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Review

An important mechanism of herb-induced hepatotoxicity: To produce RMs based on active functional groups-containing ingredients from phytomedicine by binding CYP450s

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ABSTRACT

Reactive metabolites (RMs) generated by hepatic metabolism are thought to play an important role in the pathogenesis of drug-induced liver injury (DILI). Like many synthetic drugs undergoing metabolic activation to form RMs which are often associated with drug toxicity, it is recognized that some herbal components may be also converted to toxic, or even mutagenetic and carcinogenic metabolites by cytochrome P450s (CYP450s). This review focuses on the metabolic activation of herbal components and its liver toxicological implications. By summarizing references, we found that hepatotoxic herbal components via producing RMs have some certain structural dependence. There is a correlation between the generation of RMs and the structures, which provides a good chance for the early discovery of toxic ingredients in Traditional Chinese medicines (TCMs): i) A potential hepatotoxic component information database based on active functional groups can be built, which might provide an early information for the basic research of hepatotoxic substances in TCMs; ii) RMs can combine with CYP450s to form a complete antigen, which eventually leads to an antigen-specific immune response. RMs-CYP450 protein complete antigen can be set up, and the potential idiosyncratic liver toxicity might be predicted by testing RMs-CYP450 protein antibody in plasma.

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1. Introduction

RUCAM (Roussel Uclaf Causality Assessment Method) or its previous synonym CIOMS (Council for International Organizations of Medical Sciences) is a well established tool in common use to quantitatively assess the causality in cases of suspected drug induced liver injury (DILI) and herb induced liver injury (HILI). In this assessment system, drug-induced liver injury (DILI) can be broadly categorized as idiosyncratic DILI and intrinsic DILI. Idiosyncratic DILI is an adverse drug reaction in the liver with a dose independency, which cannot be detected in preclinical testing or clinical trials, while intrinsic DILI is predictable dues to drug-induced direct hepatotoxicity with clear dose dependency. Idiosyncratic DILI is divided into two categories including metabolic type and immunologic type. Metabolic type occurs after one week to several months in individuals, which is differentiated from immunologic type by lack of hypersensitivity features and delayed response to reexposure (weeks). On the country, liver injury is induced after few weeks in immunologic type (Cavalieri & D'Agostino, 2017; Danan & Teschke, 2015; Sasaki & Yokoi, 2018; Teschke, Schulze, Eickhoff, & Danan, 2017).

Researches have shown that drugs cause liver injury mainly by two mechanisms. The first is the direct toxic effect of prototype drugs on the liver. Hepatotoxicity of most drugs is cumulative, and this depends on their concentration and exposure duration as well as the liver susceptibility and tolerance to the drug (Usui, Mise, Hashizume, Yabuki, & Komuro, 2009). The second is the toxic effect of reactive metabolites (RMs) on liver (Fig. 1). The human cytochrome P450 superfamily, containing at least 57 functional genes, can metabolize a variety of therapeutic drugs, carcinogens, steroids and eicosanoids. Drugs produce electrophilic RMs through cytochrome P450 enzymes. RMs can cause cell stress changes through a variety of mechanisms including depletion of glutathione (GSH), and binding to enzymes and other cell structures. Moreover, RMs may specifically inhibit other liver cell functions, in that case the subsequent intracellular accumulation of substances may cause secondary liver damage (Amacher, 2012).

Herbal medicines have often been increasingly used worldwide. However, the adverse reactions and side effects associated with the use of herbal medicines, especially their potential damaging effects on the liver, have increasingly been reported. Teschke and Eickhoff (2015) reported that there are 65 kinds of commonly used herbal drugs/supplements which had been linked with liver disease. On the other hand, coadministration of herbal medicines with conventional drugs, especially for drugs with narrow therapeutic indices (*e.g.* warfarin, theophylline and digoxin) and drugs that are substrates of CYPs and P-glycoprotein, raises the potential of herb-drug interactions which may cause altered drug elimination, under treatment and/or toxicity (Liu et al., 2011).

RMs generated by hepatic metabolism are thought to play an important role in the pathogenesis of DILI. A study of 207 of the most commonly prescribed oral medications in the US revealed that 62% to 69% of compounds with RM formation are associated with DILI. Like many synthetic drugs undergoing metabolic activation to form RMs which are often associated with drug toxicity, multiple herbal constituents from a number of herbal medicines/supplements can also bind to cytochrome P450 enzymes to produce RMs.

This review focuses on the metabolic activation of herbal components and their toxicological implications.

2. An indicator of RM formation based on mechanism-based inhibition (MBI)

RMs have several possible fates during liver metabolism. They can bind to cytochrome P450 enzymes to induce mechanism-based inhibition (MBI) through different mechanisms: 1) RMs can react with nucleophilic amino acids in the active site, 2) react with the porphyrin nitrogen atoms, or 3) coordinate with Fe (II) to form a metabolite intermediate (MI) complex.

In our previous paper, we summarized a regular pattern for RMs-induced hepatotoxicity from 29 mechanism-based synthetic drugs with clinical hepatotoxicity (Feng & He, 2013): i) formation of RM-protein adducts that trigger immune responses; ii) covalent binding of RMs to intracellular macromolecules (mitochondria is a common victim) which may lead to reactive oxygen metabolites (ROS) overproduction, respiratory chain dysfunction, cell stress, and so on; and iii) RM overproduction, resulting in GSH depletion. This paper suggested that mechanism-based inhibition is an indicator of RM formation and may thus be used to identify drugs with RM-induced hepatotoxic potential, particularly idiosyncratic drug-induced liver injury.

3. Common sub-structures in mechanism-based inhibitors that might produce RMs

Fontana, Dansette and Poli (2005) summarized 11 common active functional groups that might produce RMs including terminal and −1 acetylenes, furans and thiophene, epoxides, dichloro- and trichloro-ethylenes, secondary amines, benzodioxoles (methylene dioxyphenyl compounds), isothiocyanates, thioamides, dithiocarbamates, conjugated structures, and terminal alkenes (Table 1). Certain functional groups that exist in a drug play an important role in the activity/toxicity of the drug, and they have been specifically engineered to increase stability, solubility, or bioavailability. Some of functional groups have proved to be able to be catalyzed to potentially toxic reactive intermediates by specific cytochrome P450 enzymes.

Similarly, multiple herbal constituents from a number of herbal medicines/supplements can also bind to cytochrome P450 enzymes to induce RMs. Our previous paper (Wang, 2016) summarized that the amines and furan heterocyclic groups might be the most potential active functional groups that might produce RMs in ingredients from phytomedicine (Table 2 and Fig. 2).

4. Examples of herbal medicines-induced liver injury based on RMs

4.1. Psoraleae fructus

Psoraleae Fructus (Buguzhi in Chinese) is the dired fruits of *Psoralea corylifolia* L., containing a variety of coumarins such as

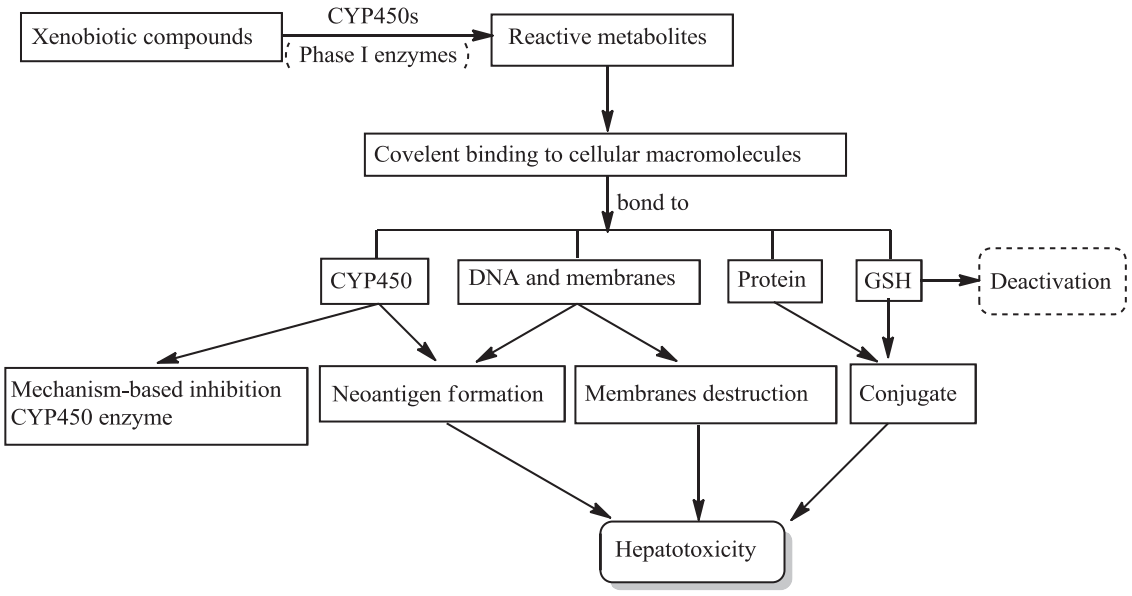


Fig. 1. Fates of RMs in liver and postulated mechanisms of RM-induced hepatotoxicity.

Table 1
Most common substructures causing mechanism-based inactivation of cytochromes P450.

