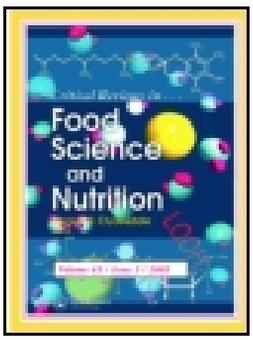

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Dietary isoflavones as modulators of drug metabolizing enzymes and transporters: Effect on prescription medicines

Isha Taneja[1,2], Kanumuri Siva Rama Raju[1,2], Wahajuddin[1,2*]

[1]Academy of Scientific and Innovative Research, New Delhi, India. [2]Pharmacokinetics and Metabolism Division, Lucknow, India.

***Corresponding author**

Dr. Wahajuddin,

Scientist, CSIR-Central Drug Research Institute, Lucknow-226031, Uttar Pradesh, India

Tel.: +91-522-2772450/2550 (Ext-4849/50); fax: +91-522-2771941

E-mail address: wahajuddin@cdri.res.in / wahajuddin@gmail.com

Abstract

Isoflavones are the most widely consumed phytoestrogens. Besides being a dietary constituent, their consumption has been increasing in the form of herbal supplements and as promising alternatives to hormonal replacement therapy, in conjunction with prescription medicines. Isoflavones are extensively metabolized by phase I and II enzymes and are substrates of drug transporters. At high concentrations isoflavones may interact with drug metabolizing enzymes and drug transporters and modulate their activity, thus, altering the absorption, metabolism, distribution, excretion and toxicity profile of the co-administered drugs. This review summarizes the up-to-date literature of isoflavone-drug interactions giving insight into the possible

mechanisms of interactions, *in vitro-in vivo* correlation and their implications on clinical outcomes.

Keywords

Drug interaction; induction; inhibition; cytochrome P450

Introduction

Isoflavones are polyphenolic compounds found exclusively in plants belonging to family, Leguminosae, particularly in subfamily, papilionoideae. These are plant secondary metabolites produced via phenylpropanoid pathway and possess a 3-phenylchromen-4-one nucleus. Isoflavones is the most frequently occurring class of isoflavonoid compounds (Veitch, 2013). Isoflavones naturally do not occur as native aglycones but rather as their glycosides and malonyl esters which are stable as such. However, upon consumption, they are converted by gut microflora into their aglycones which are then absorbed. The absorbed isoflavones may undergo phase I or phase II metabolism either in intestinal epithelia or in liver. The absorption and metabolism of isoflavones have been discussed elsewhere (Wahajuddin et al., 2013).

Many new isoflavones are discovered each year (Veitch, 2013). The most important and widely studied isoflavones are those found in soy (*Glycine max*) viz. genistein, daidzein and glycetein. Besides this, red clover is also a rich source of isoflavones formononetin and biochanin A. Although, the isoflavones content in plants vary among species and region, on an average, soyabean contains 26-120 mg genistein and 10-85 mg daidzein per 100 gram dry weight (Vincent and Fitzpatrick, 2000). Table 1 shows the classification of isoflavones based on their structure and the common source of a few commonly known isoflavones.

Isoflavones possess a variety of important pharmacological properties such as estrogenic, anticancer, antioxidant, anti-inflammatory, anti-apoptotic, anti-angiogenesis and lipid-lowering activites. They have been proposed as potential agents for treating osteoporosis, cardiovascular diseases and cancer (Arliss and Biermann, 2002; Nestel et al., 2004). Structurally resembling

estrogens, isoflavones are the most widely studied group of phytoestrogens. Various animal and clinical studies have shown cancer protective action of isoflavones owing to their estrogen-like actions. Isoflavones act as selective estrogen receptor modulators (SERMs) like raloxifene and faslodex. They exihibit greater binding affinity for ER_β than ER_α. ER_β is highly expressed in prostate, uterus, ovary, testis, bone, lung and brain while ER_α is expressed in uterus, testis, ovary and kidney. The relative estrogen receptor binding affinities of isoflavones has been found to be of the order: genistein > daidzein > biochanin A. The glycosides genistin and daidzin have been found to exert lesser estrogen-dependent transcription expression than their aglycones. In general, methylation or glycosidation of isoflavones reduces their ER binding affinity. High dietary intake of isoflavones has been associated with reduced incidence of breast cancer and prostate cancer. (Cos et al., 2003; Vitale et al., 2013). In addition, the non-hormonal effects of isoflavones such as inhibition of tyrosine kinases and DNA topoisomerase I and II and their anti-angiogenesis and anti-oxidant activity have also been suggested to contribute to their cancer preventive role (Cos et al., 2003). The antioxidant activity of isoflavones is attributed to their ability to scavenge peroxyl radicals in hydrophilic environment and inhibit lipid peroxidation in lipophilic environment. Genistein and daidzein were shown to be most potent antioxidant while formononetin was the least potent of the tested isoflavones (genistein, daidzein, glycitein, biochanin A, formononetin and prunetin) (Rufer and Kulling, 2006). These antioxidant effects of isoflavones have been attributed to increase in heme-oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO1) via promoting the binding of transcription factors Nrf2 (nuclear factor-erythroid 2-related factor 2) and c-Jun to ARE (antioxidant response element) on HO-1 and NQO-1 promoters. These effects involve phosphatidylinositol 3-kinase

(PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways as well (Park et al., 2011). The stimulation of nuclear erythroid 2-related factor 2 -- Antioxidant response elements (Nrf-2-ARE) signaling pathway has been implicated in cancer prevention as well (Zhang and Gordon, 2004). It has also been proposed that the health promoting effects of isoflavones in many disease conditions are due to modulation of estrogen receptor/ peroxisome proliferator-activated receptor (ER/PPAR) signaling. Isoflavones can activate PPARα and PPARγ as well as ER. It has been suggested that isoflavones regulate a complex cross-talk between ER_β and PPAR signaling that is responsible for their activity (Patel and Barnes, 2010).

Isoflavones are been considered as the most potential alternative for conventional hormone replacement therapy (HRT) in postmenopausal women for preventing/reducing postmenopausal symptoms including hot flashes; osteoporosis; and cardiovascular diseases. Ipriflavone is a synthetic isoflavones that is being clinically used for its efficacy against breast cancer and as an anti-osteoporotic agent (Agnusdei and Bufalino, 1997; Attila, 1995; Gennari et al., 1997; Zhang et al., 2010). Considering the increasing interest in the clinical use of isoflavones for therapeutic indication for which prescription medicines are already available, there are potential chances of polypharmacy. This may lead to possible drug-drug interactions.

Drug-herb interactions

In addition to the dietary intake of isoflavones, their use as herbal preparations is increasing both for therapeutic as well as preventive measures as mentioned above. It is a common conception that herbal preparations are devoid of side effects and safe to consume. However, several well documented herb-drug interaction clinical reports are available in literature (Fugh-Berman,

2000). Most of these interactions are pharmacokinetic in nature occurring due to alterations in ADME properties and are channeled through modulation of drug metabolizing enzymes (DMEs), both Phase I and Phase II, and drug transporters. Likewise for isoflavones, the metabolic pathways involved in their disposition are commonly shared by many clinically used drugs. Thus, when taken concomitantly for a long period of time with therapeutic drugs either as a component of daily diet or as herbal supplements, there is a probability that these isoflavones may alter the pharmacokinetics of coadministered prescription medicines. The importance of drug-isoflavone interaction has been proven in the clinical studies conducted in Japan and China. The probable outcomes of such interactions are presented in figure 1. Moreover, the major circulating forms of isoflavones in biological system are their phase II glucuronides and sulfates. Thus, these may also affect the activity of DMEs and drug transporters in the same way as their isoflavones aglycones. Furthermore, in diet, these isoflavones are present as glycosides in high amounts which may also exert some effect on DMEs and drug transporters expressed in intestine. Thus, it is imperative to examine the effects that isoflavones as well as their glycosides, glucuronides and sulfates may exert on DMEs and drug transporters and the possible impact on prescription drugs thereof.

This review centers on the modulation of drug metabolizing enzymes and drug transporters by isoflavones as well as their glycosides and metabolites and the possible drug interactions when administered with prescription medicines. The review covers the *in vitro* and *in vivo* data from clinical and preclinical studies conducted during past 25 years.

The tissue gene expression of various DMEs and drug transporters is modulated by a group of nuclear receptors, mainly comprising of pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor γ (PPARγ), nuclear erythroid 2-related factor 2 (Nrf2), hepatocyte nuclear factor 4α (HNF 4α), farnesoid X receptor (FXR), vitamin D receptor (VDR) and glucocorticoid receptor (GR). These ligand-activated transcription factors act in a co-ordinated manner to bring about the regulation of Phase I and II DMEs as well as drug transporters (Bogacz et al., 2012; Faber et al., 2003; Geier et al., 2007; Tolson and Wang, 2010; Urquhart et al., 2007).

Interaction with drug metabolizing enzymes:

The Phase I metabolism is catalyzed by a group of hemoproteins known as cytochrome P450 (CYP) enzymes The Phase II metabolism is catalyzed by uridine diphosphate glucuronosyl transferases (UDPGT/UGTs), sulfotransferases (SULTs), glutathione-S-transferase (GSTs), N-acetyl transferases (NAT) or methyl transferases. Figure 2 represents the major phase I and phase II enzymes present in humans. Being polyphenolic compounds, isoflavones are favorable substrates for enzymatic biotransformation *in vivo*. In general, CYP--mediated metabolism has little contribution to their overall metabolism when compared to phase II metabolism. Hence, being substrates of both phase I and phase II enzymes, they have high affinity for these enzymes and thus, could possibly inhibit/induce these enzymes as well.

Effect on CYP enzymes:

Contrasting results are available explaining the effect of soyabean extract and its isoflavones on CYP genes, mRNA, protein expression and activity. Genistein did not modulate hepatic CYP1A2, 2E1 and 3A activity even after a 40 mg/kg intraperitoneal treatment for 4 days in mice. The response elements for human CYP1A1, GST λa and xenobiotic response element (XRE) were also not activated by genistein in HepG2 cells (Helsby et al., 1997). Studying the inductive effects of genistein and daidzein on CYP enzymes, Kishida et al reported that feeding mice with fermented soyabean or fermented soyabean extract increased the CYP content of hepatic liver microsomes while feeding them with the aglycones genistein and daidzein did not, thus, indicating that genistein and daidzein are not responsible for CYP induction by soy (Kishida et al., 2000). Further, exploring these results *in vivo*, they showed that feeding rats with a high isoflavones diet (155 mg/kg genistein and 127 mg/kg daidzein) for 4 weeks did not modulate the hepatic mRNA levels of Cyp1a1, 1a2, 2b1/2, 2c11, 2e1, 3a1, 3a2 and 4a1 (Kishida et al., 2004). While on the other hand, it has also been shown that soyabean extract and genistein increase CYP1a1, 1a2 and 2d1 activity in rats (Bogacz et al., 2014; Mrozikiewicz et al., 2010; Perepechaeva and Grishanova, 2012). Peurarin caused induction of rat CYP2A1, 1A1/2, 3A1 and 2C11 (Guerra et al., 2000).

