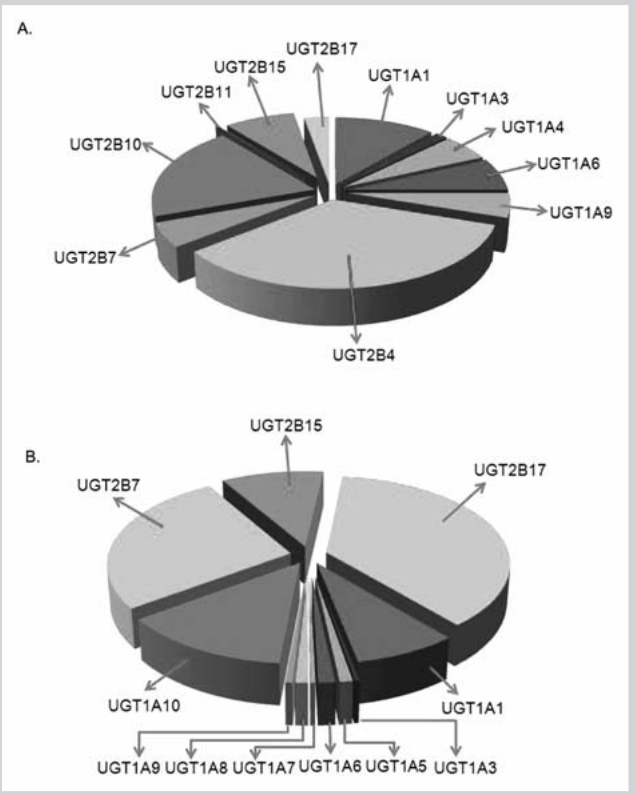

Fig. 2 Expression of UGT enzymes in the liver and the small intestine. **A** Relative expression of hepatic UGT enzyme based on 20 human liver samples. Adapted from Izukawa et al. [28]. **B** Relative expression of UGT enzymes in the small intestine based on 3 human intestine samples. Adapted from Ohno and Nakajin [27].

Search strategy

Systematic literature searches were conducted in MEDLINE (through PubMed) and Google Scholar databases through March 2010. The search terms used were each of the top-selling herbal supplements in the US and Europe (**Table 1**) or their main secondary metabolites in combination with the terms "glucuronidation" or "UGT". Only articles written in English were included. No other restrictions were imposed. When interactions are reported from *in vitro* or animal studies, the possibility of an *in vivo* interaction is discussed based on expected *in vivo* concentrations of phytochemicals, the conditions used in the experiments, and the observed inhibition potency (**Table 2**).

Herbal Medicines Containing Substrates or Modulators of UGT Enzymes

Aloe

Aloe vera leaf extract is used as an herbal supplement due to its attributed biological benefits, including antiviral, antibacterial, laxative, and immunostimulatory effects [36]. It contains several classes of phytochemicals that have been thoroughly described [37]. Among the different classes, *Aloe vera* extract is rich in anthracene derivatives including aloe-emodin. There is evidence that glucuronidation is the primary route of metabolism of aloe-

Table 2 Summary of studies on glucuronidation of phytochemicals and modulation of UGT enzymes by phytochemicals and herbal extracts.