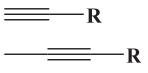
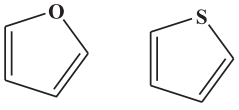
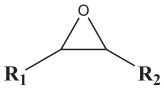
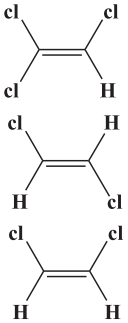
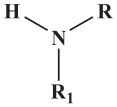
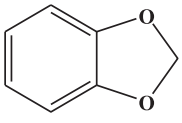
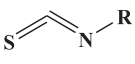
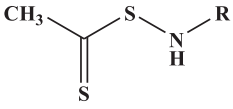
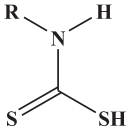
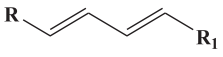
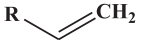
No.	Names	Chemical structures	References
1	Terminal (ω) and ω−1 acetylenes		Foroozesh (1997) and He, Woolf and Hollenberg (1999)
2	Furans and thiophenes		1998; O'Donnell; O'Donnell, Dalvie, Kalgutkar and Obach (2003)
3	Epoxides		Guo, Fukuda, Ohta and Yamazoe (2000)
4	Dichloro- and trichloro-ethylenes		Bourdi et al. (1996)
5	Secondary amines		Jones et al. (1999)
6	Benzodioxoles		Usia, Watabe, Kadota and Tezuka (2005)
7	Isothiocyanates		Nakajima Yoshida and Shimada (2001), Koudriakova et al. (1998)
8	Thioamides		Guengerich and Kim (1991), Kharasch, Hankins, Baxter and Thummel (1998), Kharasch, Hankins and Fenstamaker (2000)
9	Dithiocarbamates		Nakajima et al. (2001)
10	Conjugated structures		Chan and Delucchi (2000)
11	Terminal alkenes		De Groene, Nijmeijer, Horbach and Witkamp (1995)

Table 2
Most potential active functional groups that might produce RMs in ingredients from phytomedicine.

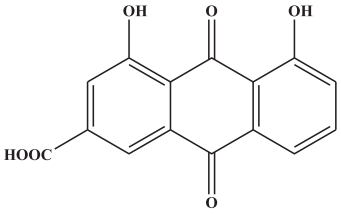
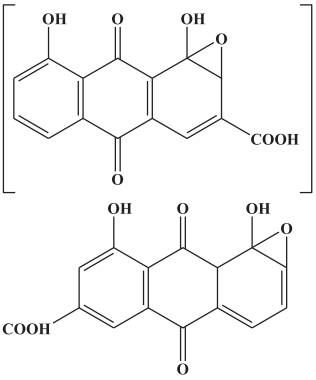
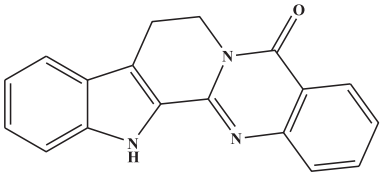
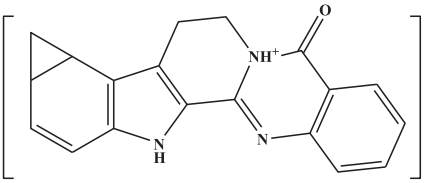
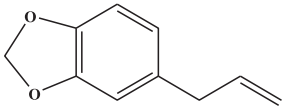
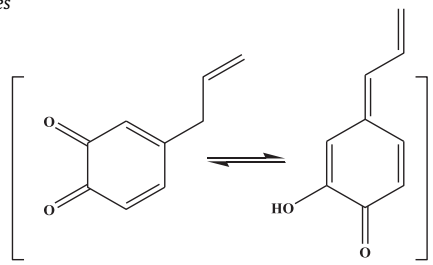
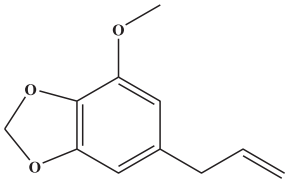
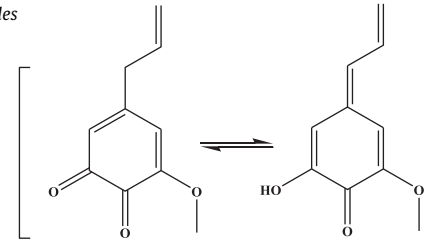
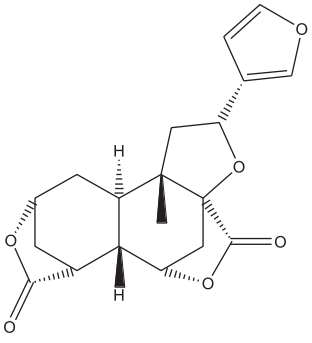
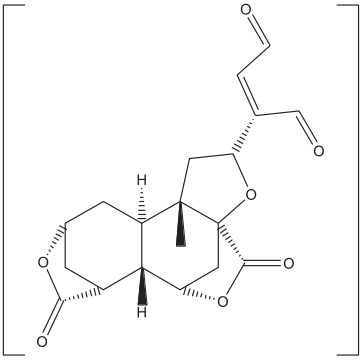
Active functional groups	Ingredients	Main medical herbs	Structures of RMs	References
 conjugated structures	 Rhein	*Polygonum multiflorum* Thunb		He et al. (2015)
	 Rutaecarpine	*Evodia rutaecarpa*		Zhang et al. (2015)
	 Safrole	*Asarumheterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag., *Zanthoxylum schinifolium* Sieb. et Zucc., *Senecio scandens* Buch. -Ham. etc.		Borchert, Wislocki, Miller and Miller (1973), Ioannides et al. (1975), Yang et al. (2017)
	 Myristicin	*Asarumheterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag., *Myristica fra grans* Houtt. etc.		Hallström et al. (1997), Murray (2012), Yang et al. (2015a)
 furan	 Diosbulbin B	Dioscorea bulbifera L.		Lin et al. (2014), Wang et al. (2017a), Li et al. (2016)

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Table 2 (*continued*)

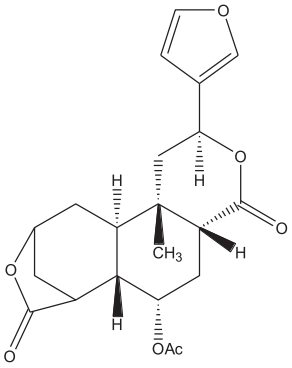
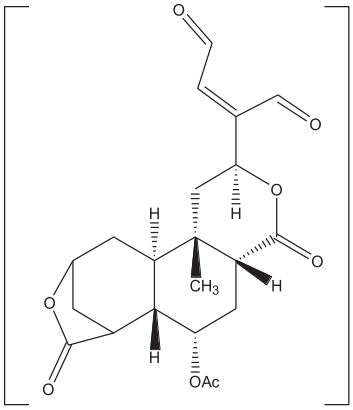
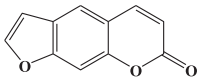
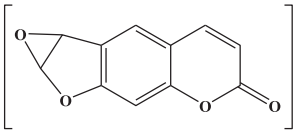
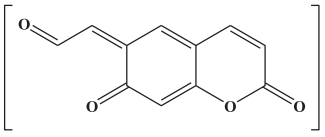
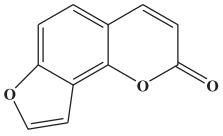
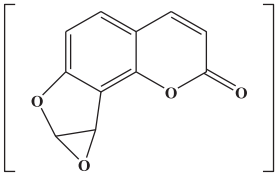
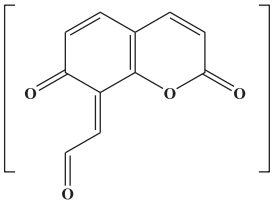
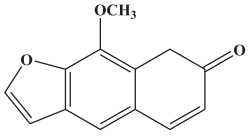
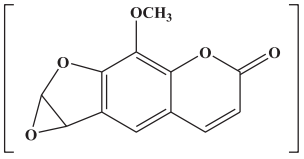
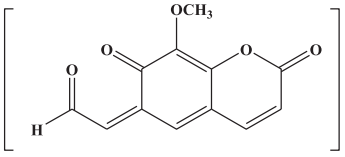
Active functional groups	Ingredients	Main medical herbs	Structures of RMs	References
	 8-epidiosbulbin E acetate			Lin et al. (2016a,b, 2015)
	 Psoralen	*Psoralea corylifolia* L.	 	Wang et al. (2012), Koenigs and Trager (1998a,b), Ji et al. (2015)
	 isopsoralen		 	Wang (2012), Lu et al. (2015)
	 8-methoxypsoralen		 	Koenigs and Trager (1998a,b), Nyfors, Dahl-Nyfors and Hopwood (1986), Pariser et al. (1980)

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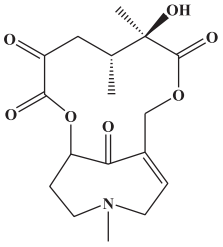
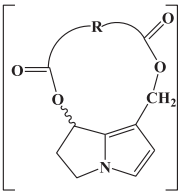
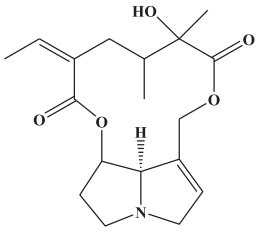
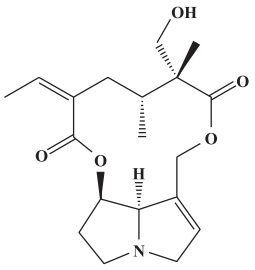
Table 2 (*continued*)

Active functional groups	Ingredients	Main medical herbs	Structures of RMs	References
	 Senkirkine	*Senecio scandens* Buch. -Ham *Senecio vulgaris* L.	 dehydro-PA	Luckert et al. (2015), Lin et al. (2009), Li et al. (2008b), Chen et al. (2016), Xiong et al. (2014)
	 Senecionine	Senecio vulgaris L.		
	 Retrorsine			