CYP inhibition has also been reported in numerous studies as shown in table 2. Peurarin was found to be an inhibitor of rat CYP2E1 and 2B1 although the exact mechanism underlying these effects was not explained (Guerra et al., 2000). For biochanin A and formononetin, it has been proposed that their inhibitory effects may be due to direct competition with substrates for active binding site or indirectly through their metabolites, daidzein and genistein (Roberts et al., 2004).

The *in vitro* parameters (K_i and IC_{50}) obtained from these studies could be used to predict the possibility of drug interaction *in vivo*. According to the US FDA guidelines for CYP interaction studies, the cut-off R value for prediction of drug-drug interaction at hepatic level (calculated as $1 + [I]/K_i$) is 1.1 while at the intestinal level, the cut-off R value (calculated as $1 + [I_{gut}]/K_i$) is 11 (US FDA, 2012). The circulating levels of total isoflavones may reach up to 15 µM (Yang et al., 2014; Takimoto et al, 2003) while that of free isoflavones may reach up to 0.5 µM in individuals consuming dietary soy supplements (Yang et al., 2014; Zhang et al., 2014; Takimoto et al., 2003; Gooderham et al., 1996; Penetar et al., 2006; Howe et al, 2002). As observed from the *in vitro* studies conducted in microsomal systems, the K_i/IC_{50} values for CYPs for most isoflavones lie above 1 µM. Thus, there are little chances of interaction of isoflavones with CYPs at the hepatic level. However, concentrations of isoflavones reached in the intestine are higher than their systemic levels and may lead to potential CYP inhibition at the intestinal level. Further, studies performed using *in vivo* preclinical models ascertains the modulatory effect of isoflavones on DMEs and transporters.

Effect on Phase II enzymes:

Effect on UGTs:

Studies conducted in rats and mouse have shown that feeding soy based diet for long duration leads to increased UGT activity (Appelt and Reicks, 1997, 1999; Mirsalis et al., 1993). The hepatic UGT activity was increased on being fed with a high isoflavone (0.81 mg/g) diet for 2 weeks (Appelt and Reicks, 1999). Genistein, daidzein, biochanin A, formononetin and pruentin were tested for their effects on UDP-glucuronosyltransferase activity upon long term treatment in

androgen-responsive human prostatic carcinoma cell line LNCaP. All isoflavones stimulated the activity of UGT. At 5 µM, biochanin A was the most potent isoflavone and increased the formation of testosterone glucuronide by 10-fold via upregulation of UGT2B15 expression (Sun et al., 1998). The release of prostate tumor marker, prostate specific antigen (PSA), was also decreased as a result of diminished testosterone availability due to which they have been suggested as useful chemopreventive agents for prostate cancers (Sun et al., 1998).

Effect on SULTs:

Isoflavones may act as inhibitors or inducers of SULTs. They have been found to be inhibitors of both human SULT1A1 and 1E1 isoforms. Isoflavones have been found to be inhibitors of human phenol sulfotransferases (encoded by gene SULT1A) (Ghazali and Waring, 1999; Jeong et al., 1999). Genistein was reported to be a potent isoflavone for inhibiting P-form phenol sulfotransferase with a Ki value of 0.10 µM. Further it was found to be a non-competitive inhibitor for this enzyme since it is not a substrate for the same (Ghazali and Waring, 1999). Studying the effect of various isoflavones on different human sulfotransferases and sulfatases, Harris et al reported that genistein and its metabolite, equol, were the most potent inhibitors of SULT1E1 (estrogen sulfotransferase) having K_i values of 0.4 and 0.5 µM at the active site, and 2 and 5 µM at an allosteric site, respectively. For SULT1A1 (phenol sulfotransferase), the most potent inhibition was shown by 3',4',7-trihydroxyisoflavone with IC_{50} value of 20 nM. Genistein and daidzein inhibited SULT1A1 with IC_{50} of 0.5 and 0.6 µM, respectively. Dopamine sulfation by SULT1A3 was only inhibited by 3',4',7-trihydroxyisoflavone at higher concentrations (IC_{50} = 2 µM). Of all the tested isoflavones, formononetin was the least potent inhibitor of SULTs

(Harris et al., 2004). Daidzein, however, did not have any inhibitory effects on SULT2A activity even at a high concentration of 25 µM (Harris and Waring, 2008). Interestingly, the sulfate conjugate of daidzein, daidzein-4,7-bisulfate, inhibited SULT1E1 exhibiting IC_{50} value of 10 µM (Harris et al., 2004). Daidzein is sulfated at 4' and 7' positions by SULT 1A1. It was observed that in human liver cytosol, these biotransformation reactions are inhibited by daidzein itself causing substrate inhibition of SULT1A1 at concentrations greater than 1µM while no such inhibition was seen for SULT1E1 (Ruan and Yan, 2014).

In contrast, genistein and biochanin A have been shown to induce sulfostransferases (Chen et al., 2008; Chen et al., 2010). Genistein has been found to increase hSULT2A1 and hSULT1A1 activity time and dose-dependently in HepG2 and Caco-2 cells. The mRNA and protein levels of both these SULT isoforms were found to be significantly elevated following 2 or 7 days treatment of genistein at 25 µM (Chen et al., 2008). In *in vivo* studies, biochanin A treatment (2, 10 and 50 mg/kg/day) for one week by intraperitoneal route resulted in significantly increased protein expression and mRNA levels of SULT1A1, SULT2A1 in liver and intestine in both male and female rats. The enzymatic activity assay results corresponded with the increased transcriptional and translational effects (Chen et al., 2010). Thus,the induction of SULTs occur at transcription level rather than translational level causing an increase in both mRNA levels as well as protein expression (Chen et al., 2008; Chen et al., 2010). This maybe mediated through nuclear receptors such as PXR, VDR and FXR which are involved in their regulation.

Effect on miscellaneous DMEs

Genistein (1 μM) was shown to modulate GST expression via ER/ARE signaling pathway. However, the effects were found to be cell-type specific. In ERα and ERβ expressing COS 1 cells, genistein repressed the GST Ya ARE-dependent gene expression while in ER-negative MCF-7 cell line derived breast cancer cells, C4-12-5 cells, genistein showed only modest GST gene induction following transfection with ERα and ERβ (Ansell et al., 2004). Wiegand et al observed that feeding rats with genistein (2 g/kg) enriched diet did not alter its total hepatic GST activity although the mRNA levels of Gsta2 were significantly increased while the mRNA levels of Gstm2 and Gstp1 were decreased (Wiegand et al., 2009).

Studying the inhibitory effect of flavonoids on N-acetyl conjugation, it was found that while flavones potently inhibited the acetylation of 5-aminosalicylic acid, isoflavones (genitsein and daidzein) only weakly inhibited Phase II N-acetylation reaction catalyzed by N-acetyl transferase (Mizoyama et al., 2004).

Isoflavones have been reported to be inhibitors of mitochondrial aldehyde dehydrogenase (ALDH2). The glycoside, daidzin, was shown to competitively inhibit human ALDH2 having K_i of 20 nM (Keung and Vallee, 1993) while prunetin was found to be its inhibitor with a K_i of 2 μM. An allosteric inhibition mechanism was proposed for the same. Prunetin also inhibited the esterase activity of ALDH enzyme competitively (Sheikh and Weiner, 1997).

The effect of isoflavones has also been investigated on phosphodiesterase (PDE) enzymes. In guinea pig lung and heart homogenate containing PDE isoforms, genistein inhibited PDE1, 2, 3, 4 and 5, daidzein inhibited PDE3 while biochanin A and prunetin preferentially inhibited PDE4 (Ko et al., 2004). Biochanin A that inhibited PDE4 with IC_{50} value of 8.5 μM was also found to

inhibit PDE4 in guinea pig brain cell membrane with EC_{50} value greater than 300 µM. This inhibition was also thought to be responsible for suppression of ovalbumin-induced airway hyper-responsiveness by biochanin A (Ko et al., 2011).

Aryl sulfatase and sterol sulfatase were not inhibited by any of the tested isoflavones. However, the phase II sulfate metabolites of isoflavones inhibited sterol sulfatase at higher concentrations in micromolar range. Sterol sulfatase activity was inhibited by daidzein-4'-O-sulfate and daidzein-7,4'-O-sulfate with Ki values of 5.9 µM and 1 µM, respectively. Both daidzein sulfoconjugates competitively inhibited DHEA sulfate metabolism while daidzein showed no such effect. Hydroxysteroid and phenol sulfotransferase were also inhibited by them at higher concentrations (Harris et al., 2004; Wong and Keung, 1997).

Interaction with drug transporters

Drug transporters have been classified into two classes: ATP-binding cassette (ABC) transporters that are efflux transporters and solute carrier (SLC) transporters that are influx transporters. All the three major ABC transporters, viz. P-glycoprotein (Pgp), breast cancer resistance protein (BCRP) and multidrug resistance protein (MRP) have been studied for the effects of isoflavones on them as shown in table 3. The expression of drug transporters in different tissues is shown in figure 3.

Effect on P-gp:

P-gp (also known as multidrug resistance protein 1/MDR1), encoded by gene ABCB1, is the most widely studied drug transporter that is involved in the ATP-powered efflux of many

unrelated hydrophobic compounds amphipathic compounds, therapeutic drugs, peptides and lipid-like comounds. Being present at the luminal intestinal surface, it contributes in limiting the absorption of orally administered drugs. It is an important component of blood brain barrier and is also expressed in many human tumors conferring multidrug resistance (Aller et al., 2009; Higgins and Gottesman, 1992).

Versantwoort et al showed that genistein (200µM) reversed the decreased accumulation of daunorubicin in five non-Pgp MDR cell lines (GLC4/ADR, SW-1573/2R120, HT1080/DR4, MCF7/Mitox and HL60/ADR) while not producing similar effects in Pgp MDR cell lines (SW-1573/2R160, MCF7/DOX40 and KBB-5). VP-16 accumulation was also increased in all the non-Pgp MDR cells by genistein. Thus, they suggested that genistein could be used as a specific probe to identify non-Pgp mediated transport (Versantvoort et al., 1993). Further, it was shown that genistein increased the apparent K_m of DNR efflux out of GLC4/ADR cells, suggesting it to be a competitive inhibitor for non-Pgp mediated daunorubicin (DNR) transport (Versantvoort et al., 1994). However, investigating the same, Castro et al found that at the same concentration, genistein elevated the intracellular accumulation of P-gp specific substrate, rhodamine 123, in human MDR1 transfected human breast cancer cell line, BC19/3, and human MDR1 transfected mouse fibroblast cell line, BALB/c-3T3-1000, by 7-fold and 10-fold, respectively. They also demonstrated that in these cells, daunorubicin intracellular concentration was also significantly increased by genistein treatment. The daunorubicin intracellular levels were also increased in MRP overexpressing MCF-7/VP cell by genistein (Castro and Altenberg, 1997). The mechanism by which isoflavones inhibit drug transporters has been studied extensively. Genistein is a potent inhibitor of tyrosine kinase activity. It was suggested that inhibition of tyrosine kinase could be

involved in inhibition of transporter activity. However, biochanin A is only a weak inhibitor of tyrosine kinase activity while causing a significant increase in DNR accumulation in non-Pgp MDR GLC4/ADR cells, thus, rejecting this possibility (Versantvoort et al., 1993). Versantwoort et al suggested competitive inhibition of MRP by genistein (Versantvoort et al., 1994). Genistein (200 µM) was found to inhibit photoaffinity labeling of Pgp by [^{3}H] azidopine, thus, indicating that genistein interacts with P-gp directly (Castro and Altenberg, 1997).