Herb	Phytochemicals studied for glucuronidation (UGT enzymes involved)	Interaction studies			References
		In vitro	Animal	Clinical	
Aloe	Aloe-emodin (uncharacterized)				[38]
Cranberry	Quercetin (UGT1A3, UGT1A9) Resveratrol (UGT1A1, UGT1A9)	Quercetin: ↑ UGT2B17 ↓ UGT1A1 ↓ UGT1A9	Quercetin: ↑ UGT (nonspecific)		[42–46, 48, 54, 65]
Diosmin	Diosmin (uncharacterized)				[61, 62]
Echinacea	Echinacoside (uncharacterized)	Echinacea extract: ↓ UGT1A1			[64, 65]
Garlic				↔ UGT1A6	[67]
*Ginkgo biloba**	Flavonoids (UGT1A3, UGT1A9)	Ginkgo extract: ↓ UGT1A9 Flavonoids: ↑ UGT2B17 ↓ UGT1A1 ↓ UGT1A9	Flavonoids: ↑ UGT (nonspecific)		[42–46, 48]
Ginseng	ND			↔ UGT2B7	[76–78]
Grape seed	Flavonoids (UGT1A3, UGT1A9) Resveratrol (UGT1A1, UGT1A9) Catechins (uncharacterized)	Flavonoids: ↑ UGT2B17 ↓ UGT1A1 ↓ UGT1A9	Flavonoids: ↑ UGT (nonspecific)		[42–46, 48, 54, 56, 80]
Green tea*	EGCG >> EGC (UGT1A1, UGT1A8, UGT1A9)	Polyphenols: ↓ UGT1A EGCG: ↓ UGT1A1	Green tea extract: ↑ UGT1A		[65, 84–86]
Hawthorn	Quercetin (UGT1A3, UGT1A9) Epicatechin (uncharacterized)	Quercetin: ↑ UGT2B17 ↓ UGT1A1 ↓ UGT1A9	Quercetin: ↑ UGT (nonspecific)		[44–46, 48, 94]
Mangosteen	α-Mangostin (uncharacterized)				[96]
Milk thistle*	Flavonolignans (uncharacterized)	Milk thistle extract: ↓ UGT1A1		↔ UGT1A1	[44, 65, 100–103]
		Silymarin & silybin: ↓ UGT1A1 ↓ UGT1A6 ↓ UGT1A9			
		Silybin: ↓ UGT2B7 ↓ UGT2B15			
Noni juice			Noni juice: ↓ UGT (nonspecific)		[105]
Soy*	Isoflavones (genistein and daidzein: UGT1A1, UGT1A4, UGT1A6, UGT1A7, 1A9; genistein: UGT1A10)	Genistein: ↓ UGT1A1 Daidzein: ↑ UGT1A1 Soy extract: ↓ UGT2B15	↔ UGT (nonspecific)		[107–108, 110, 112]
St. John's wort*	Quercetin (UGT1A3, UGT1A9)	SJW extract: ↓ UGT1A1 Quercetin: ↑ UGT2B17 ↓ UGT1A1 ↓ UGT1A9 Hypericin: ↓ UGT1A6	SJW extract: *Long-term*: ↓ Irinotecan & SN-38 C_{max} *Short-term:* ↓ SN-38 glucuronide $AUC_{0-\infty}$ & $t_{1/2}$ Quercetin: ↑ UGT (nonspecific)		[42–46, 48, 115, 117]
Valerian*		Valerian & valerenic acid: ↓ UGT1A1 ↓ UGT2B7 ↓ UGT (nonspecific)			[121]

* Effects on glucuronidation by these herbal extracts could potentially translate *in vivo* – further studies are warranted. Uncharacterized: there is evidence that the phytochemicals are glucuronidated; however, the UGT enzymes involved have not been characterized. ND: Metabolism of the herb or its phytochemical was studied, but no glucuronides detected. ↓, inhibition of UGT; ↑, activation or induction; ↔, no effect on UGT activity

emodin in rats [38]. *In vitro* characterization of aloe-emodin glucuronidation has not been performed.

Cranberry

Cranberry (*Vaccinium macrocarpon*) is often used to prevent urinary tract infections and has potential antibacterial and anticancer activity [39]. Cranberry juice has a high content of flavonoids, catechins, and other phenolic compounds. Although no studies investigated the effects of cranberry juice on UGT enzyme activities, some information is available on the effects of quercetin – the most abundant flavonoid in cranberry [39].

Quercetin content in cranberry is estimated to be between 83 and 121 mg/kg (about 50 µg in a 500 mg cranberry supplement capsule) [40]. Quercetin is conjugated by UGT1A9 and, to a lesser extent, UGT1A3 [41–43]. Studies on the activity of quercetin on UGT enzymes show mixed effects. In two independent studies using human liver microsomes (HLM), quercetin inhibited UGT1A1 (IC_{50} value > 50 µM) and UGT1A9 activities (IC_{50} value = 19.1 µM) [44,45]. On the other hand, treatment with 5 µM quercetin increased testosterone glucuronidation (primarily catalyzed by UGT2B17) by almost 2.5-fold in a prostate cancer cell line [45–47]. In addition, a study in rats showed that 2-week intake of quercetin (1% w/w in diet) induced p-nitrophenol glucuronidation by 1.5- to 4-fold in rat liver and different parts of the intestine [48]. p-Nitrophenol is a nonselective UGT substrate; therefore, it is not clear which UGT enzymes were induced by quercetin and whether this effect may translate to humans [49–51].