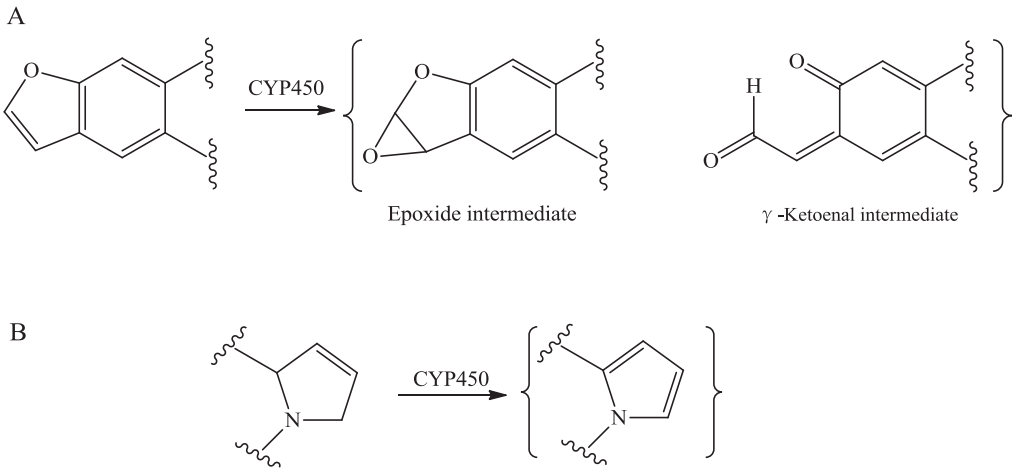


Fig. 2. Reaction of furan heterocycle containning (A) and amine-containning (B) ingredients producing RMs.

psoralen isopsoralen, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and bergamottin. It is generally accepted that they require metabolic activation before exerting toxic effects, *e.g.*, methimazole, ticlopidine, and tienilic acid. The furan ring structure is one of the active functional groups that may produce RMs via CYP450s. The oxidation of the double bond of the furan ring to give the reactive furanoepoxide or γ-ketoenal intermediate, resulting in the modification of CYP450s, has been well-documented during the irreversible inactivation (also called MBI) of CYP450s by the psoralen analogues 8-MOP, 5-MOP, and bergamottin (Koenigs & Trager, 1998a,b; Mays et al., 1990; Wang et al., 2012). Recently, psoralen and isopsoralen have also been found to give the irreversible inactivation of CYP1A2 in human and rat liver microsomes (Lin, Kenaan, & Hollenberg, 2012), and the formation of RMs has been reported (Ji et al., 2015) (Zhuang, Zhong, Xiao, Li, & Lu, 2013) Figs. 3 and 4.

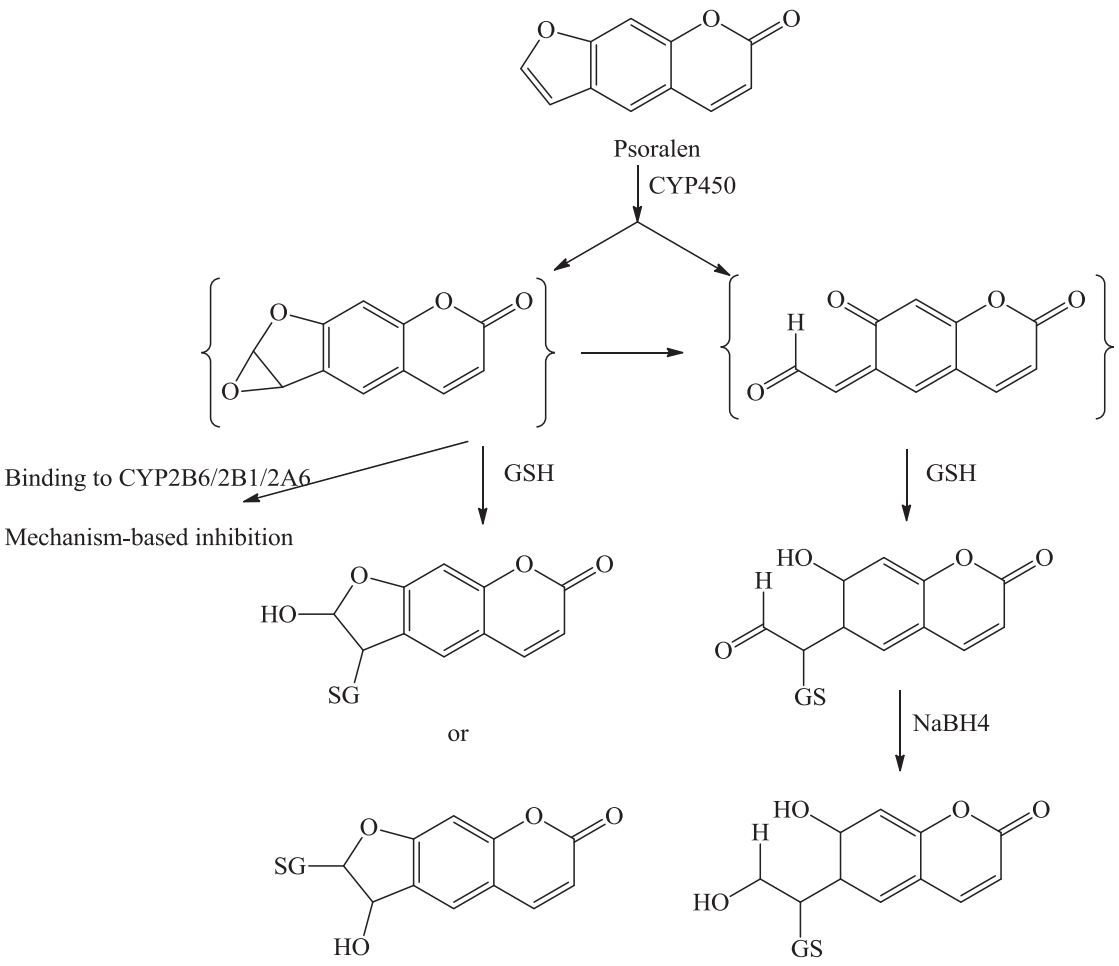


Fig. 3. Metabolic activation pathway for psoralen.

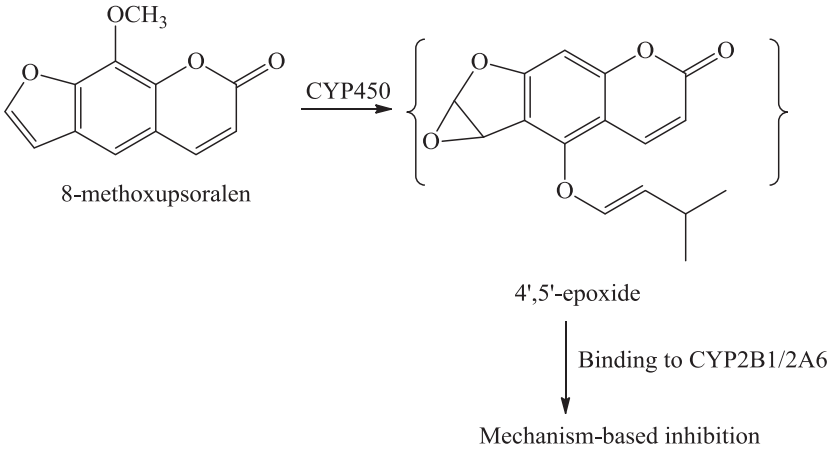


Fig. 4. Metabolic activation pathway for 8-methoxypsoralen.

4.2. *Euodiae fructus*

Euodiae Fructus (Wuzhuyu in Chinese) is the dry and ripe fruits of *Euodia rutaecarpa* (Juss.) Benth., *Euodia rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang, and *Euodia rutaecarpa* (Juss.) Benth. var. *bodinieri* (Dode) Huang. The responsible compound rutaecarpine can be metabolized by CYP450s in rat liver microsomes and produce nine metabolites (Lee et al., 2004). Huang et al. found that rutaecarpine can be metabolized in rat liver microsomal incubation system and inhibit the metabolism of berberine hydrochloride. The pharmacokinetics of berberine significantly impacted the inhibition of rat liver enzyme ERD and ADM activity. Ueng et al. (2002) found that rutaecarpine from *E. rutaecarpa* showed selective inhibition on rats and human CYP450s. Rutaecarpine can induce the formation of liver metabolic enzyme P450. After continuously administration of rutaecarpine for 3 d in male mice, the up-regulation of P4501A and P4502B in hepatic metabolism was detected by Western blotting (Iwata, Tezuka, Kadota, Hiratsuka, & Watabe, 2005). Postulated mechanism of rutaecarpine-induced hepatoxicity was showed in Fig. 5.