Genistein, daidzein, biochanin A and peurarin have been shown to be inhibtitors of Pgp *in vitro*. In a study in Caco-2 cells, the accumulation of digoxin (583%) and vinblastine (640%) was significantly increased by biochanin A dose-dependently. In bidirectional transport studies, the mean transport ratio of digoxin was decreased from 43.4 to 1.62 in presence of 150 µM of biochanin A. The inhibition constant (Ki) values of biochanin A for apical to basolateral (AP-to-BL) and basolateral to apical (BL-to-AP) were 33.2 µM and 32.7 µM, respectively (Zhang and Morris, 2003a). Thus, it could be argued that the effect isoflavones may exert of transporters *in vivo* could be dependent on the type of cells and thus, cannot be taken as an alternative to *in vivo* scenario. Apart from inhibition of P-gp by biochanin A, An et al showed that biochanin A stimulated P-gp in P-gp expressing, BCRP negative MCF-7/ADR cells. Upon co-incubation with 10 µM biochanin A, the intracellular accumulation of mitoxantrone, a P-gp as well as BCRP substrate, was decreased by 31.9% while 50 µM biochanin A apparently had no effect on mitoxantrone accumulation, suggesting the concentration dependent inhibitory and stimulatory effects of biochanin A on P-gp (An and Morris, 2010). Such concentration dependent, biphasic effects have also been seen for other flavonoids such as quercetin and kaempferol, on vincristine efflux (Mitsunaga et al., 2000).

Hien et al observed that in human breast cancer MDR cancer cell line (MCF-7/adr), puerarin (100 μM) pretreatment significantly increased Adriamycin cytotoxicity and increased the intracellular fluorescence of known MDR1 substrate, Rhodamine 123. The levels of MDR1 mRNA were decreased dose and time-dependently following puerarin exposure. Puerarin was also found to decrease the activity of NF-κB and MDR1 promoter via inhibiting the phosphorylation of IκB-α. They also reported that in MCF-7/adr cells, puerarin significantly increased the phosphorylation of AMP-activated protein kinase (AMPK), glycogen synthase kinase-3β (GSK-3β) and acetyl-CoA carboxylase (ACC) while decreasing cAMP responsive element-binding protein (CREB) activity. They proposed AMPK activation and the downregulation of CRE transcriptional activity through GSK-3β in addition to the inhibition of NF-κB as probable mechanisms for the reversal of MDR in MCF-7/adr cells (Hien et al., 2010).

Effect on BCRP:

BCRP (also known as mitoxantrone resistance associated protein), encoded by gene ABCG2, is highly expressed in the apical membranes of secretory organs such as small intestine, liver, kidney and in the apical membranes of blood vessels of blood-brain barrier, blood-testis barrier, placental barrier limiting the transport of many anticancer drugs, other therapeutic agents and hydrophilic conjugated organic anions. Its expression is induced during lactation causing secretion of many drugs into milk. It has been proposed that there are non-overlapping or partially overlapping multiple substrate binding sites in BCRP conferring resistance against various anticancer drugs (Natarajan et al., 2012; Ni et al., 2010).

Genistein has been shown to have BCRP inhibitory effects stronger than estrone. In K562/BCRP cells, genistein (3 μM) markedly increased the cytotoxicity of SN-38 and topotecan with mean reversal indexes of 7.23 and 6.23, respectively (Imai et al., 2004). The inhibitory action of genistein has been described by its competitive transport at substrate binding site of anticancer agents since it is also a substrate of BCRP (Imai et al., 2004). However, in a study conducted in BCRP overexpressing human cell lines, biochanin A was found to be the most potent BCRP inhibitor among all the isoflavones tested. At 1 μM, it produced 216% increase in mitoxantrone accumulation in MCF-7 MX100 cells and 166% increase NCI-H460 MX20. Both biochanin A and genistein significantly lowered the IC_{50} values of mitoxantrone in BCRP transfected cells compared to the parent cell lines (Zhang et al., 2004b). Merino et al studied the effects of genistein and daidzein on nitrofurantoin transport in human BCRP and murine Bcrp1 transfected MDCKII cell lines (MDCK/BCRP and MDCK/Bcrp1). The BL-to-AP transport and the mean transport ratio of nitrofurantoin were decreased in both the cell lines. To confirm these results in vivo, they determined the inhibitory effects of genistein and daidzein on nitrofurantoin disposition in Bcrp1 knockout mice. The mixture of genistein/daidzein (100 mg/kg each) was administered intragastrically 5 minutes before nitrofurantoin dosing. The plasma concentration of nitrofurantoin was significantly higher in isoflavone-treated wild type mice while its mean concentration in milk was significantly lower in isoflavone-treated knockout mice. The mean milk/plasma ratio decreased from 7.1 to 4.2 in isoflavone treated knockout mice than control knockout mice (Merino et al., 2010).

Soyabean extract significantly reduced the BCRP-mediated transport of mitoxantrone (MTX) with IC_{50} value of 16.5 μg/mL. Genistein, daidzein, glycitin, genistin, daidzin, prunetin,

biochanin A, formononetin, sissotrin and other isoflavones inhibited MTX uptake into vesicles in a concentration dependent manner. Comparing the inhibitory effects of different isoflavones, Tamaki and colleagues inferred that the presence of hydroxyl moiety at C5, methylation at C6 potentiated inhibitory effects while 7-O-glucosidation decreased the inhibitory potency (10 to 100 fold) (Tamaki et al., 2010). Even the phase II metabolites of daidzein, daidzein-7-glucuronide inhibited MTX transport with IC_{50} value 100-fold greater than daidzein while daidzein-4'-sulfate had comparable inhibitory potency as daidzein (Tamaki et al., 2010). The increased cellular accumulation of anticancer agents as a result of inhibition of BCRP-mediated efflux could restore the sensitivity of MDR cancer cells. The postulated mechanisms of inhibition of BCRP includes interaction with either nucleoside binding site or with substrate binding site stimulating ATPase activity and causing competitive inhibition (Alvarez et al., 2010; Cooray et al., 2004).

Effect on MRP:

MRP, encoded by gene ABCC, has seven members. Of these, MRP1/ABCC1, MRP2/ABCC2/cMOAT/cMRP and MRP3/ABCC3/MOAT-D/cMOAT-2 have been studied most extensively. These are involved in the transport of anionic molecules such as anionic drugs and neutral drugs conjugated to acidic ligands like glutathione (GSH), glucuronate or sulfate. They are known to cause resistance to neutral drugs by transporting them together with free GSH (Borst et al., 2000; Jiang and Hu, 2012).

Isoflavones have been found to be inhibitors of MRP1. Biochanin A and genistein at 100µM increased daunomycin and vinblastine accumulation in MRP1 expressing human Panc-1 cells

(Nguyen et al., 2003). Explaining the mechanism of interaction of isoflavones with MRP, Hooijberg et al demonstrated that in plasma membrane vesicles of MRP overexpressing GLC_4/ADR cells, the ATPase activity of MRP is stimulated by genistein and flavopiridol and hypothesized that isoflavones affect the transport of anticancer drugs like daunorubicin by direct interaction with MRP. Genistin was not found to exert any influence on the ATPase activity (Hooijberg et al., 1997). Likewise, it did not inhibit the transport of daunorubicin (DNR) in MRP-overexpressing tumour cells (Hooijberg et al., 1999). Further, they showed that genistein and flavopiridol specifically stimulated the ATPase activity associated with MRP1 at as low a concentration as 1 µM which was inhibited by MRP1 specific antibody, MIB6. They also demonstrated that contrary to the hypothesis that reduced glutathione (GSH) is required for the transport of many uncharged and basic compounds by MRP (Toyoda et al., 2008), the presence of GSH did not affect ATPase induction by flavopiridol and genistein. Flavopiridol also significantly increased daunorubicin accumulation at even 100nM concentration while genistein did not modulate DNR accumulation at low micromolar concentration (Hooijberg et al., 1999). However, the increase in MRP1 mediated accumulation of daunomycin and vinblastine in human Panc-1 cells was accompanied by a decrease in cellular GSH concentrations, suggesting a probable role of GSH in modulating MRP1 activity (Nguyen et al., 2003).

Jager et al showed that the inhibition of biliary excretion of bilirubin and bromsulphthalein conjugates is inhibited by both genistein and daidzein via inhibition of Mrp2. Since daidzein does not have tyrosine kinase inhibitory activity, they proposed that isoflavones decrease Mrp2 activity of competitive inhibition rather than by inhibiting tyrosine kinase (Jager et al., 1997). In addition, it has been proposed that human MRP2 has non-identical ligand binding sites, one from

which the substrate is transported and the other site that regulates the affinity of the transport site for substrate. Thus, this may cause allosteric inhibition (Zelcer et al., 2003). Sergent and colleagues showed that commonly achieved intragastric levels of genistein and biochanin A (0.75 nM and 7.5 nM) can inhibit the efflux of ochratoxin A (OTA) across Caco-2 cells. OTA is a common fungal contaminant found in food that had earlier been observed to be effluxed out by MRP-2 (Berger et al., 2003). The AP-to-BL transport of OTA increased significantly in presence of 7.5 nM genistein and biochanin A, indicating that these isoflavones are inhibitors of MRP-2 (Sergent et al., 2005).

Effect on other transporters:

Isoflavones have been found to be inhibitors of OATP-B influx transporter of SLC class. Genistien and biochanin A decreased dehydroepiandrosterone sulfate (DHEAS) uptake in OATP1B1 expressing HeLa cells while in rats, biochanin A was found to decrease the bioavailavailability of OATP substrate, fexofenadine (Pend et al., 2006; Wand et al., 2005). Genistein and biochanin A competitively and dose-dependently inhibited the transport of deoxyglucose and dehydroascorbic acid by directly interacting with GLUT1 transporter while puerarin did not show any such effect (Vera et al., 2001). Genistein inhibited glucose influx and efflux by GLUT1 in human erythrocytes (Afzal et al., 2002; Martin et al., 2003). Genistein and daidzein also significantly inhibited GLUT-mediated transport of glucose across plasma membrane in isolated rat liver lysosomes with IC_{50} value of 45 µM and 71 µM. They also inhibited sulfate uptake in the same test system (Chou et al., 2010).