In vivo concentrations of quercetin following cranberry intake are unlikely to reach inhibitory levels. A pharmacokinetic study showed that the maximum plasma concentration (C_{max}) of quercetin aglycone was 15.4 ng/mL (equivalent to 51 nM) following oral intake of 500 mg quercetin [52]. Taking into account the relatively low content of quercetin in cranberry supplements (about 50 µg in a 500 mg capsule), *in vivo* concentrations of quercetin from cranberry are not expected to be close to the reported IC_{50} values [44,45].

In addition to flavonoids, cranberry juice contains resveratrol, which is also found in grapes and red wine [45,53]. *In vitro* studies show that resveratrol is glucuronidated to two major glucuronide conjugates, resveratrol-3′-glucuronide and resveratrol-4′-glucuronide. The major enzymes that catalyze resveratrol glucuronidation are UGT1A1 and UGT1A9 [54–56]. No studies on the effects of resveratrol on UGT enzyme activities were found.

Diosmin

This glycosylated flavonoid is found in citrus, hyssop, and rosemary and is commonly used for its venotonic effects [57–59]. In Europe, it is prescribed for treatment of venous insufficiency while in the US, it is marketed as a food supplement [60,61]. A study in rat liver perfusate showed that diosmin was mainly excreted in the bile as a glucuronide conjugate [62]. Glucuronides of diosmetin (diosmin aglycone) were detected on the apical side of Caco-2 cell culture [61]. Specific UGT enzymes associated with diosmin glucuronidation have not been reported. Effect of diosmin on drug glucuronidation is yet to be determined.

Echinacea

Echinacea products refer to herbs or roots of *Echinacea purpurea, Echinacea angustifolia*, or *Echinacea pallida*, or a combination thereof [63]. The herbs and roots of these different species have different composition and medicinal properties. Among the common compounds in *Echinacea* are polyphenolic compounds including cichoric acid and echinacoside. Jia et al. studied phase II metabolites of echinacoside in rats and isolated two glucuronide metabolites for echinacoside [64]. More *in vitro* studies using HLM or expressed UGT enzymes are needed to characterize the relative contribution of individual UGT enzymes to *Echinacea* metabolism. We recently showed that *Echinacea* extract weakly inhibited UGT1A1 with an IC_{50} of 211.7 µg/mL [65]. Such weak inhibition is not expected to have clinical significance on the metabolism of UGT1A1 substrates.

Garlic

Garlic (*Alium sativum*) bulbs have been used for over 4000 years as a medicinal plant to treat a variety of ailments including headache, bites, intestinal worms, and tumors [66]. Garlic is rich in organo-sulphur compounds such as alliin, and γ-glutamylcysteines, diallyl sulphide, diallyl disulphide, and others [66]. These compounds are not known to be substrates for glucuronidation. Gwilt et al. [67] studied the effect of garlic on acetaminophen metabolism in healthy subjects. Subjects were given 10 mL garlic extract daily (equivalent to six to seven cloves of garlic) for three months. Garlic consumption did not have a significant effect on acetaminophen or acetaminophen glucuronide pharmacokinetic parameters.

Ginkgo

Ginkgo (*Ginkgo biloba)* leaf extract is commonly used for its perpetual benefits on memory and circulation. The primary active constituents of ginkgo are terpene lactones (ginkgolides and bilobalide) and flavone glycosides, which are hydrolyzed *in vivo* to flavone-aglycones (e.g., quercetin, kaempferol, and isorhamnetin) [68]. Ginkgo flavonoids are substrates for intestinal and hepatic UGT enzymes, primarily UGT1A9 and, to a lesser extent, UGT1A3 [41–43].