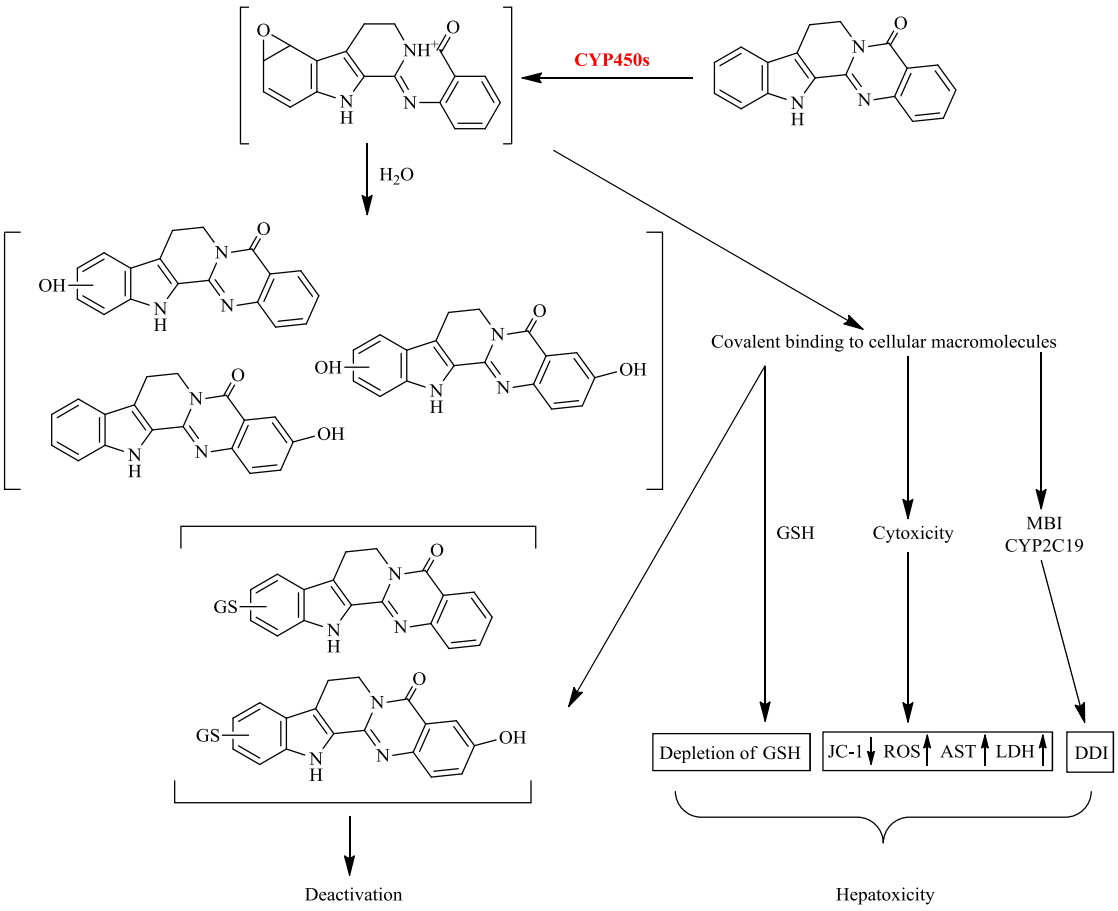


Fig. 5. Postulated mechanism of rutaecarpine-induced hepatoxicity: major metabolic pathways.

4.3. Polygoni multiflori radix and polygoni multiflori radix praeparata

Polygoni Multiflori Radix (Heshouwu in Chinese) is dry roots of *Polygonum multiflorum* Thunb., which induces liver injury accounting for 1.06% of all drug liver damage cases and ranking No. 15. The drug accounts for 5.69% of all cases of liver damage in TCMs and ranks No. 1. The British Drug and Health Products Authority (MHRA) issued in 2006 records it as follows: *P. multiflorum* preparations related adverse reactions manifested as having a variety of signs and symptoms of liver disease, including jaundice, dark urine, nausea, vomiting, fatigue, weakness, stomach pain, abdominal pain, loss of appetite and et al (Wu & Gan, 2014).

Both *Polygoni Multiflori Radix* and *Polygoni Multiflori Radix Praeparata* can cause livers in rats with early liver cholestasis and accompanied by mild inflammatory damage, and free anthraquinone components are the most toxic to hepatocytes. Moreover, the first is more serious than the later. Anthraquinones such as chrysophanol components may be responsible for hepatocyte apoptosis.

Zhang et al. found that 95% ethanol extract of *P. multiflorum* containing anthraquinones and emodin monomer had significant hepatotoxicity to human normal liver cell line L-02 *in vitro* (Zhang, Liu, Sun, & Xu, 2010). Emodin can promote hepatocyte growth at low doses but inhibit it at high doses. Postulated mechanism of rhein-induced hepatoxicity was shown in Fig. 6.

4.4. Asari Radox et Rhizoma

Asari Radox et Rhizoma (Xixin in Chinese) is dried roots and rhizomes of *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag., *Asarum sieboldii* Miq. var. *seoulense* Nakai and *Asarum sieboldii* Miq. The major ingredients of Xixin are the volatile oil including α-pinene, β-pinene, asarinine estragole, eucarvone, kakuol, methyl eugenol, myristicin and safrole responsible for its toxicity (Drew et al., 2002). Methyl eugenol is one of the major effective components in volatile oils, and safrole is the toxic component (Wang & Hong, 1987).

A study showed that the long-term toxicity of Xixin can cause pathological damage to the liver, which get worse at high dose (Li et al., 2008a). Hepatocyte necrosis with different degrees of lobular or portal area inflammation is the main feature. The results of liver function test showed that ALT and total bilirubin (TBIL) were significantly increased, but there was no significant difference between total proteins (TP), albumin (ALB), globulin (GELO) and albumin/ globulin (A/G). That means long-term use of large dose of Xixin can cause liver cell injury in rats, including increasing cell membrane permeability, the desmoenzyme leakage entering the blood, increasing serum liver enzymes, affecting the uptake of bilirubin, binding and excretion function, appearance of bilirubin, but there was no obvious effect on metabolism of protein and could not damage the hepatic synthesis.

Recently, increasing number of people begin to pay attention to liver microsomes and benzodioxole compounds, particularly cytochrome P450. Safrole and myristicin both contain benzodioxole that has been studied for a long time (Hodgson & Philpot, 1975). Methylenedioxyphenyl (1,3-benzodioxole) derivatives have been used as insecticide synergists or insect chemosterilants (Jurd, Fye, & Morgan, 1979). Induction of rat-hepatic microsomal cytochrome P450 and aryl hydrocarbon hydroxylase by 1,3-benzodioxole derivatives was also studied (Murray, Wilkinson, & Dube, 1985).

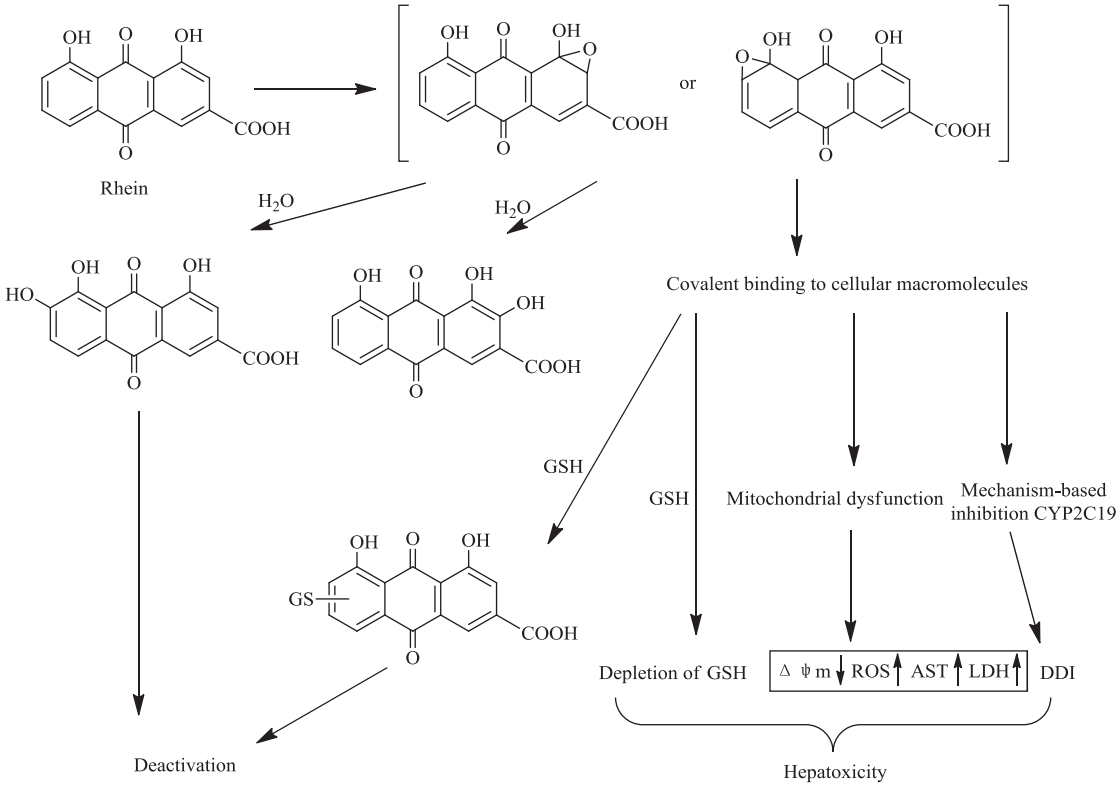


Fig. 6. Postulated mechanism of rhein-induced hepatoxicity: major metabolic pathways.

Methylenedioxyphenyl compounds can form a metabolite intermediate carbene at the methylene group, and the carbene may bind to the CYP heme iron and generate an MI complex (Murray, 2000). Carbene formation has been reported to be a mechanism of inhibition of P450s by 1,3-benzodioxoles (Simonneaux & Maux, 2006). Fontana et al. (2005) summarized the most common substructures causing the MBI of CYPs, including benzodioxole-containing compounds such as myristicin and safrole. Therefore, a new mechanism of hepatotoxicity caused by Xixin is proposed.

For example, an earlier report showed that safrole was a hepatocarcinogen in mice and rats (Wislocki, Borchert, Miller, & Miller, 1976). The metabolite 4-allyl-*O*-quinones of safrole associated with hepatotoxicity has been mentioned (Bolton, Acay, & Vukomanovic, 1994). The hepatotoxicity of safrole and related compounds has been attributed to the electrophilic compounds generated from the oxidation (Sugumaran & Bolton, 1995). Safrole, similar to benzodioxole in chemical substructure, is estimated that the hepatotoxicity may be relevant to the mechanism-based inactivation of CYPs, which was verified by Yang et al. (2017). The data of CYPs cocktail screening, GSH capture, the IC_{50} and the enzyme activity recovery showed that safrole had a strong inhibition ability on CYP1A2 and the high level of RMs was detected. Safrole can be oxidized to form a quinone (4-allyl-*O*-quinone) that can be converted into its tautomeric form, (Z)-4-allylidene-2-hydroxy-cyclohexa-2,5-dien-1-one (Fig. 7A).