Interplay of DMEs/drug transporters and lack of *in vitro* and *in vivo* correlation:

The clinically used drugs may not be metabolized or transported by just one DME or transporter but could be substrates of a multitude of Phase I/Phase II enzymes and influx/efflux transporters. Isoflavones, on the other hand, may modulate the activity of more than one DME or transporter simultaneously. Additionally, the isoflavones may get converted to metabolites that may inhibit/induce another isoform of DME or another transporter. Thus, this interplay of different transporters and DMEs among themselves and with each other is an important factor affecting the outcome of isoflavonoid-drug interaction. Several studies are reported that demonstrate that the *in vitro* results for DME/drug transporter interactions cannot be simply extrapolated to *in vivo* conditions since the biological system involve many factor such as the co-expression of DMEs and drug transporters that the *in vitro* systems, at present, does not mimic. Moreover, the overlapping substrate specificity of DMEs and transporters, especially P-gp and CYP3A4, makes the prediction of isoflavonoid-drug interaction outcome highly complex and variable (Lin and Yamazaki, 2003). Even the *in vivo* studies conducted in preclinical studies may not truly reflect the scenario that may be encountered in human body due to species differences in the isoform alleles, expression level and substrate specificity of DMEs and transporters. Apart from these, the differences in the formulation development and administration strategies used in different studies may lead to different observations.

This DME/transporter interplay has been suggested to be responsible for the unexpected outcomes in many studies. Genistein was demonstrated to inhibit Mrp2 (canalicular multispecific organic anion transporter/cmao) and P-gp. Also it is a substrate for UGTs. In a series of experiments conducted in isolated perfused rat liver of TR^+ (control) and TR^- (mutant strain expressing P-gp but not cmao) Wistar rats, it was observed in control rats that genistein inhibited

the biliary excretion of bilirubin, bromsuphthalein and rhodamine conjugates, all of which are cmao substrates. In mutant rats, the secretion of cationic rhodamine was increased. It was suggested that the competitive inhibition of cmao caused the former results while the inhibition of glucuronidation of rhodamine due to presence of genistein exposed greater concentrations of rhodamine available for P-gp mediated efflux (Jager et al., 1997). Similar results were obtained with flavopiridol. Flavopiridol (30 µM) reversibly inhibited the biliary elimination of bilirubin and bromsulphthalein conjugates mediated by Mrp2 by 54 and 51% in Wistar rats (Jager et al., 2003).

Peng et al had reported the interaction of P-gp substrates paclitaxel, digoxin and fexofenadine with oral biochanin A (100mg/kg) coadministration in *Sprague-dawley* rats. Biochanin A increased the oral bioavailability of paclitaxel and digoxin by 3.77 and 1.75-fold while their peak plasma concentrations (C_{max}) were increased by 2.04 and 1.71-folds, respectively. In rats, paclitaxel is a substrate for P-gp and CYP3A while digoxin is a substrate for Oatp2, P-gp and CYP3A. For fexofenadine, however, the oral bioavailability and C_{max} reduced by 0.69 and 0.42-fold upon co-administration with biochanin A. Fexofenadine is a substrate of P-gp, Oatp1, Oatp2 and Oatp3. They proposed preferential inhibition of Oatp over P-gp by biochanin A as a possible explanation for the results obtained (Peng et al., 2006).

Biochanin A, a P-gp inhibitor, potentiated daunomycin accumulation by 4-fold in P-gp overexpressing MCF-7/ADR cells. To investigate its *in vivo* interaction potential, Zhang et al characterized the pharmacokinetics of three known P-gp substrates viz. doxorubicin, cyclosporine A and paclitaxel in *Sprague-dawley* rats with/without the coadministration of

biochanin A. Biochanin A had no effect on their systemic exposure and systemic clearance of doxorubicin or cyclosporine. However, it decreased the AUC and increased the clearance of intravenously administered paclitaxel by 0.78 and 1.53-folds while not affecting the pharmacokinetics of orally administered paclitaxel (Zhang et al., 2010). Genistein (10 mg/kg), on the other hand, was found to increase the AUC (54.7%) and decrease the plasma clearance (35.2%) of orally administered paclitaxel (Li and Choi, 2007). This discrepancy in the effects of different isoflavones on paclitaxel may be due to its poor bioavailability, rapid clearance or the involvement of other transporters and metabolizing enzymes in the disposition of probe substrates (Zhang et al., 2010). Genistein has been reported to be an inhibitor of CYP3A4 activity which metabolizes paclitaxel. For biochanin A, no such reports are available. Our experimental data (unpublished data) has shown that biochanin A does not appreciably inhibit rat CYP3A activity. Thus, the differences in the results may be explained based on the differential inhibition of drug metabolizing enzymes by isoflavones rather than their effect on drug transporters. Also, in an earlier report, the oral bioavailability and C_{max} were found to increase by 3.77 and 2.04-folds on coadministration of 100 mg/kg biochanin A orally (Peng et al., 2006). The differences in the results obtained with paclitaxel and biochanin A were suggested to be due to strain/species differences, the doses and dosing methods used in the experiments and formulation excipients (Zhang et al., 2010).

An et al also demonstrated the species difference and the contrasting dose-dependent induction and inhibitory effects of isoflavones. In a study conducted to investigate the effect of biochanin A on BCRP, the transport of MTX was studied *in vitro* in human MDCK/BCRP and murine MDCK/Bcrp1 cells (at 2.5 and 25 µM biochanin A) and *in vivo* in mice (at 10 mg/kg biochanin

A). Biochanin A showed more potent inhibition of human BCRP than murine Bcrp. Although the BL-to-AP of MTX was decreased leading to increased accumulation in transfected BCRP over-expressing cells, there was no significant effect on its concentration in plasma and most tissues. On the contrary, its concentration in kidney and spleen were decreased. They attributed this ambiguity of results to the finding that at low concentrations biochanin A (10 μM) could stimulate P-gp expression. Since MTX is also a substrate of P-gp, thus, this stimulatory effect of biochanin A on P-gp, in addition to its inhibitory effects on OATP1B1 and rat Oatp3 could compensate its stimulatory effects on BCRP *in vivo* (An and Morris, 2010).

Clinical studies

Although the effects of isoflavones on DMEs and drug transporters have been researched widely, till date, only two clinical studies have been reported determining the effect of isoflavones on 1DMEs in humans. In one study, human volunteers were given 200 mg daidzein twice for ten days and the pharmacokinetics of theophylline were compared before and after daidzein treatment. Caffeine metabolic ratio (CMR) used as an indicator of CYP1A2 activity significantly decreased in daidzein treated group by -50% to 20% while no such changes were seen in the control group. Also$_{0\text{-}48}$, C_{max} and $t_{1/2}$ of theophylline increased significantly by 33.57, 23.54 and 41.3% upon daidzein treatment (Peng et al., 2003). The inhibitory effects of isoflavones on human CYP2A6 activity have also been investigated. In this study, human volunteers were abstained from soy foods for one week. On the eighth day, they were asked to chew nicotine gum. The conitine/nicotine plasma ratio, taken as marker for CYP2A6 activity, significantly

decreased following abstinence from soy food (Nakajima et al., 2006). Thus, the administration of isoflavones should be carefully monitored when concurrently with therapeutic drugs.

Conclusions:

Isoflavones exert potent modulatory effects on the activity of DMEs and drug transporters. The same isoflavones may concentration dependently inhibit or induce a particular DME/transporter and also depending upon cell-type. The *in vivo* extrapolation of effects of isoflavones from *in vitro* or preclinical data is in adequate. The *in vivo* disposition of drugs involves an interplay of a multitude of DMEs and transporters which is not replicated in *in vitro* systems. Thus, the exact clinical outcome for isoflavones-drug interactions cannot be predicted from experimental data alone. Generally, the glycosides have less affinity and thus, show inconspicuous effects on transporter activity than aglycones. The phase II metabolites, on the other hand, also potently inhibit some DMEs and their systemic concentrations should also be taken into consideration when predicting *in vivo* interaction probability. Most isoflavones show competitive inhibition mechanism for DMEs/transporters, however, the involvement of transcription factors have also been shown for some. Since, isoflavones such as biochanin A inhibit both CYP3A4 and P-gp, these could be used as excipients in formulations to increase the oral absorption of drugs that are substrates of both P-gp and CYP3A4 as shown for paclitaxel and digoxin. More mechanistic studies are, nonetheless, required to increase our predictive tools in order to get an accurate *in vivo* projection.

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Afzal, I., et al. (2002). Interactions of ATP, oestradiol, genistein and the anti-oestrogens, faslodex (ICI 182780) and tamoxifen, with the human erythrocyte glucose transporter, GLUT1. *Biochem. J.*, **365**, 707-719.

Agnusdei, D., et al. (1997). Efficacy of ipriflavone in established osteoporosis and long-term safety. *Calcif. Tissue Int.,* **61 Suppl 1**, S23-27.

Aller, S. G., et al. (2009). Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science,* **323**, 1718-1722.

Alvarez, A. I., et al. (2010). Modulation of the activity of ABC transporters (P-glycoprotein, MRP2, BCRP) by flavonoids and drug response. *J. Pharm. Sci.*, **99**, 598-617.

An, G., et al. (2010). Effects of the isoflavonoid biochanin A on the transport of mitoxantrone in vitro and in vivo. *Biopharm. Drug Dispos.*, **31**, 340-350.

Anderson, G. D., et al. (2003). Drug interaction potential of soy extract and Panax ginseng. *J. Clin. Pharmacol.*, **43**, 643-648.

Ansell, P. J., et al. (2004). In vitro and in vivo regulation of antioxidant response element-dependent gene expression by estrogens. *Endocrinology,* **145**, 311-317.

Appelt, L. C., et al. (1997). Soy feeding induces phase II enzymes in rat tissues. *Nutr. Cancer,* **28**, 270-275.

Appelt, L. C., et al. (1999). Soy induces phase II enzymes but does not inhibit dimethylbenz[a]anthracene-induced carcinogenesis in female rats. *J. Nutr.*, **129**, 1820-1826.

Arliss, R. M., et al. (2002). Do soy isoflavones lower cholesterol, inhibit atherosclerosis, and play a role in cancer prevention? *Holist. Nurs. Pract.*, **16**, 40-48.

Attila, B. K. (1995). Overview of clinical studies with ipriflavone. *Acta Pharm. Hung.,* **65**, 223-228.

Berger, V., et al. (2003). Interaction of ochratoxin A with human intestinal Caco-2 cells: possible implication of a multidrug resistance-associated protein (MRP2). *Toxicol. Lett.,* **140-141**, 465-476.

Bobrowska-Hagerstrand, M., et al. (2001). Monitoring of MRP-like activity in human erythrocytes: inhibitory effect of isoflavones. *Blood Cells Mol. Dis.,* **27**, 894-900.

Bogacz, A., et al. (2012). Molecular mechanisms of regulation of CYP enzymes of phase I metabolism of xenobiotics--synthetic drugs and herbal preparations. *Herba Pol.,* **58**, 46-59.

Bogacz, A., et al. (2014). The influence of soybean extract on the expression level of selected drug transporters, transcription factors and cytochrome P450 genes encoding phase I drug-metabolizing enzymes. *Ginekol Pol.,* **85**, 348-353.

Borst, P., et al. (2000). A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl. Cancer Inst.,* **92**, 1295-1302.

Castro, A. F., et al. (1997). Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein. *Biochem. Pharmacol.,* **53**, 89-93.

Chen, J., et al. (2004). Potential beneficial metabolic interactions between tamoxifen and isoflavones via cytochrome P450-mediated pathways in female rat liver microsomes. *Pharm. Res.,* **21**, 2095-2104.

Chen, Y., et al. (2008). Genistein induction of human sulfotransferases in HepG2 and Caco-2 cells. *Basic Clin. Pharmacol. Toxicol.,* **103**, 553-559.