There is *in vitro* and animal evidence that ginkgo and its flavonoids modulate UGT enzymes. As mentioned under cranberry, quercetin showed inhibition *in vitro* of UGT1A1 and 1A9 in addition to induction of p-nitrophenol glucuronidation in rats [44,45,48]. In a prostate cell line, both quercetin and kaempferol induced testosterone glucuronidation *in vitro* by 2.5- and 4-fold, respectively [46]. Testosterone is metabolized primarily by UGT2B17, which plays an important role in androgen metabolism but not in drug metabolism [69]. We recently showed that unhydrolyzed and acid-hydrolyzed ginkgo extracts, quercetin, and kaempferol inhibit mycophenolic acid (MPA) glucuronidation in human liver and intestine microsomes [45]. Inhibition of intestinal glucuronidation was 4- to 12-fold more potent than hepatic glucuronidation. MPA is an immunosuppressive drug that is metabolized in the liver by UGT1A9 and in the intestine by UGT1A7, 1A8, 1A9, and 1A10 [70–72]. In HLM, IC_{50} values for inhibition by quercetin and kaempferol were 19.1 and 23 µM which are many fold higher than the expected plasma C_{max} of flavonoids [52]. Therefore, inhibition of systemic MPA metabolism *in vivo* is unlikely. On the other hand, IC_{50} values in human intestine microsome incubations were 5.8 µM and 7.6 µM for quercetin and kaempferol, respectively. These concentrations are attainable in the intestine based on an estimated intestine fluid volume of 0.5 to 5.0 L [73]. Therefore, concomitant intake of ginkgo extract with MPA may result in inhibition of first-pass MPA metabolism, which accounts for clearance of about 30% of the dose of mycophenolate sodium [74]. Further clinical studies are warranted to evaluate the clinical significance of this *in vivo* interaction.

Ginseng

Ginseng typically refers to roots of *Panax ginseng* or *Panax quinquefolium*, which are used as general tonics and adaptogens [75]. The most important bioactive components contained in ginseng are a group of saponins called ginsenosides [75]. No reports of ginsenosides glucuronidation were found in the literature. In a pharmacokinetic study in which ginsenoside Rd was administered intravenously to volunteers, no glucuronide conjugates were detected in plasma [76]. Another *in vitro* study on metabolism of ginsenoside Rg3 using rat S9 liver fraction did not detect any glucuronidated metabolites [77]. In a pharmacokinetic interaction study, 10 healthy volunteers received 300 mg of zidovudine, a UGT2B7 substrate, orally before and after 2 weeks of treatment with 200 mg American ginseng extract twice daily. American ginseng did not significantly affect the pharmacokinetic parameters of zidovudine or zidovudine glucuronide [78].

Grape seed

Grape seed extract (*Vitis vinifera*) contains phenolic and polyphenolic compounds including flavonoids (kaempferol, quercetin, and myricetin), resveratrol, (+)-catechins, (−)-epicatechin, and (−)-epicatechin-3-O-gallate [79]. As mentioned, quercetin and kaempferol are substrates of UGT1A3 and UGT1A9 and *in vitro* modulators for UGT1A1, UGT1A9, and UGT2B17 [42–46,48]. However, based on flavonoid content in grape seeds of 4 to 5%, *in vivo* quercetin and kaempferol concentrations are not likely to reach inhibitory levels [79]. Resveratrol is also a substrate for UGT1A1 and UGT1A9 enzymes, but no information is available on its potential to modulate UGT enzymes [54,55]. In rats, catechin glucuronides were the primary existing form of catechins in plasma following oral grape seed extract administration [80]. Taken together, grape seed extract is rich in phytochemicals that are substrates for UGT enzymes. No studies were found on the potential of grape seed extract to modulate UGT-mediated drug metabolism.

Green tea

Green tea (*Camellia sinensis*) has gained increased popularity as a beverage and an herbal supplement with many attributed health benefits including reduction in the risk of cardiovascular disease and certain cancers [81]. Green tea extract is rich in polyphenolic compounds – mainly catechins. The major green tea catechins are: (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate, (−)-epigallocatechin (EGC), (−)-epicatechin, (+)-gallocatechin, and (+)-catechin [82]. EGCG is believed to be the most biologically active and most abundant catechin in green tea extract [83]. *In vitro*, animal, and human studies provide evidence that green tea catechins are metabolized by methylation, sulfation, and glucuronidation [83]. Lu et al. reported that EGCG was conjugated by UGT1A1, 1A8, and 1A9 and that glucuronidation of EGCG was much higher than EGC [84].