Another example is myristicin (Yang et al., 2015a), which has also been confirmed that it can be metabolited by CYP1A2 into RMs similar to safrole thus producing toxic effects. The highly RMs (a quinone and its tautomer) were identified by UPLC-MS^2 through a detailed characterization of the myristicin cleavage processes and the GSH-M1 adduct (Fig. 7B), which was concluded that toxicity of myristicin was also produced by activation of metabolites. In virtue of the structure containing benzodioxole functional groups, it is speculated to be the MI coordination mechanism (Lee et al., 2012).

1,3-Benzodioxoles (Li, Sun, & Gao, 2012), one of the major subclasses of many natural products such as safrole, leucettamine B (Chan et al., 1993), steganacin (Kupchan et al., 1973), and egonol (Akgul & Anil, 2003), exhibited a wide variety of biological activities. the structure–activity relationships showed that the 1,3-benzodioxole moiety is fundamental for the cytotoxic activity because it can be metabolized by CYP to form metallo-carbene intermediates which could be responsible for the antitumor activity of lignans (Castro, Corral, Gordaliza, & Grande, 2003; Imperio et al., 2007).

4.5. *Tripterygium wilfordii Hook F*

The preparations of *Tripterygium wilfordii* is used to treat rheumatoid arthritis, nephrotic syndrome, in which triptolide, celastrol, alkaloids are the major active components. Triptolide is the hepatotoxicity component (Li, Du, Liu, Ji, & Xing, 2015; Qu et al., 2015). There are many cases of liver injury, such as enhancement of the expansion of Th17 cells and suppression of the production of Tregs (Wang et al., 2014b,c), P450 inhibition/inactivation. Xue et al. (2011) reported that inactivation of hepatic P450s abolishes the ability in metabolism of triptolide in the liver, subsequently resulting in an increase in bioavailability and toxicity of triptolide *in vivo*. It is the important causes for the hepatotoxicity that triptolide could inhibit the activity of CYP and produce the RMs via CYP (Shen et al., 2014; Zhang, Ya, & Rui, 2016). According to the report, triptolide has potential inhibitory effect on CYP3A, 1A2, 2C9, 2C19, and 2E1 by inhibiting their activity and protein expression, and CYP3A4 is the primary isoform responsible for hydroxylation. Triptolide inhibited the actively of CYP1A2 with $K_i(\mu mol/L)$ and $K_{inact}(min^{-1})$ of is 7.32 and 0.024, respectively. The

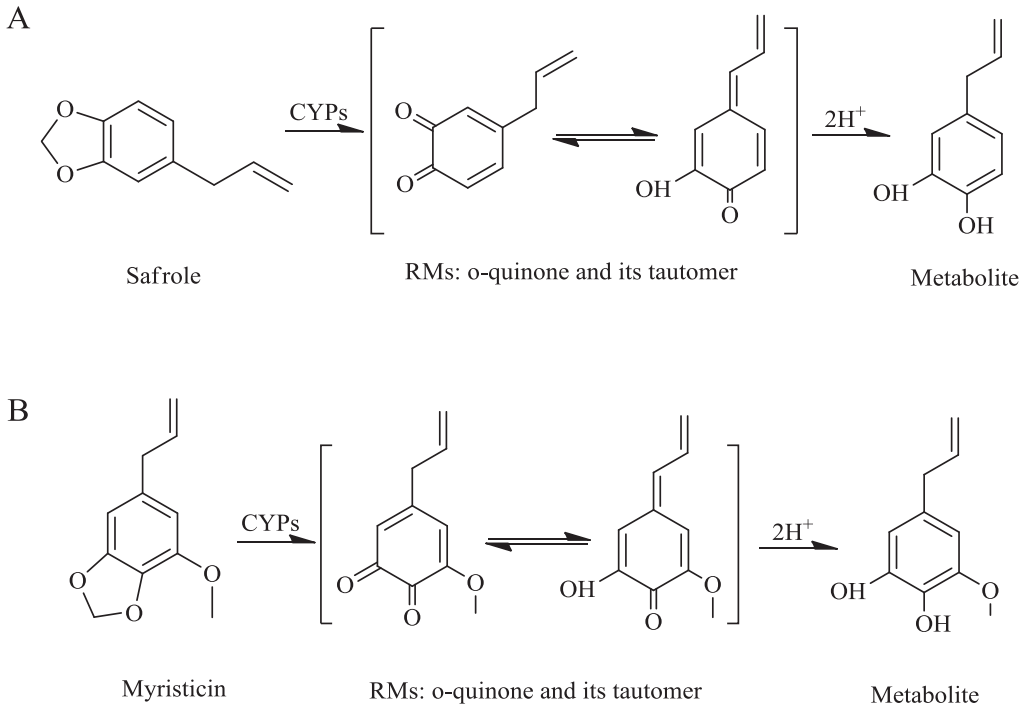


Fig. 7. Metabolic activation pathway for safrole (A) and myristicin (B).

IC_{50} values of CYP 1A2 and 3A4 were 14.18 and 8.36 μmol/L, respectively (Zhang et al., 2016). It can cause hepatotoxicity by reducing the substrate affinity, activity, and expression at the transcriptional and protein levels of the CYP (Li et al., 2008c; Lu et al., 2017; Xi, Peng, Wu, Zhou, & Zhou, 2017; Zhang et al., 2016). Epoxy groups of triptolide are closely relative to hepatotoxicity. The metabolic reactions of triptolide that we observed *in vivo* were hydrolysisreaction, hydroxylationreaction, and the conjugate reaction with sulfate, glucuronide and GSH, respectively (Du, Liu, Li, & Xing, 2014).

Peng, Wang, Du and Chen (2012) identified the structure of metabolites of triptolide *in vivo* and *in vitro* using liquid chromatography–tandem mass spectrometry. They found that triptolide was metabolized *in vivo* by hydrolysis and hydroxylation reactions, and conjugated with sulfate, glucuronide and GSH (Fig. 8), respectively. The biotransformation of triptolide via C2-H hydroxylation, 12,13-epoxyethane hydrolysis and C14-OH conjugate reaction were found, which might have an important influence on the toxicity of triptolide (Peng et al., 2012).

Su et al. reported that wilfortrine as a type of sesquiterpene alkaloids was the most abundant components in Tripterygium glycosides tablets that were mainly used in clinical (Su, Zhou, Lan, Di, & Hang, 2015). Furthermore, wilfortrine can induce HepG2 cell apoptosis by up-regulating expression of the apoptotic protein Bax and down-regulating expression of Bcl-2 (Li & Lei, 2016; Yue et al., 2015).

In our previous study, wilfortrine was a potent CYP3A4 mechanism-based inhibitors by pharmacophore model together with molecular docking method with fit values of 3.49. Additionally, wilfortrine could bound to the active cavities of CYP3A4 with the CDOCKER interaction energies of 73.3 kcal/mol, and showed concentration- and NADPH-dependent inhibitory activity against CYP3A4. Therefore, wilfortrine might be a mechanism-based inhibitor of CYP3A4. Also, two metabolites of wilfortrine can be detected at 10.00 (M1) and 10.07 min (M2), with a *m/z* of 891 (+16 Da) and 833 (−42 Da), respectively. They speculated that the oxidation (+O) and deacetylation ($-C_2H_2O$) of wilfotrine were the metabolites (Wang et al., 2017b).

The bioactivation of wilfortrine might involve the formation of an electrophilic epoxide intermediate metabolite, and then it formed GSH-wilfortrine adduct (Fig. 9) when GSH was added and monohydroxy wilfortrine after the addition of GSH and H_2O (Wang et al., 2017b). GSH, the intracellular nonprotein thiol for cellular antioxidant defense systems, serves as the key mediator in many cellular events, such as anti-apoptotic effect (Han, Song, Yu, & Chen, 2017). Therefore, hepatotoxicity of wilfortrine may be caused by the molecular bonding of metabolites and GSH.

Celastrol has the potential to inhibit cytochrome P450 activities (Jin et al., 2015) and this inhibition was concentration-dependent. It inhibited the metabolism of CYP1A2, 2C and 3A substrates in rat liver *in vitro* with a different mode of inhibition (Sun, Tang, Ding, Liu, & Wang, 2014).

4.6. *Dioscorea bulbifera*

Dioscorea bulbifera (Huangdu in Chinese) has been widely used to treat carbuncles, lung abscesses, breast lumps, and thyroid disease and cancer. However, hepatotoxicity hinders its clinical application. A number of liver injury cases have been demonstrated to be associated with consumption of Huangdu or its remedies (Niu, Wang, Ji, & Wang, 2014; Sheng, Ma, Deng, Wang, & Ji, 2014). Also, there are numerous reports mentioned its hepatotoxicity in animal models and cell study; The mechanism of hepatotoxicity induced by Huangdu may due to liver oxidative stress injury (Ma, Niu, Wang, Ji, & Wang, 2013; Qu et al., 2017; Yang et al., 2016). The definite hepatotoxic mechanism and mainly toxic compounds of Huangdu induced liver injury are still needed to study up to now. Few reports showed that diosbulbin B (DIOB) is the main hepatotoxic chemical compound in Huangdu, and the reactive intermediate of DIOB play a critical role in hepatotoxicity. The reports documented the cis-enedial reactive metabolite of DIOB metabolized by P450 3A reacted with cysteine and lysine residues produce the