Chen, Y., et al. (2010). Biochanin A induction of sulfotransferases in rats. *J. Biochem. Mol. Toxicol.*, **24**, 102-114.

Chou, H. F., et al. (2010). Genistein inhibits glucose and sulphate transport in isolated rat liver lysosomes. *Br. J. Nutr.*, **103**, 197-205.

Cooray, H. C., et al. (2004). Interaction of the breast cancer resistance protein with plant polyphenols. *Biochem. biophys. res. commun.*, **317**, 269-275.

Cos, P., et al. (2003). Phytoestrogens: recent developments. *Planta Med.*, **69**, 589-599.

Eaton, E. A., et al. (1996). Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. *Drug Metab. Dispos.*, **24**, 232-237.

Evans, W. E., et al. (1999). Pharmacogenomics: translating functional genomics into rational therapeutics. *Science*, 286, 487-491.

Faber, K. N., et al. (2003). Drug transport proteins in the liver. *Adv. Drug Deliv. Rev.*, **55**, 107-124.

Fuchikami, H., et al. (2006). Effects of herbal extracts on the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab. Dispos.*, **34**, 577-582.

Fugh-Berman, A. (2000). Herb-drug interactions. *Lancet*, **355**, 134-138.

Geier, A., et al. (2007). Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. *Biochim. Biophys. Acta*, **1773**, 283-308.

Gennari, C., et al. (1997). Effect of chronic treatment with ipriflavone in postmenopausal women with low bone mass. *Calcif. Tissue Int.*, **61 Suppl 1**, S19-22.

Ghazali, R. A., et al. (1999). The effects of flavonoids on human phenolsulphotransferases: potential in drug metabolism and chemoprevention. *Life sci.*, **65**, 1625-1632.

Giacomini, K. M., et al. (2010). Membrane transporters in drug development. *Nat. Rev. Drug Discov.*, 9, 215-236.

Gooderham, M. H., et al. (1996). A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. *J Nutr.* **126**, 2000-2006.

Guerra, M. C., et al. (2000). Comparison between chinese medical herb Pueraria lobata crude extract and its main isoflavone puerarin antioxidant properties and effects on rat liver CYP-catalysed drug metabolism. *Life sci.*, **67**, 2997-3006.

Harris, R. M., et al. (2008). Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. *Curr. Drug Metab.*, **9**, 269-275.

Harris, R. M., et al. (2004). Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. *J. Clin. Endocrinol. Metab.*, **89**, 1779-1787.

Helsby, N., et al. (1997). The isoflavones equol and genistein do not induce xenobiotic-metabolizing enzymes in mouse and in human cells. *Xenobiotica*, **27**, 587-596.

Hien, T. T., et al. (2010). Molecular mechanism of suppression of MDR1 by puerarin from Pueraria lobata via NF-kappaB pathway and cAMP-responsive element transcriptional activity-dependent up-regulation of AMP-activated protein kinase in breast cancer MCF-7/adr cells. *Mol. Nutr. Food Res.*, **54**, 918-928.

Higgins, C. F., et al. (1992). Is the multidrug transporter a flippase? *Trends biochem. sci.*, **17**, 18-21.

Hong, J., et al. (2003). Involvement of multidrug resistance-associated proteins in regulating cellular levels of (−)-epigallocatechin-3-gallate and its methyl metabolites. *Biochem. Biophys. Res. Commun.,* **310**, 222-227.

Hooijberg, J., et al. (1997). Modulation by (iso) flavonoids of the ATPase activity of the multidrug resistance protein. *FEBS lett.*, **413**, 344-348.

Hooijberg, J., et al. (1999). Potent interaction of flavopiridol with MRP1. *Br. J. Cancer,* **81**, 269.

Howes, J., et al. (2002). Long-term pharmacokinetics of an extract of isoflavones from red clover (Trifolium pratense). *J. Altern. Complement. Med.* **8**, 135-142.

Imai, Y., et al. (2004). Phytoestrogens/flavonoids reverse breast cancer resistance protein/ABCG2-mediated multidrug resistance. *Cancer Res.,* **64**, 4346-4352.

Jager, W., et al. (2003). Biliary excretion of flavopiridol and its glucuronides in the isolated perfused rat liver: role of multidrug resistance protein 2 (Mrp2). *Life sci.*, **73**, 2841-2854.

Jager, W., et al. (1997). Modulation of liver canalicular transport processes by the tyrosine-kinase inhibitor genistein: implications of genistein metabolism in the rat. *Hepatology,* **26**, 1467-1476.

Janneh, O., et al. (2009). P-glycoprotein, multidrug resistance-associated proteins and human organic anion transporting polypeptide influence the intracellular accumulation of atazanavir. *Antivir. Ther.,* **14**, 965-974.

Jeong, H. J., et al. (1999). Inhibition of aromatase activity by flavonoids. *Arch. Pharm. Res.,* **22**, 309-312.

Jiang, W., et al. (2012). Mutual interactions between flavonoids and enzymatic and transporter elements responsible for flavonoid disposition via phase II metabolic pathways. *RSC Adv.*, **2**, 7948-7963.

Keung, W. M., et al. (1993). Daidzin: a potent, selective inhibitor of human mitochondrial aldehyde dehydrogenase. *Proc. Natl. Acad. Sci. U S A*, **90**, 1247-1251.

Kishida, T., et al. (2004). Lack of an inducible effect of dietary soy isoflavones on the mRNA abundance of hepatic cytochrome P-450 isozymes in rats. *Biosci. Biotechnol. Biochem.*, **68**, 508-515.

Kishida, T., et al. (2000). Soy isoflavonoid aglycons genistein and daidzein Do not increase the cytochrome P-450 content of the liver microsomes of mice. *J. Agric. Food Chem.*, **48**, 3872-3875.

Ko, W. C., et al. (2011). Biochanin a, a phytoestrogenic isoflavone with selective inhibition of phosphodiesterase 4, suppresses ovalbumin-induced airway hyperresponsiveness. *Evid. Based Complement. Alternat. Med.*, **2011**, 635058.

Ko, W. C., et al. (2004). Inhibitory effects of flavonoids on phosphodiesterase isozymes from guinea pig and their structure-activity relationships. *Biochem. Pharmacol.*, **68**, 2087-2094.

Li, X., et al. (2007). Effect of genistein on the pharmacokinetics of paclitaxel administered orally or intravenously in rats. *Int. J. Pharm.*, **337**, 188-193.

Limtrakul, P., et al. (2005). Inhibition of P-glycoprotein function and expression by kaempferol and quercetin. *J. Chemother.*, **17**, 86-95.

Lin, J. H., et al. (2003). Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin. Pharmacokinet.,* **42**, 59-98.

Martin, H. J., et al. (2003). The inhibitory effects of flavonoids and antiestrogens on the Glut1 glucose transporter in human erythrocytes. *Chem. Biol. Interact.,* **146**, 225-235.

Merino, G., et al. (2010). In vivo inhibition of BCRP/ABCG2 mediated transport of nitrofurantoin by the isoflavones genistein and daidzein: a comparative study in Bcrp1 (-/-) mice. *Pharm. Res.,* **27**, 2098-2105.

Mesia-Vela, S., et al. (2003). Inhibition of rat liver sulfotransferases SULT1A1 and SULT2A1 and glucuronosyltransferase by dietary flavonoids. *Xenobiotica,* **33**, 1211-1220.

Mirsalis, J. C., et al. (1993). Effects of soya bean flakes and liquorice root extract on enzyme induction and toxicity in B6C3F1 mice. *Food Chem. Toxicol.,* **31**, 343-350.

Mitsunaga, Y., et al. (2000). Effect of bioflavonoids on vincristine transport across blood-brain barrier. *Eur. J. Pharmacol.,* **395**, 193-201.

Mizoyama, Y., et al. (2004). Inhibitory effect of flavonoids on N-acetylation of 5-aminosalicylic acid in cultured rat hepatocytes. *Biol. Pharm. Bull.,* **27**, 1455-1458.

Mrozikiewicz, P. M., et al. (2010). The influence of a standardized soybean extract (Glycine max) on the expression level of cytochrome P450 genes in vivo. *Ginekol Pol.,* **81**, 516-520.

Nakajima, M., et al. (2006). Isoflavones Inhibit Nicotine C-Oxidation Catalyzed by Human CYP2A6. *J. Clin. Pharmacol.,* **46**, 337-344.

Natarajan, K., et al. (2012). Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance. *Biochem. Pharmacol.,* **83**, 1084-1103.

Nestel, P., et al. (2004). A biochanin-enriched isoflavone from red clover lowers LDL cholesterol in men. *Eur. J. Clin. Nutr.,* **58**, 403-408.

Nguyen, H., et al. (2003). Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. *J Pharm. sci.,* **92**, 250-257.

Ni, Z., et al. (2010). Structure and function of the human breast cancer resistance protein (BCRP/ABCG2). *Curr. Drug Metab.*, **11**, 603-617.

Park, J. S., et al. (2011). Antioxidant mechanism of isoflavone metabolites in hydrogen peroxide-stimulated rat primary astrocytes: critical role of hemeoxygenase-1 and NQO1 expression. *J. Neurochem.*, **119**, 909-919.

Patel, R. P., et al. (2010). Isoflavones and PPAR Signaling: A Critical Target in Cardiovascular, Metastatic, and Metabolic Disease. *PPAR Res.,* **2010**, 153252.

Paul, S., et al. (1996). Structure and in vitro substrate specificity of the murine multidrug resistance-associated protein. *Biochemistry*, **35**, 13647-13655.

Penetar, D. M. et al. (2006). Pharmacokinetic profile of the isoflavone puerarin after acute and repeated administration of a novel kudzu extract to human volunteers. *J Altern. Complement. Med.* **12**, 543-548.

Peng, S. X., et al. (2006). Altered oral bioavailability and pharmacokinetics of P-glycoprotein substrates by coadministration of biochanin A. *J. Pharm. Sci.*, **95**, 1984-1993.

Peng, W. X., et al. (2003). Effect of daidzein on CYP1A2 activity and pharmacokinetics of theophylline in healthy volunteers. *Eur. J. Clin. Pharmacol.,* **59**, 237-241.

Perepechaeva, M. L., et al. (2012). In vivo effects of genistein, herbimycin a and geldanamycin on rat hepatic cytochrome P4501A.

Perez, M., et al. (2013). Inhibition of ABCG2/BCRP transporter by soy isoflavones genistein and daidzein: Effect on plasma and milk levels of danofloxacin in sheep. *Vet. J.*, **196**, 203-208.

Perez, M., et al. (2009). Milk secretion of nitrofurantoin, as a specific BCRP/ABCG2 substrate, in assaf sheep: modulation by isoflavones1. *J. Vet. Pharmacol. Ther.*, **32**, 498-502.

Roberts, D. W., et al. (2004). Inhibition of extrahepatic human cytochromes P450 1A1 and 1B1 by metabolism of isoflavones found in Trifolium pratense (red clover). *J. Agric. Food Chem.*, **52**, 6623-6632.