In terms of interactions, there is evidence from animal and *in vitro* studies that green tea extract modulates UGT enzyme activity. A study in rats showed that consumption of green tea extract (at concentrations of 2.5, 5.0, and 7.5%) for four weeks enhanced hepatic glucuronidation of 2-aminophenol [85]. However, the effect was not dose-dependent and 2-aminophenol is not a selective substrate for any specific UGT enzyme. So, it is not clear what impact this effect may have in humans. Zhu et al. investigated the effect of administration of green tea extract for 18 days on hepatic glucuronidation activity in female Long-Evans rats. Green tea extract stimulated liver microsomal glucuronidation of estrone, estradiol, and 4-nitrophenol by 30–37%, 15–27%, and 26–60%, respectively [86]. The same authors reported that green tea polyphenols, including EGCG, inhibited estradiol and estrone glucuronidation *in vitro* using rat liver microsomes with IC_{50} values of 10–20 µg/mL [86]. In agreement with these findings, we recently showed that EGCG inhibited estradiol-3-*O*-glucuronidation, an index for UGT1A1 activity, in HLMs [65,87]. The IC_{50} value was 7.8 µg/mL, which is similar to the IC_{50} reported earlier in rat microsomes [86]. To understand the potential clinical significance of this *in vitro* interaction on drugs metabolized by UGT1A1, the reported IC_{50} value should be compared to expected *in vivo* concentrations following green tea intake. EGCG C_{max} following a green tea dose containing 800 mg EGCG was 2.5 µg/mL which is about threefold lower than the *in vitro* IC_{50} [88]. On the other hand, intestinal concentrations of EGCG are expected to be much higher than the observed IC_{50} concentrations. Therefore, inhibition of first pass metabolism of UGT1A1 substrates is more likely than inhibition of systemic metabolism. Examples of drugs cleared primarily by intestinal first pass UGT1A1 include raloxifene and ezetimibe [89]. Mirkov et al. investigated the effects of green tea catechins on the glucuronidation of SN-38, the active metabolite of the anticancer drug irinotecan, and a UGT1A1 substrate [90]. In the latter study, green tea catechins inhibited the glucuronidation of SN-38 in HLM incubations in a concentration-dependent manner. However, in human hepatocytes, a significant decrease in SN-38 glucuronide was observed in only 33% (EGCG), 44% (ECG), and 44% (EGC) of the hepatocyte preparations. Therefore, the authors concluded that at pharmacologically relevant concentrations, catechins are unlikely to inhibit the formation of inactive irinotecan metabolites when administered concomitantly [90].

Hawthorn

Hawthorn (*Crataegus oxyacantha*) extract is used in Europe for its cardiotonic effects [91]. Hawthorn leaf, flower, and berry extracts are rich in flavonoids and oligomeric catechins that are thought to be responsible for pharmacologic activity [91]. Flavonoids include quercetin, isoquercitrin, rutin, hyperoside, and vitexin; and epicatechin [92,93]. Quercetin and epicatechin are known to be substrates for glucuronidation [41–43,83,94]. Quercetin is an *in vitro* modulator of UGT1A1, 1A9, and 2B17 enzymes as described above [44–46,48]. No studies were found on the effects of hawthorn extract on UGT enzymes.

Mangosteen juice

Mangosteen (*Garcinia mangostana*) juice is well-known for its anti-inflammatory properties and is traditionally used in the treatment of skin infections and wounds [95]. Mangosteen juice is rich in phenolic compounds called xanthones, mainly *α*, *β*, and *γ*-mangostin [95]. Bumrungpert et al. showed that *α*-mangostin was conjugated by phase II enzymes in caco-2 cells [96]. In their study, one third of *α*-mangostin was conjugated after 4–6 hours of incubation with cells. Conjugation was measured by hydrolysis using a *Helix pomatia*-derived enzyme that possesses both glucuronidase and sulfatase activity. Therefore, it was not possible to determine the relative contribution of glucuronidation and sulfation. No studies were found on the effects of mangosteen juice or its phytochemicals on drug glucuronidation.