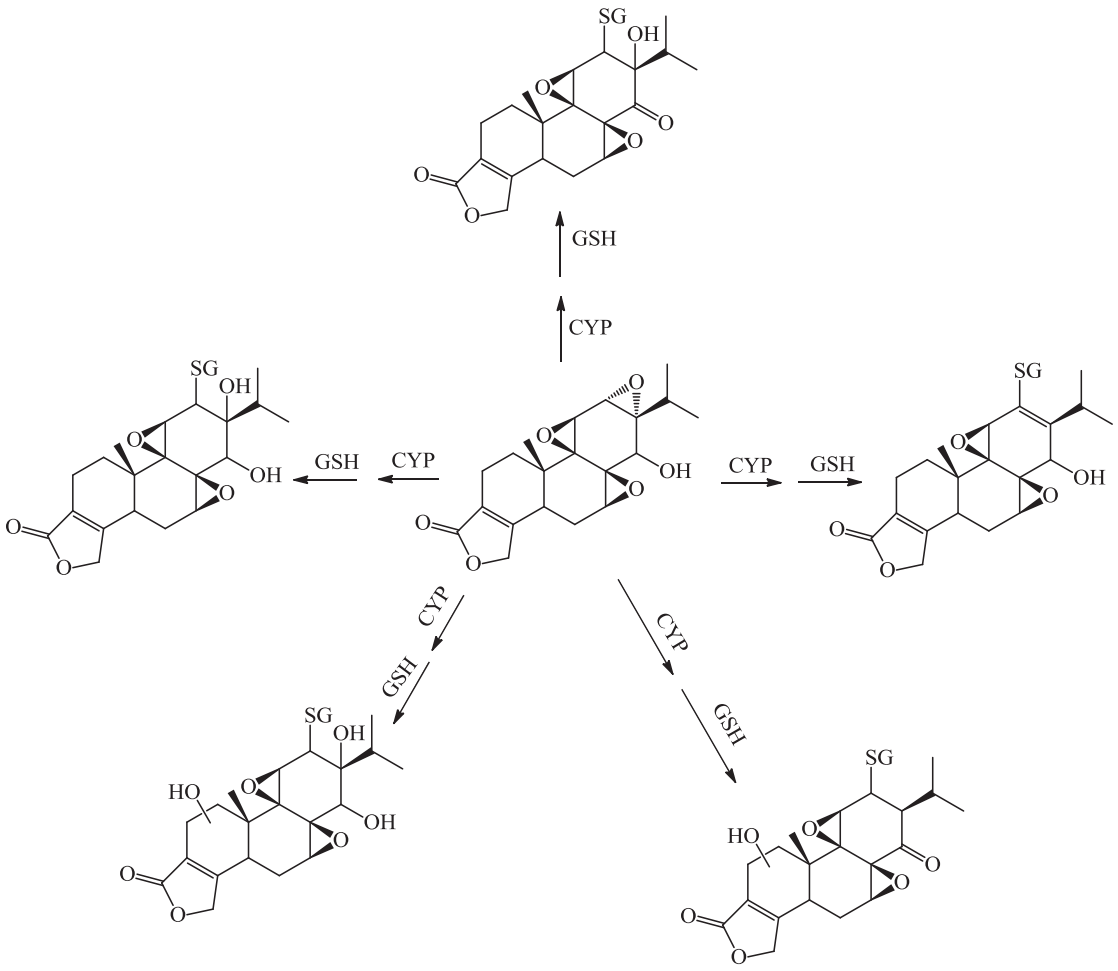


Fig. 8. Metabolic activation pathway for triptolide.

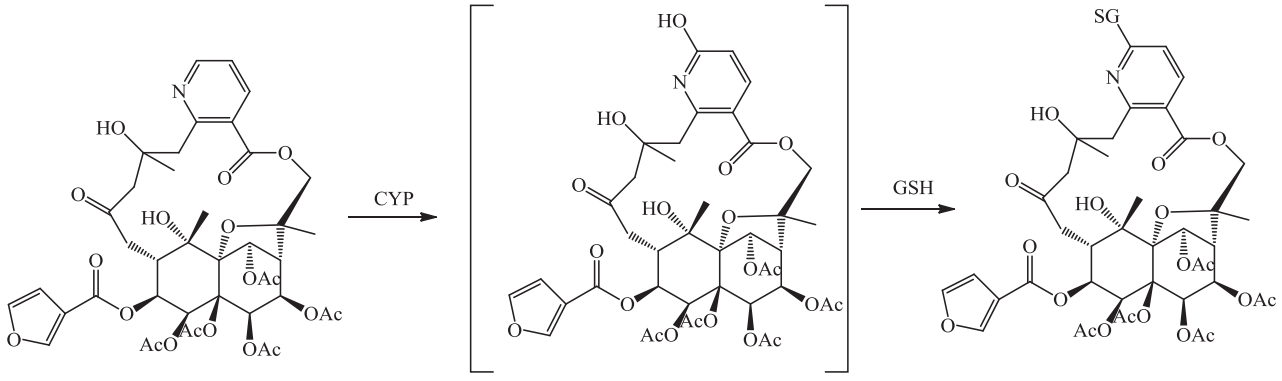


Fig. 9. Metabolic activation pathway for wilfortrine.

corresponding adductions which may form crosslink that destroy the critical proteins correlated with the serious hepatotoxicity (Fig. 10) (Lin, Li, Peng, Gao, & Zheng, 2014; Wang et al., 2017a; Yang et al., 2016). 8-Epidiosbulbin E acetate (EEA) is another abundant diterpenoid lactone in some Huangdu demonstrated by some reports. It possesses the same furan ring as DIOB, and EEA-derived *cis*-enedial metabolized by P450 3A4 can react with cysteine and lysine residues which form corresponding adductions and cause severe hepatotoxicity which have been verified *in vitro* and *in vivo* (Fig. 11) (Lin et al., 2016a,b, 2015).

4.7. Senecionis Scandentis Hebra

Senecionis Scandentis Hebra (Qianliguang in Chinese) is dired herbs of *Senecio scandens* Buch.-Ham, recorded in Chinese Pharmacopeia, which has a variety of biological activities. Li et al. found that a single overdose of water extract of Qianliguang (6 g/kg) could produce typical PA-induced hepatotoxicity in rats (Lin et al., 2009). Pyrrolizidine alkaloids (PAs) are the main active ingredients of this drug, responsible for the toxicities (Xiong et al., 2014). PAs can produce active metabolites through CYP metabolism and then

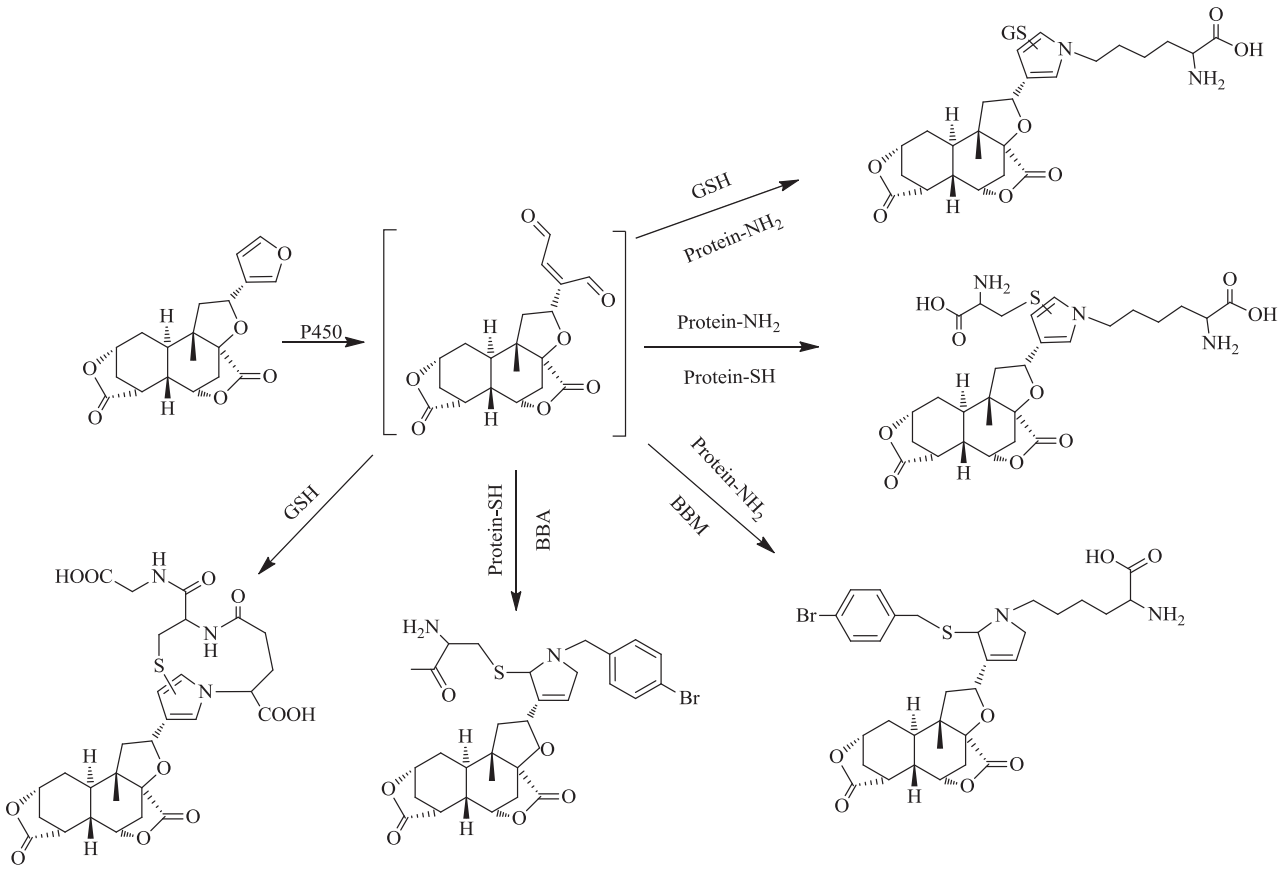


Fig. 10. Metabolic activation pathway for diosbulbin B.

combine with DNA, GSH and protein. The hepatotoxicity could be induced by these conjugates (Fig. 12) (Chen et al., 2016; Fu et al., 2010; He, Xia, Woodling, Lin, & Fu, 2017; Li, Xia, Ruan, Fu, & Lin, 2011; Ruan, Yang, Fu, Ye, & Lin, 2014; Xia, Ma, He, Cai, & Fu, 2015; Yang, Ruan, Fu, & Lin, 2015b; Zhao et al., 2012, 2014; Zhu, Xue, Xia, Fu, & Lin, 2016). Both dehaloperoxidase (DHP) and the PA pyrroles are strong electrophiles capable of alkylating cellular nucleophiles, including DNA, GSH and proteins.