Ruan, J. Q., et al. (2014). Regioselective glucuronidation of the isoflavone calycosin by human liver microsomes and recombinant human UDP-glucuronosyltransferases. *Chem. Biol. Interact.*, **220**, 231-240.

Rufer, C. E., et al. (2006). Antioxidant activity of isoflavones and their major metabolites using different in vitro assays. *J. Agric. Food Chem.*, **54**, 2926-2931.

Sergent, T., et al. (2005). Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. *Toxicol. Lett.*, **159**, 60-70.

Sheikh, S., et al. (1997). Allosteric inhibition of human liver aldehyde dehydrogenase by the isoflavone prunetin. *Biochem. Pharmacol.*, **53**, 471-478.

Sun, X. Y., et al. (1998). Increased UDP-glucuronosyltransferase activity and decreased prostate specific antigen production by biochanin A in prostate cancer cells. *Cancer Res.*, **58**, 2379-2384.

Takimoto, C. H., et al. (2003). Phase I pharmacokinetic and pharmacodynamic analysis of unconjugated soy isoflavones administered to individuals with cancer. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 1213-1221.

Tamaki, H., et al. (2010). Inhibitory effects of herbal extracts on breast cancer resistance protein (BCRP) and structure-inhibitory potency relationship of isoflavonoids. *Drug Metab. Pharmacokinet.*, **25**, 170-179.

Tolson, A. H., et al. (2010). Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv. Drug Deliv. Rev.*, **62**, 1238-1249.

Toyoda, Y., et al. (2008). MRP class of human ATP binding cassette (ABC) transporters: historical background and new research directions. *Xenobiotica*, **38**, 833-862.

US FDA. (2012). Guidance for industry drug interaction studies---study design, data analysis, implications for dosing, and labeling recommendations. Food and Drug Administration: Rockville, MD.

Urquhart, B. L., et al. (2007). Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J. Clin. Pharmacol.*, **47**, 566-578.

Veitch, N. C. (2013). Isoflavonoids of the leguminosae. *Nat. Prod. Rep.*, **30**, 988-1027.

Vera, J. C., et al. (2001). Direct inhibition of the hexose transporter GLUT1 by tyrosine kinase inhibitors. *Biochemistry*, **40**, 777-790.

Versantvoort, C., et al. (1996). Acceleration of MRP-associated efflux of rhodamine 123 by genistein and related compounds. *Br. J. Cancer*, **74**, 1949.

Versantvoort, C., et al. (1993). Genistein modulates the decreased drug accumulation in non-P-glycoprotein mediated multidrug resistant tumour cells. *Br. J. Cancer,* **68**, 939.

Versantvoort, C. H., et al. (1994). Competitive inhibition by genistein and ATP dependence of daunorubicin transport in intact< i> MRP</i> overexpressing human small cell lung cancer cells. *Biochem. Pharmacol.,* **48**, 1129-1136.

Vincent, A., et al. (2000). Soy isoflavones: are they useful in menopause? *Mayo Clin. Proc.,* **75**, 1174-1184.

Vitale, D. C., et al. (2013). Isoflavones: estrogenic activity, biological effect and bioavailability. *Eur. J. Drug Metab. Pharmacokinet.,* **38**, 15-25.

Wahajuddin, et al. (2013). Disposition of pharmacologically active dietary isoflavones in biological systems. *Curr. Drug Metab.,* **14**, 369-380.

Wang, Q., et al. (2007). Flavonoids modulate monocarboxylate transporter-1-mediated transport of gamma-hydroxybutyrate in vitro and in vivo. *Drug Metab. Dispos.,* **35**, 201-208.

Wang, X., et al. (2005). Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators. *Drug Metab. Dispos.,* **33**, 1666-1672.

Wiegand, H., et al. (2009). Effect of dietary genistein on Phase II and antioxidant enzymes in rat liver. *Cancer Genomics Proteomics,* **6**, 85-92.

Wong, C. K., et al. (1997). Daidzein sulfoconjugates are potent inhibitors of sterol sulfatase (EC 3.1.6.2). *Biochem. Biophys. Res. Commun.,* **233**, 579-583.

Yang, Z., et al. (2012). Bioavailability and pharmacokinetics of genistein: mechanistic studies on its ADME. *Antican. Agents Med. Chem.,* **12**, 1264-1280.

Zelcer, N., et al. (2003). Evidence for two interacting ligand binding sites in human multidrug resistance protein 2 (ATP binding cassette C2). *J. Biol. Chem.*, **278**, 23538-23544.

Zhang, S., et al. (2003a). Effect of the flavonoids biochanin A and silymarin on the P-glycoprotein-mediated transport of digoxin and vinblastine in human intestinal Caco-2 cells. *Pharm. Res.*, **20**, 1184-1191.

Zhang, S., et al. (2003b). Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J. Pharmacol. Exp. Ther.*, **304**, 1258-1267.

Zhang, S., et al. (2010). Interactions between the flavonoid biochanin A and P-glycoprotein substrates in rats: in vitro and in vivo. *J. Pharm. Sci.*, **99**, 430-441.

Zhang, S., et al. (2004a). Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. *Pharm. Res.*, **21**, 1263-1273.

Zhang, S., et al. (2004b). Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. *Mol. Pharmacol.*, **65**, 1208-1216.

Zhang, X., et al. (2010). Effects of ipri fl avone on postmenopausal syndrome and osteoporosis. *Gynecol. Endocrinol.*, **26**, 76-80.

Zhang, Y., et al. (2004). A strategy for cancer prevention: stimulation of the Nrf2-ARE signaling pathway. *Mol. Cancer Ther.*, **3**, 885-893.

Zhang, Y., et al. (2014). LC-MS/MS determination and pharmacokinetics study of puerarin and daidzein in rat plasma after oral administration of Gegenqinlian decoction and Radix Puerariae extract. *Pharmacogn. Mag*. **10,** 241-248.

Zheng, J., et al. (2010). The effects of puerarin on CYP2D6 and CYP1A2 activities in vivo. *Arch Pharm. Res.*, **33**, 243-246.

Table 1: Classification and common sources of isoflavones.

INLINEFIG#3-phenylchromen-4-one (backbone of isoflavones)		
Isoflavone	**Substitutions**	**Source**
Genistein	5-OH, 7-OH, 4'-OH	*Glycine max*
Daidzein	7-OH, 4'-OH	*Glycine max*
Irilone	9-OH, 4'-OH, [1,3]dioxolo	*Iris unguicularis, Iris germanica*
Orobol	5-OH, 7-OH, 3'-OH, 4'-OH	*Aspergillus niger*
Pseudobaptigenin	7-OH, 3',4'-methylenedioxy	*Trifolium pratense*
O-methylated isoflavones		
Biochanin A	5-OH, 7-OH, 4'-OCH_3	*Trifolium pratense*
Formononetin	7-OH, 4'-OCH_3	*Trifolium pratense*
Glycitein	7-OH, 4'-OCH_3, 6-OCH_3	*Glycine max*
Cajanin	5-OH, 2'-OH, 4'-OH, 7-OCH_3	*Butea monosperma*
Isoformononetin	7-OCH_3, 4'-OH	*Butea monosperma*
Calycosin	7-OH, 3'-OH, 4'-OCH_3	*Trifolium pratense, Astralagus membranaceus*
Irigenin	5-OH, 7-OH, 6-OCH_3, 3'-OH, 4'-OCH_3, 5'-OCH_3	*Belamcanda chinensis*
Pratensein	5-OH, 7-OH, 3'-OH, 4'-OCH_3	*Trifolium pratense*
Prunetin	5-OH, 7-OCH_3, 4'-OH	*Prunus emarginata*

Isoprunetin	5-OCH_3, 7-OH, 4-OH	*Ormosia excels*
Tectorigenin	5-OH, 7-OH, 6-OCH_3, 4'-OH	*Belamcanda chinensis*
Psi-tectorigenin	5-OH, 7-OH, 8- OCH_3, 4-OH	*Belamcanda chinensis, Dalbergia sissoo*
Retusin	7- OH, 8-OH, 4-OCH_3	*Dipteryx odorata*
Glycosides		
Genistin	5-OH, 4'-OH, 7-O-glucoside	*Glycine max*
Daidzin	4'-OH, 7-O-glucoside	*Glycine max*
Glycitin	7-OH, 4'-OCH_3, 6-OCH_3, 7-O-glucoside	*Glycine max*
Iridin	5-OH, 6-OCH_3, 3'-OH, 4'-OCH_3, 5'-OCH_3, 7-O-glucoside	*Iris florentina, Iris versicolor*
Ononin	4'-OCH_3, 7-O-glucoside	*Glycine max*
Puerarin	7-OH, 4'-OH, 8-C-glucoside	*Pueraria lobata*
Sissotrin	5-OH, 4'-OCH_3, 7-O-glucoside	*Cicer mogoltavicum*
Sophoricoside	5-OH, 7-OH, 4'-O-glucoside	*Sophora japonica*
Tectoridin	5-OH, 6-OCH_3, 4'-OH, 7-O-glucoside	*Pueraria thunbergiana*
Prenylated isoflavones		
Derrubone	5-OH, 7-OH, 3',4'-methylenedioxy, 6-prenyl	*Derris robusta*
Bidwillol A	7-OH, 2'-CH_3, 4'-OH, 3'-prenyl	*Erythrina orientalis*
Luteone	5-OH, 7-OH, 2'-OH, 4'-OH, 6-prenyl	*Laburnum anagyroides*

7-O-Methylluteone	5-OH, 7-OCH_3, 2'-OH, 4'-OH, 6-prenyl	*Erythrina burttii*
Wighteone	5-OH, 7-OH, 4'-OH, 6-prenyl	*Maclura aurantiaca*
Licoisoflavone A	5-OH, 7-OH, 2'-OH, 4'-OH, 3'-prenyl	*Glycyrrhiza eurycarpa*
Pyranoisoflavones		
Alpinumisoflavone	2'-OCH_3, 4'-OCH_3, 5'-OCH_3, 6",6"-dimethylpyrano(2",3":6,7)	*Rinorea welwitschi*
Barbigerone	2'-OCH_3, 4'-OCH_3, 5'--OCH_3, 6",6"-dimethylpyrano(2",3":7,8)	*Millettia pachycarpa*
Sophoraisoflavone	6-OH, 2'-OH,4'-OH,6",6"-dimethylpyrano(2",3":7,8)	*Sophra mooracroftiana*
Synthetic		
Ipriflavone	7-$CH(CH_3)_2$	