Milk thistle

Milk thistle (*Silybum marianum*) is used to treat hepatotoxicity [97]. Extract of milk thistle is rich in flavonolignans, primarily silybin, silydianin, and silychristine, which are collectively known as silymarin [98]. There is evidence on glucuronidation of silymarin flavonolignans from both animal and human studies. In a study in rats, silybin A, silychristin, and silydianin were excreted as glucuronides [99]. Moreover, silibinin mono- and di-glucuronides were detected in human plasma following ingestion of silibinin phytosome capsules in colorectal carcinoma patients [100]. *In vitro* experiments showed inhibitory effects of milk thistle compounds on UGT enzymes. In human hepatocytes, silymarin inhibited glucuronidation of 4-methylumbelliferone, a substrate for all UGT1A and 2B enzymes except UGT1A4, by about 80% and 90% at concentrations of 100 and 250 µM, respectively [101]. However, the use of a nonselective substrate and relatively high concentrations of silymarin limit the clinical utility of this finding. In another study, silybin inhibited recombinant UGT1A1, 1A6, 1A9, 2B7 and 2B15 with IC_{50} values of 1.4, 28, 20, 92, and 75 µM, respectively using 7-hydroxy-4-(trifluoromethyl)coumarin as a substrate for the different UGT enzymes [102]. In an *in vitro* study using HLM and estradiol-3-*O*-glucuronidation as an index for UGT1A1 activity, silymarin inhibited UGT1A1 at estradiol concentrations of 50 and 100 µM, while results at lower concentrations showed mixed inhibition and activation [44]. We recently showed that milk thistle extract inhibited estradiol-3-*O*-glucuronidation in HLM with IC_{50} value of 30.4 µg/mL which is equivalent to 11.5 µg/mL flavonolignans [65].

To understand the potential *in vivo* effects of milk thistle on drug glucuronidation, inhibitory concentrations should be compared to expected *in vivo* levels. C_{max} of total flavonolignans was 24 ng/mL following intake of 600 mg milk thistle extract. Thus, plasma concentrations of flavonolignans are not expected to reach IC_{50} concentrations reported by our group and others [65, 102]. Therefore, milk thistle intake is not expected to affect systemic metabolism of UGT substrates. In agreement with this conclusion, 4-day and 12-day administration of milk thistle showed no significant effects on the pharmacokinetics of the intravenous anticancer drug irinotecan in cancer patients [103]. In contrast, intestinal concentrations of milk thistle flavonolignans are likely to be higher than the observed IC_{50}. Based on a range of intestinal volume of 0.5 to 5.0 L, the expected intestinal concentration of milk thistle extract is 40 to 1200 µg/mL following intake of 200 to 600 mg of milk thistle [73]. Therefore, further research is warranted on the effect of milk thistle on drugs cleared primarily by first pass glucuronidation – particularly substrates of UGT1A1, 1A6, and 1A9, which showed the lowest IC_{50} values [65, 102].

Noni juice

Noni juice (*Morinda citrifolia*) has a long history of being used for a wide range of indications including hypertension, menstrual cramps, gastric ulcers, and many others [104]. Noni juice contains several classes of secondary metabolites, including polysaccharides, fatty acid glycosides, iridoids, anthraquinones, and flavonoids [104]. Many of these are phenolic compounds that could be substrates for UGT enzymes and may compete with the metabolism of drugs. However, no studies were found regarding the glucuronidation of compounds in noni juice. In a study in rats, noni juice inhibited *ex vivo* p-nitrophenol glucuronidation by 39% at a dose of 21 mg/kg following 1 day of treatment and by 35% and 49% after 14 days of treatment at doses of 2.1 and 21 mg/kg, respectively. However, there was no inhibition at a higher dose of 210 mg/kg [105]. Further research is warranted to investigate the potential of noni juice to alter drug metabolism in humans.