Senkirkine (SK) and senecionine (SN), two kinds of PAs, were detected in the aqueous extract and water extract of Qianliguang (Li et al., 2008b; Lin et al., 2009). SK can lead to gene expression changes of human hepatocytes, and downregulate CYP3A5, 4A11, 7A1, 8B1 and 2D6 but upregulate CYP3A4 (Luckert, Hessel, Lenze, & Lampen, 2015).

The C26 atom of SK was closest to the catalytic heme Fe that is chemically the most susceptible location, which contributes to the electrophilic oxidizing reactions. Therefore, the C26 of SK is the most active sites of hydroxylation leading to the production of the toxic metabolites. Moreover, it benefits the predicted orientation of SK in the active site of the CYP3A4 crystal structure. An *in vitro* metabolism study verified that both DHP, toxic metabolites, formation and N-oxidation were catalyzed by CYP3A4 (Fig. 13) (Fashe et al., 2015). Hartmann et al. (2005) found that SK can be bioactivated by CYP450 and easily form adducts with biological nucleophiles.

Furthermore, a total of 18 and nine metabolites of SK and SN in rats were identified respectively, and the main biotransformation routes of SK and SN were identified as demethylation, N-methylation, oxidation and reduction (Cheng, Liao, Diao, Sun, & Zhang, 2017).

Compared with the toxicity of five PAs in HepG2 cells, the potency order of hepatotoxicity was retrorsine>SN>riddelliine>seneciphylline>isatidine by BrdU assay and retrorsine>riddelliine>SN>isatidine>seneciphylline by MTT assay (Li, Kan, Li, & Lin, 2013).

The majority of regulated phase I enzymes, including CYP4A11, 7A1 and 8B1, were downregulated by SN. Furthermore, aminotransferases AGXT and GLYAT, epoxide hydrolase EPHX2, HNMT and methyltransferases NNMT, N-acetyltransferase ACSM3 and sulfotransferase SULT1E1 also can be downregulated by SN. Nevertheless, dehydrogenase NQO1, methyltransferases GNMT and TPMT, N-acetyltransferase SAT1, and sulfotransferase SULT1A3 were upregulated by SN (Luckert et al., 2015).

PPARs are involved in the regulation of intracellular lipid metabolism. Transcription regulator state of PPAR α, δ, γ can be significantly inhibited by SN (Li et al., 2013). Wang et al. (2014a) found that DHPs can induce photocytotoxicity and photogenetic toxicity mediated by ROS and lipid peroxidation in skin. Therefore, SN interferes with lipid metabolism and associated signaling pathways.

Huan et al. found that there were two forms of the metabolites of SN, DHP and SN N-oxide, in hamsters and sheep. Members of CYP3A subfamily play an important role in the metabolism of PA. However, CYP2B isoforms have a limited capacity, which illustrated CYP2B are less efficient in biotransformation of PA (Huan, Miranda, Buhler, & Cheeke, 1998).

The pharmacokinetics of two PAs including SN and adonifoline were compared with their main metabolites in rats after intravenous and oral administration (Wang et al., 2011). The high N-oxygenation activity and extensive toxicity of SN in rats might be related to its potent toxicity.

The metabolic profile of SN was obtained and 36 metabolites, including SN N-oxides (SN–NO), SN-M2 to SN-M5 (hydroxylation products of SN–NO and its isomerides), DHP, and GSH conjugates

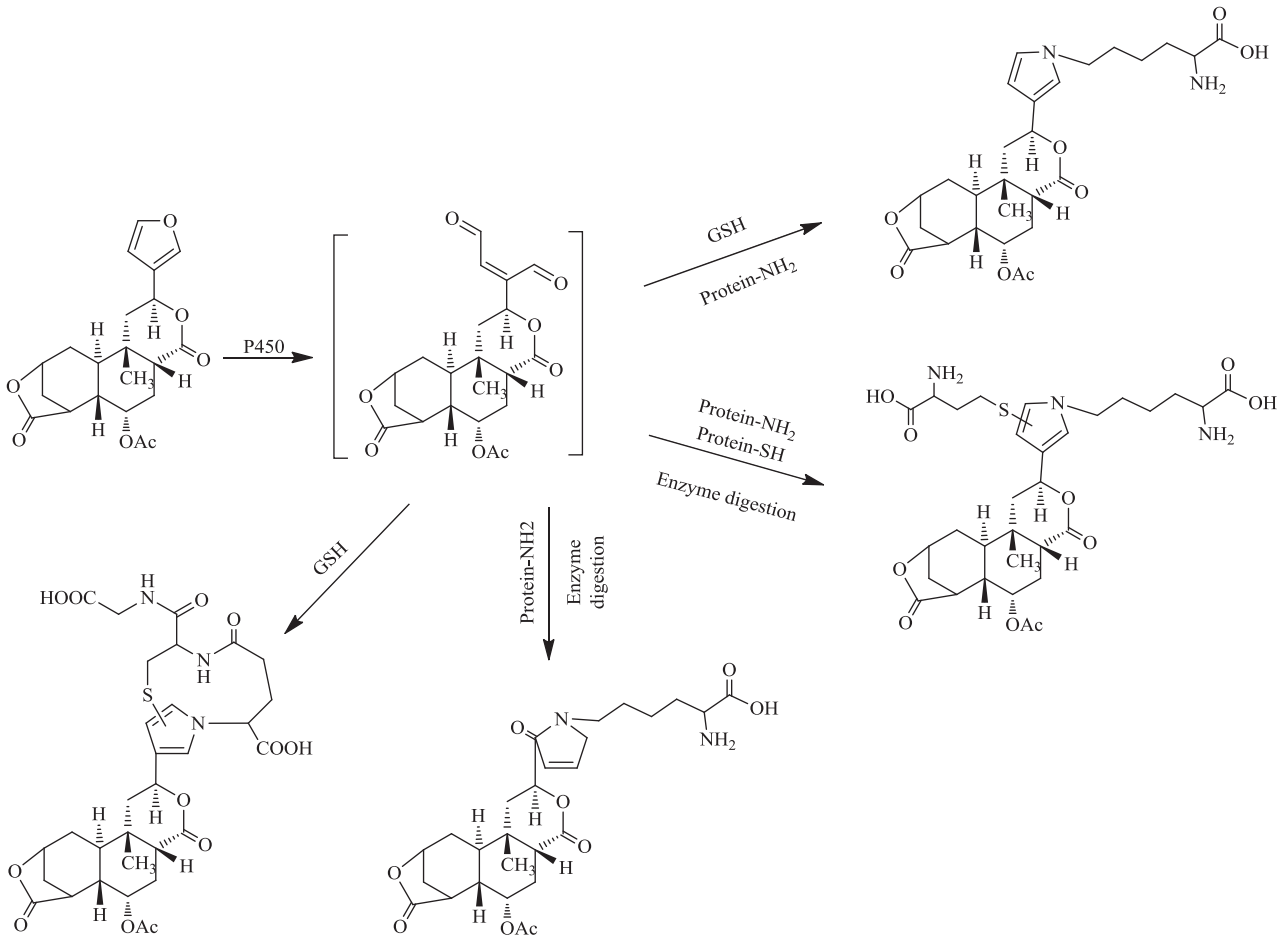


Fig. 11. Metabolic activation pathway for 8-epidiosbulbin E acetate.

of different metabolites, were identified from urine, feces, and bile of rats (Wang et al., 2011).

GSH reacts readily with RMs of SN at physiological conditions, and forms the DHP-GSH conjugate (Fig. 14) (Reed, Miranda, Kedzierski, Henderson, & Buhler, 1992).

5. Methods for identifying formation of RM_S

5.1. GSH-depleted animal models for identifying the formation of RM_S

Most RMs are naturally electrophilic and react with nucleophiles, *e.g.*, GSH, the most important biomolecule for protecting against chemical-induced cytotoxicity (Masini, Gallesi, Giovannini, Trenti, & Ceccarelli, 1997). An overproduction of these RMs depletes GSH, leading to aggravation of cytotoxicity due to the lack of a deactivation pathway. A GSH-depleted animal model is normally used to demonstrate the mechanism of hepatotoxicity caused by the RMs of xenobiotic compounds. For example, administering ticlopidine alone does not cause hepatotoxicity, however combining ticlopidine with L-buthionine-(*S,R*)-sulfoxinine, a GSH-depletion agent, induces centrilobular necrosis in the liver and increases plasma alanine aminotransferase (ALT) activity (Shimizu et al., 2011).

5.2. Computer simulation methods for identifying the formation of RMS

Over the past years, numerous reviews (Cheng et al., 2012) have described computational modeling and how such in silico methods can be used for absorption, distribution, metabolism, excretion (ADME)/Tox predictions. These methods have played an important role in various stages of drug discovery and development (Ekins, 2014). In silico models have been constructed to predict drug-drug interaction (DDI) and assist in decision-making since it is not feasible to test all possible combinations of drug interactions experimentally. In addition, some methods are ideally suited to working with molecules as they are designed.