Table 2: Interactions of isoflavones with drug metabolizing enzymes

Mechanism	Interacting isoflavone	Object drug	Study model	Outcome	Reference
Inhibition of CYP19A1 (aromatase)	Biochanin A, Pruneti n	Androstenedione	Human placental microsomes	Decreased radioactivity	(Jeong et al., 1999)
Induction of CYP1A2, 3A1, 2B1 and	Radix Puerariae	p-nitrophenol (CYP2E1), ethoxyresorufin (CYP1A1), methoxyresorufin	Wistar rats	Increase in the activity of methoxy-O-demethylase, ethoxycoumarin-O-deethylase, 6α-testosterone hydroxylase, 2α-testosterone hydroxylase, 16α-	(Guerra et al., 200

inactivation of CYP2E1, 2B1	crude extract	(CYP1A2), pentoxyresorufin (CYP2B1), testosterone (CYP2A1, 3A1, 2C11, 2B1)		testosterone hydroxylase, 6β-testosterone hydroxylase, 16β-testosterone hydroxylase, 2β-testosterone hydroxylase,	0)
Induction of CYP2A1, 1A1/2, 3A1, 2C11 and inactivation of CYP2E1, 2B1	Puerarin			Decrease in the activity of p-nitrophenol hydroxylase, ethoxyresorufin-O-deethylase, petnoxyresorufin-O-dealkylase, 7α-testosterone hydroxylase,	
Inhibition of CYP1A2	Daidzein	Theophylline	Human volunteers	Significant decrease in caffeine metabolic ratio	(Peng et al., 2003)
Inhibition	Hy	(S)-Warfarin	Human	Inhibition of (S)-Warfarin	(An

of CYP2C9	dro lys ed soy extr act		liver microsomes	hydroxylation (IC_{50} = 2.6 µM)	ders on et al., 200 3)
Inhibition of CYP3A4		Testosterone		Inhibition of testosterone hydroxylation (IC_{50} = 12.2 µM)	
Inhibition of CYP1B1	Bio cha nin A, For mo non etin	7-Ethoxyresorufin	Recombina nt hCYP1B1	Inhibition of 7-Ethoxyresorufin deethylation	(Ro bert s et al., 200 4)
Inhibition of CYP1A2	Ge nist ein	Tamoxifen	Rat liver microsomes	Inhibition of tamoxifen-α-hydroxylation (K_i = 10.6 µM)	(Ch en et al., 200 4)
Inhibition	Ge	Nicotine	Recombina	Inhibition of CYP2A6 (K_i = 0.7	(Na

of CYP2A6	nistein, Daidzein, Glycitein		nt hCYP2A6	μM for genistein, 1.3 μM for daidzein, 5.2 μM for glycitein)	kajima et al., 2006)
			Human CYP2A6*1 homozygotes	Significantly reduced cotinine/nicotine plasma ratio	
Inhibition of CYP3A4/ P-gp	Genistein	Paclitaxel	Rats	Greater AUC and lower clearance	(Li and Choi, 2007)
Inhibition of CYP2D6	Puerarin	Metoprolol	Human volunteers	Decreased urinary metabolic rate of metoprolol to α-hydroxymetoprolol	(Zheng et al., 2010)
Induction of CYP1A2		Caffeine		Increased plasma paraxanthin/caffeine ratio	
Induction	Bio	Testosterone	LNCaP	Increased formation of	(Su

of UDPGT	chaninA		cells	gtestosterone glucuronides	n et al., 1998)
Inhibition of UGT2B15	Unhydrolysed soy extract	Dihydrotestosterone	Human liver microsomes	Inhibition of dihydrotestosterone glucuronidation (IC_{50} = 4.6 µM)	(Anderson et al., 2003)
	Hydrolysed soy extract			Inhibition of dihydrotestosterone glucuronidation (IC_{50} = 6.1 µM)	
Inhibition of human	Genist	p-Nitrophenol, Acetaminophen	Recombinant P-form	Inhibition of sulfation (IC_{50} = 2 µM)	(Eaton

P-form phenol sulfotransferase	ein		phenol sulfotransferase, partially purified P-form phenol sulfotranferase		et al., 1996)
Inhibition of human phenol sulfotransferase	Genistein	p-Nitrophenol	Human platelet homogenate	Decreased formation of p-nitrophenol sulfate (K_i = 0.21 µM)	(Ghazali and Waring, 1999)
	Daidzein			Decreased formation of p-nitrophenol sulfate (K_i = 0.34 µM)	
Inhibition of SULT1A1, SULT2A	Genistein	Oestradiol	Recombinant rat SULT1A1, Rat liver cytosol	Decreased formation of oestrogen sulfate (K_i = 1.6 µM)	(Mesia-Vela and
	Daidze			Decreased formation of oestrogen sulfate (K_i = 0.7 µM)	

1	in				Kauffman, 2003)
Inhibition of human SULT1A1	Genistein, daidzein	4-Nitophenol, Dopamine	Human platelet cytosol	Inhibition of sulfation (IC_{50} = 0.5 µM for genistein, 0.6 µM for daidzein)	(Harris et al., 2004)
Inhibition of human SULT1A3	3',4',7-Trihydroxyisoflavone	Dopamine	Human platelet cytosol	Inhibition of sulfation (IC_{50} = 2 µM)	
Inhibition	Ge	Estradiol	Human	Inhibition of sulfation (K_i = 0.5	

of human SULT1E1	nistein		liver cytosol	μM)	
Induction of SULT1A1, SULT2A1	Genistein	--	HepG2, Caco-2 cells	Time and dose-dependent induction of mRNA and protein expression	(Chen et al., 2008)
Induction of rSULT1A1	Biochanin A	2-Naphthol	Rat liver cytosol	Inhibition of sulfation	(Chen et al., 2010)
Induction of rSULT2A1		DHEA	Rat intestine cytosol		
Inhibition of sterol sulfatase	Daidzein-4'-	Dehydroepiandrosterone sulfate	Hamster liver microsomes	Inhibition of DHEA sulfate hydrolysis (K_i = 5.9 μM)	(Wong and Keu

	O-sulfate				ng, 1997)
	Daidzein-7,4'-O-sulfate			Inhibition of DHEAS sulfate hydrolysis (K_i = 1 µM)	
Inhibition of mitochondrial aldehyde dehydrogenase (ALDH1 and ALDH 2)	Daidzin	Acetaldehyde	Purified human ALDH 1 and ALDH 2 mixture	Inhibition of acetaldehyde metabolism (K_i = 20 nM)	(Keung and Vallee, 1993)

Inhibition of mitochondrial aldehyde dehydrogenase (ALDH2)	Prunetin	--	Recombinant human aldehyde dehydrogenase	Inhibition of ALDH2 (K_i = 2 µM)	(Sheikh and Weiner, 1997)
Inhibition of phosphodiesterase 4	Biochanin A	--	Isolated guinea pig trachealis	Inhibition of OVA-induced contractions and suppression of OVA-specific IgE serum levels (IC_{50} = 8.5 µM; EC_{50} > 300 µM)	(Ko et al., 2011)

Table 3: Interactions of isoflavones with drug transporters

Mechanism	Interacting isoflavone	Object drug	Study model	Outcome	Reference
Non-Pgp mediated inhibition of drug transporter	Genistein, Biochanin A	Daunorubicin (DNR)	GLC4/ADR, SW-1573/2R120, HT1080/DR4, MCF7/Mitox, HL60/ADR cells	Increased accumulation	(Versantvoort et al., 1993)
Inhibition of P-gp	Genistein	Rhodamine 123 (Rh123), DNR	BC19/3 cells	Increase in Rh123 accumulation Elevated DNR accumulation.	(Castro and Alt
			MCF-7/VP cells	Elevated DNR accumulation.	

			BALB/c-3T3-1000	Increased Rh123 accumulation. Elevated DNR accumulation.	enberg, 1997)
	Biochanin A	Digoxin	Caco-2 cells	Mean transport ratio of digoxin decreased	(Zhang and Morris, 2003a)
	Biochanin A	Daunomycin	MCF-7/ADR	Increased accumulation	(Zhang and
		Doxorubicin	MDA435/LCC6 MDR1	Increased cytotoxicity	

					Morris, 2003b)
	Genistein, Daidzein	Vinblastine, Paclitaxel	KB-V1 cells	Dose dependent increased senstivity to vinblastine and paclitaxel	(Limtrakul et al., 2005)
	Biochanin A	Paclitaxel	Rats	Increase in oral bioavailability and peak plasma concentration	(Peng et al., 2006
		Digoxin		Increase in oral bioavailability and peak plasma	

				concentration	)
		Doxorubicin		Increased cytotoxicity	
	Biochanin A	Mitoxantrone (MTX)	P-gp expressing MCF-7/ADR cells	Significantly decreased (31.9%) intracellular flourescence of MTX	(An and Morris, 2010)
	Puerarin	Adriamycin	MCF-7/adr	Increased cytotoxicity	(Hien et al., 2010)
		Rh123	MCF-7/adr	Increased flourescence	
Stimulation of P-gp	Genistein	MTX, SN-38	K562/BCRP cells	Increased cytotoxicity	(Imai et

					al., 2004)
Inhibition of BCRP	Genistein	DNR	MRP over expressing GLC4/ADR cells	Decreased efflux	(Versantvoort et al., 1994)
	Biochanin A, Genistein	MTX	MCF-7 MX100 cells	Increased cytotoxicity	(Zhang et al., 2004a)

	Biochanin A	MTX	MCF-7 MX100 cells, NCI-H460 MX20 cells	Increased accumulation and cytotoxicity	(Zhang et al., 2004b)
	Daidzein	MTX, Bodipy-FL-prazosin	MCF7/MR cells, K562/BCRP cells	Increased accumulation	(Cooray et al., 2004)
	Modified isoflavone diet	Nitrofurantoin	Assaf sheep	Decreased secretion in milk	(Perez et al., 20

					09)
	Genistein, Daidzein	Nitrofurantoin	MDCK-Bcrp1	Significantly decreased mean transport ratio	(Meri no et al., 20 10)
			Bcrp-/- mice	Signifcantly higher plasma levels, lower milk secretion and decreased bile excretion in wild type	
	Biochanin A	MTX	MDCK/hBCRP cells	Significantly increased intracellular levels	(An and Morris, 20 10)
			MDCK/murine Bcrp cells		
			MDCK/Bcrp1 cells	Decreased $P_{app,B-A}$ value.	
	Soyabean extract	MTX	BCRP-expressing membrane	Inhibition of MTX transport	(Tam

			vesicles		aki et al., 2010)
	Genistein, Daidzein	Danofloxacin	MDCK/BCRP cells, MDCK/Bcrp cells	Decreased transport	(Perez et al., 2013)
			Ewes	Decreased milk/plasma ratio	
Inhibition of Pgp/MRP	Genistein, Daidzein	Atazanavir	CEMVBL cells	Decreased accumulation	(Jannehet al., 2009)

Inhibition of MRP	Genistein	DNR	COR-L23/R	Increased accumulation	(Versantvoort et al., 1996)
		Rh123	MOR/R	Decreased accumulation.	
Inhibition of MRP	Genistein, Biochanin A	Daunomycin, Vinblastine	Panc-1 cells	Increased accumulation of DNM and VBL	(Nguyen et al., 2003)
Inhibition of MRP	Genistein, Biochanin A	Ochratoxin A (OTA)	Caco-2 cells		(Sergent

					et al., 2005)
Inhibition of MRP1	Genistein	DNR	pSRα-muMRP-66-16-6 NIH3T3 membrane vesicles	Decreased uptake	(Paul et al., 1996)
Inhibition of MRP1	Flavopiridol	DNR	GLC4/ADR cells	Increased accumulation	(Hooijberg et al., 1999)
Inhibition	Genistein,	2',7'-bis-	Human	Decreased efflux of	(B

of MRP1	Daidzein, Sophoraisoflavone, Licoisoflavone	(carboxypropyl)-5(6)-carboxyfluorescein (BCPCF)	erythrocytes	BCPCF	obrowska-Hagerstrand et al., 2001)
Inhibition of MRP1/2	Genistein, Daidzein, Biochanin A	(-)-epigallocatechin-3-gallate (EGCG)	MDCKII/MRP1, HT-29 cells	Significantly increased accumulation of EGCG	(Hong et al., 2003)