Soy

There has been increasing interest in soy isoflavones, especially genistein and daidzein, due to their wide range of potential biological activities [106]. *In vitro* and clinical studies provide evidence that soy isoflavones are substrates for UGT enzymes. Despite being structurally similar, genistein and daidzein conjugates exhibit preferences for different UGT enzymes. UGT1A1, 1A4, 1A6, 1A7, and 1A9 catalyzed 7- and 4′-glucuronidation of both genistein and daidzein, while UGT1A10 was selective for genistein. The authors also reported that genistein, but not daidzein, was conjugated in human colon microsomes [107]. The glucuronide was the predominant circulating form for both genistein (69–98%) and daidzein (40–62%), with smaller amounts of the aglycone and sulfate. This indicates that glucuronidation is the primary route of metabolism for these soy isoflavones.

Pfeiffer et al. reported that daidzein and genistein as well as several structurally related isoflavones modulated UGT1A1 activity *in vitro* using HLM [108]. Daidzein (25 µM) stimulated estradiol-3-*O*-glucuronidation, a marker for UGT1A1 activity, by about 50%; however, inhibition was observed at higher daidzein concentrations [87]. In contrast, genistein (25 µM) inhibited the 3-*O*-glucuronidation by about 80%. The 17-glucuronidation of E2 – which is catalyzed by several UGT enzymes – was not affected by either compound. The observed modulatory effects on estradiol metabolism generated interest in the anticancer properties of soy isoflavones [108, 109]. However, implications of these effects on the metabolism of UGT1A1 drug substrates have not been explored. The potency of estradiol-3-*O*-glcuruonidation inhibition by genistein was higher than the activation by daidzein. Thus, it would be expected that the net effect of soy extract on UGT1A1 activity will be inhibition. An earlier study by Anderson et al. showed that soy extract was a weak inhibitor of estradiol glucuronidation (IC_{50} > 100 µg/mL) [110]. However, it is important to note that the authors investigated the effect of soy extract on formation of all estradiol glucuronides, which is not a selective measure for UGT1A1 activity. Therefore, further studies are warranted to determine the effect of soy isoflavones on the metabolism of UGT1A1 drug substrates. Since soy is being studied for use in cancer patients, its effects on metabolism of anticancer drugs like irinotecan is especially important. In the latter study by Anderson et al., unhydrolyzed and hydrolyzed soy extracts inhibited dihydrotestosterone glucuronidation, an index for UGT2B17 activity, with IC_{50} values for soy isoflavones of 4.6 and 6.1 µg/mL, respectively [47, 110]. Although these concentrations are close to reported *in vivo* concentrations of genistein (16.3 µM; equivalent to 4.4 µg/mL), UGT2B17 is not known to play an important role in drug metabolism [69, 111].

In a study in mice, genistein and daidzein only slightly decreased UGT activities in some tissues in a sex- and duration-dependent manner [112]. In this study, genistein and daidzein inhibited glucuronidation of 3-methyl-2-nitrophenol in the small intestine of male mice after five days of isoflavone administration by about 50% and 40%, respectively. This effect was not reproducible in the liver and the kidneys, or in female mice. Glucuronidation of the substrate used in the study (3-methyl-2-nitrophenol) has not been characterized; therefore, the clinical applicability of this information is limited.

St. John's wort

St. John's wort (*Hypericum perforatum*) extract is used for insomnia and depression [113]. Flavonol glycosides are the major class of compounds found in St. John's wort extract, with rutin, hyperoside, isoquercitrin, quercitrin (quercetin 3-rhamnoside), and miquelianin being the main compounds. Other components include hypericin, pseudohypericin, and hyperforin [114]. As explained above, quercetin is a known substrate and modulator of UGT1A enzymes [42–46,48]. No studies regarding glucuronidation of other St. John's wort components were found.

In vitro and animal studies show that St. John's wort could modulate UGT enzyme activity. In a recent study, Volak reported that hypericin inhibited UGT1A6-mediated glucuronidation of acetaminophen in human colon cells and serotonin in UGT1A6-expressing insect cells with IC_{50} values of 7.1 and 0.59 µM, respectively (equivalent to 13.6 µg/mL and 0.3 µg/mL, respectively) [115]. The authors concluded that the mechanism of this interaction was through inhibition of UGT1A6 phosphorylation by protein kinase C, which is considered a novel mechanism of drug-drug interaction. The observed IC_{50} values are much higher than the reported plasma C_{max} of hypericin following oral intake of 900 mg St. John's wort extract (C_{max} = 3.8 ng/mL) [116]. Therefore, the translation of this observation into a clinical *in vivo* interaction is unlikely.

In an animal study, the effects of St. John's wort on irinotecan pharmacokinetics were measured after 3 and 14 days of daily St. John's wort administration [117]. Long-term (14-day) exposure to St. John's wort significantly decreased C_{max} of irinotecan by 39.5% and SN-38 by 38.9%, but did not significantly affect SN-38 glucuronide plasma concentrations. On the other hand, short-term (3-day) administration of St. John's wort did not significantly alter the pharmacokinetics of irinotecan and SN-38, but decreased the $AUC_{0-\infty}$ and the elimination $t_{1/2}$ of SN-38 glucuronide by 31.2% and 25.8%, respectively [117]. In the same study, St. John's wort extract (5 µg/mL) decreased SN-38 glucuronidation by 45% in rat liver microsomes, while preincubation of St. John's wort extract in hepatoma cells significantly increased SN-38 glucuronidation. Although rat UGT enzymes differ from human enzymes in substrate affinity, these results indicate that St. John's wort may affect pharmacokinetics of SN-38 in humans [118,119]. This may lead to increased exposure to irinotecan and SN-38 and, consequently, increased risk of adverse reactions including neutropenia and thrombocytopenia.

Valerian

Valerian (*Valeriana officinalis*) extract is used to treat sleeping disorders, restlessness, and anxiety [120]. Alkaloids, organic acids, terpenes, and valepotriates are among the major classes of phytochemicals found in valerian extract. In terms of interactions with UGT enzymes, valerian methanolic extract inhibited UGT1A1 and UGT2B7 in HLM using estradiol and morphine as probe substrates, respectively. In the same study, valerenic acid, a monoterpene in valerian extract, inhibited glucuronidation of acetaminophen, estradiol, and morphine with both HLM and expressed UGT enzymes [121]. IC_{50} values for inhibition with valerenic acid were 9.24 µM for acetaminophen glucuronidation, 8.79 µM for estradiol-3-*O*-glucuronidation, 2.33 µM for estradiol-17-*O*-glucuronidation, 4.96 µM for morphine-3-glucuronidation, and 47.31 µM for testosterone glucuronidation. All the observed IC_{50} values were higher than the reported C_{max} following a single dose of valerian of 600 mg (C_{max} = 2.3 ng/mL) [122]. Based on intestinal fluid volume of 0.5 to 5.0 L, valerenic acid concentrations in the intestine could fall between 0.8 to 16 µg/mL following intake of 500 to 1000 mg of valerian extract. Thus, IC_{50}-equivalent concentrations are more likely to be attained in the intestine rather than the blood following valerian intake. Therefore, the effects of valerian extract on intestinal glucuronidation warrant further studies.

Conclusion and Summary

The studies reviewed provide evidence on the potential for modulation of UGT-mediated drug metabolism by commonly used herbal supplements and highlight the need for further studies. Flavonoid compounds were the most studied class of phytochemicals for metabolism by and interactions with UGT enzymes. Based on *in vitro* and animal studies, flavonoid-rich supplements may affect metabolism of UGT drug substrates. Many phytochemicals are known to be substrates for glucuronidation; however the UGT enzymes involved in their metabolism are not characterized. These include aloe-emodin, resveratrol, diosmin, echinacoside, α-mangostin, and milk thistle flavonolignans.

Characterization of UGT enzymes involved in the metabolism of phytochemicals would help identify the potential for competitive inhibition of drug glucuronidation if catalyzed through the same enzymatic pathway. Despite many *in vitro* and animal studies on potential modulatory effects of herbal supplements on UGT enzymes, the clinical significance of these effects is poorly understood. Only three published clinical studies investigated the potential of herbal extracts to affect pharmacokinetics of drugs metabolized primarily by UGT enzymes [67,78,103].

Considering the worldwide popularity of herbal supplements and the development of herbal formulations with enhanced bioavailabilities, an increase in incidence of herb-drug interactions is predicted [123]. This review highlights the lack of sufficient information to assess the safety of taking herbal supplements with drugs metabolized primarily by UGT enzymes. Further studies are needed to characterize the glucuronidation of phytochemicals and their potential to interact with UGT-mediated drug metabolism.

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