Inhibition of MRP1/2	Genistein, Daidzein	Atazanavir	CEME1000 cells	Increases uptake	(Jannehet al., 2009)
Inhibition of MRP2	Genistein, Daidzein	Glucuronides of bilirubin, Glutathione conjugates of bromsuphthalein	Isolated perfused TR+ and TR- rat liver	Decreased biliary excretion	(Jageret al., 1997)
Inhibition of Mrp2	Flavopiridol	Glucuronides of bilirubin	Isolated perfused TR+ and TR- rat liver	Decreased biliary excretion	(Jageret al., 2003)

Inhibition of Oatp3	Biochanin A	Fexofenadine	Male sprague dawley rats	Decrease in oral bioavailability and peak plasma concentration	(Peng et al., 2006)
Inhibition of OATP1B1	Genistein, Biochanin A	Dehydroepiandrosterone sulfate (DHEAS)	OATP1B1-expressing HeLa cells	Decrease in uptake	(Wang et al., 2005)
Inhibition of OATP-B	Soyabean extract	Estrone-3-sulfate	HEK 293/OATP-B cells	Inhibition of uptake	(Fuchikami et al.,

					2006)
Inhibition of monocarboxylate transporter 1 (MCT1)	Genistein, Biochanin A	γ-hydroxybutyrate	Rat MCT1 gene transfected MDA-MB231 cells	Significantly decreased uptake of GHB	(Wang and Morris, 2007)
Inhibition of GLUT1	Genistein	Glucose	Human erythrocytes	Inhibition of glucose exit	(Afzal et al., 2002)
	Genistein	Dehydroascorbi	Human HL-60	Inhibition of transport	(V

		c acid, Deoxyglucose, Methylglucose	cells		era et al., 2001)

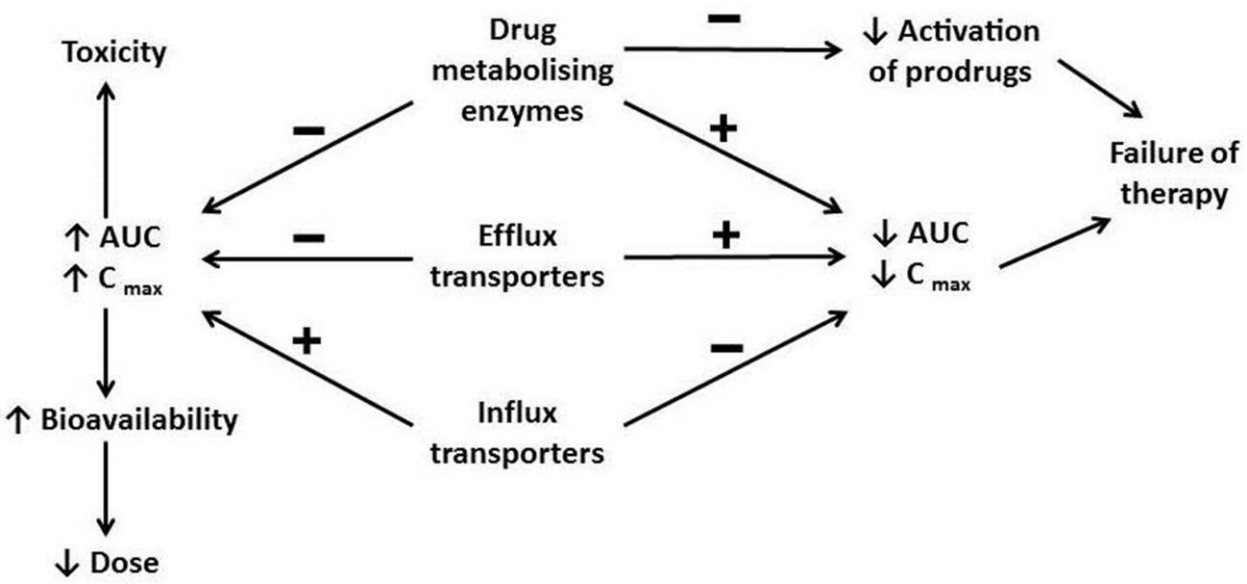

Figure 1: Probable outcomes of pharmacokinetic drug interactions

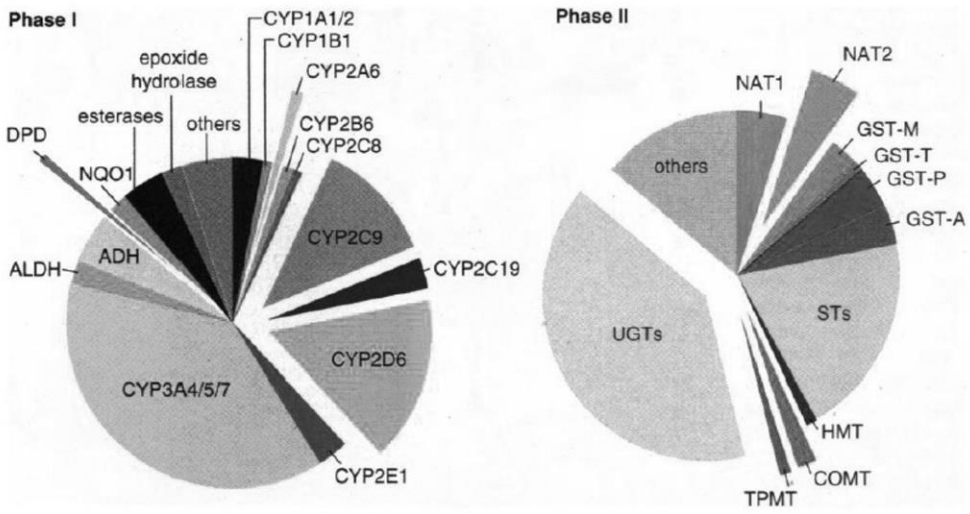

Figure 2: Drug metabolizing enzymes. The percentage of phase I and phase II metabolism of drugs that each enzyme contributes is estimated by the relative size of each section of the corresponding chart. ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; CYP: cytochrome P450; DPD: dihydropyrimidine-dehidrogenase; NQOI: NADPH:quinone oxidoreductase or DT: diaphorase; COMT: catechol O-methvltransferase; GST: glutathione S-transferase; HMT: histamine methvl-transferase; NAT: N-acetyl transferase; STs: sulfotransferases/SULTs; TPMT: thiopurine methyltransferse; UGTs: uridine 5'-triphosphate glucuronosyltransferases. (Adapted from Evans, W. E., et al. (1999). Science, 286, 487-491.)

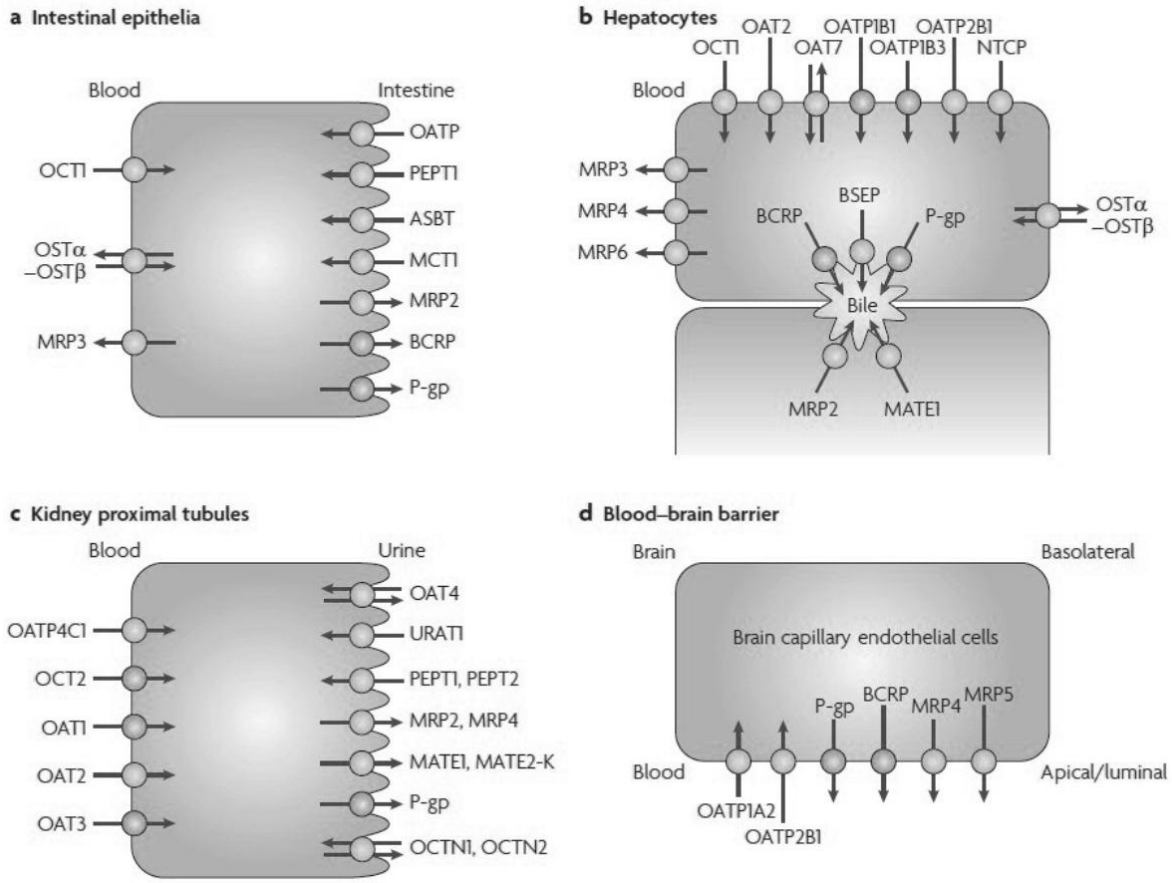

Figure 3: Transporters present in plasma membrane of intestinal epithelia, hepatocytes, kidney proximal tubules and brain capillary endothelial cells. ABC transporters (efflux transporters) present are P-gp/MDR1/ABCB1: P-glycoprotein; MRP2/ABCC2: multidrug resistance protein 2; MRP3/ABCC3: multidrug resistance protein 3; BCRP/ABCG2: breast cancer resistance protein; BSEP/SPGP/ABCB11: bile-salt export pump and SLC transporters (influx transporters) present are OATP: organic anion transporting polypeptide; OCT: organic cation transporter; OAT: organic anion transporter; PEPT: peptide transporter 1; MCT1: monocarboxylic acid transporter 1; ASBT: ileal apical sodium/bile acid co-transporter; OSTα--OSTβ: heteromeric organic solute

transporter; NTCP: sodium-taurocholate co-transporting peptide; MATE: multidrug and toxin extrusion protein; URAT: urate transporter. (Adapted from Giacomini, K. M., et al. (2010). Nat Rev Drug Discov, 9, 215-236.)