5.2.1. Rapid virtual screening MBI inhibitors from phytomedicine based on pharmacophore models

Pharmacophores were established by Lemont Kier, who first mentioned the concept in 1967 (Kier, 1967) and usesd the term in a publication in 1971 (Kier, 1971). Pharmacophore has been defined to be "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response (Wermuth, Ganellin, Lindberg, & Mitscher, 1998). Therefore, a pharmacophore is an abstract description of molecular features that are necessary for molecular recognition of a ligand by a biological macromolecule, and explains how structurally diverse ligands can bind to a common receptor site.

Pharmacophore models have been extensively employed towards understanding and predicting the possibilities of enzyme inhibition. Our previous paper suggested that mechanism-based inhibition is an indicator of RM formation. Wang et al. (2017b) focused on studying and predicting the possibilities of CYP450s MBI with computational methods. In this study a mechanism-based inhibition on CYP3A4 of pharmacophore model of *T. wilfordii* has been

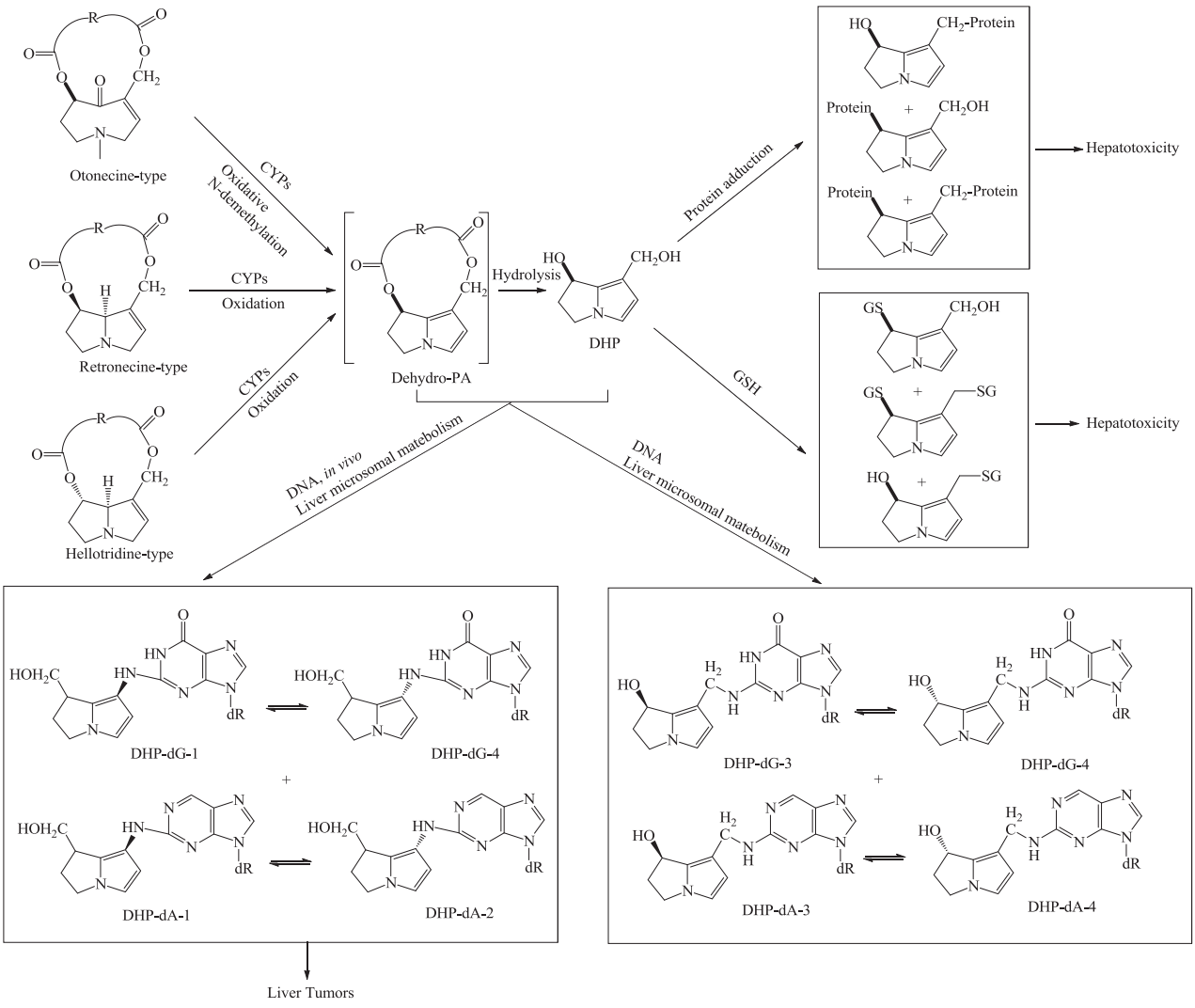


Fig. 12. Metabolic activation pathway for three kinds of pyrrolizidine alkaloids.

derived, which can be used to search for other mechanism-based inhibition on CYP3A4 molecular that contains the same pharmacophore features which might be also active. In addition, a combination of computational approaches including pharmacophore models together with molecular docking are used to rapidly virtual screen the potential CYP3A4 mechanism-based inhibitors from *T. wilfordii*, and *in vitro* experiments are conducted to validate the computational data.

5.2.2. Mode interaction between RMs with CYP450 based on molecular docking simulation

Molecular docking simulation technology has been widely used to study the interaction drug-drug prototype bond with protein receptors. There is a special meaning that the RMs was undertaken in molecular docking simulation to understand the coordination reaction that plays a significant role in the combination of unstable intermediates and CYP enzymes (Hai et al., 2017) (Fig. 15). In the present study (Hai et al., 2017), psoralen and isopsoralen were oxidized by CYP450s, causing the MBI of CYP3A4. Although the mechanism of MBI is unknown for now, we can find some clues from the molecular docking study. On the one hand, psoralen and isopsoralen γ-ketoenal intermediates bound to human CYP3A4 more tightly than psoralen/isopsoralen and the furanoepoxide intermediates. On the other hand, molecular docking result suggests that the γ-ketoenal intermediates of psoralen/isopsoralen have the possibility to coordinate to bond to CYP3A4 and exists a π backbinding (Fig. 16) (Cotton, Wilkinson, & Murillo, 1999; Miessler & Tarr, 1999). These findings may account for the mechanism of coordination binding as a vital role in MBI between the γ-ketoenal intermediates and CYP3A4. Consistently, high-dose 5-MOP (560 mg/kg daily for 12 days) has been shown to cause hepatocyte changes in animals (Cheung et al., 2009). In humans, transient elevations of liver enzymes were found in 5% of patients (4 of 80) within 3.5–20 months of commencement of PUVA 5-MOP (Choi, Kim, Hann, & Park, 1995). However, there was no liver injury report associated with 8-MOP till now. In the present and previous studies, psoralen, isopsoralen, and 5-MOP were found to form furanoepoxide or γ-ketoenal intermediate via CYP450s, while 8-MOP was only found to form furanoepoxide (Koenigs & Trager, 1998a; Lin et al., 2012). It is then implied that γ-ketoenal intermediate may be the more plausible cause for the hepatotoxicity of psoralen, isopsoralen, and 5-MOP.

Chinese materia medica (CMM), characterized with the multi-component, multi-target, and multi-pathway and coincided with the theory of network pharmacology, may misdirect the further predication on effects of relevant metabolic pathways and the specific toxicity manifestation. Therefore, applying network pharmacology combined with moleculer docking to Chinese materia

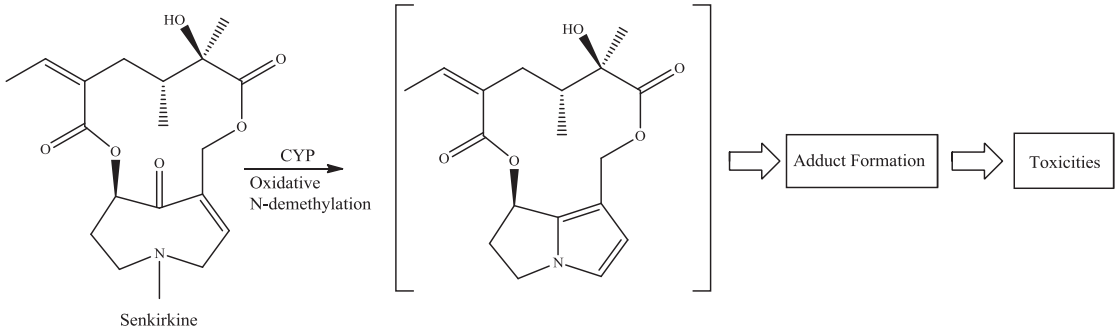


Fig. 13. Metabolic activation pathway for senkirkine.

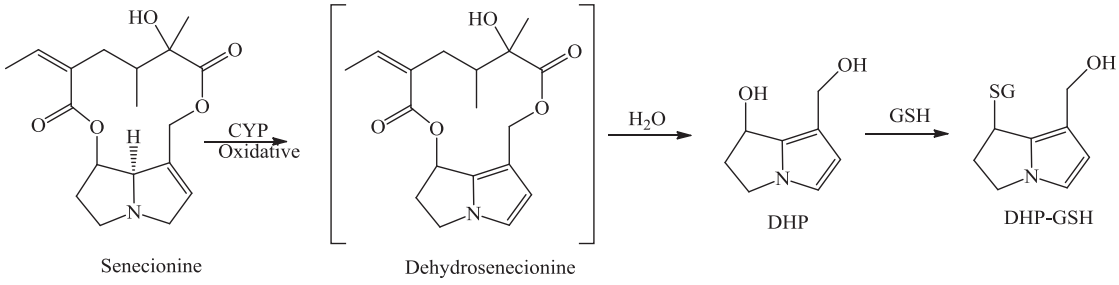


Fig. 14. Metabolic activation pathway for senecionine.

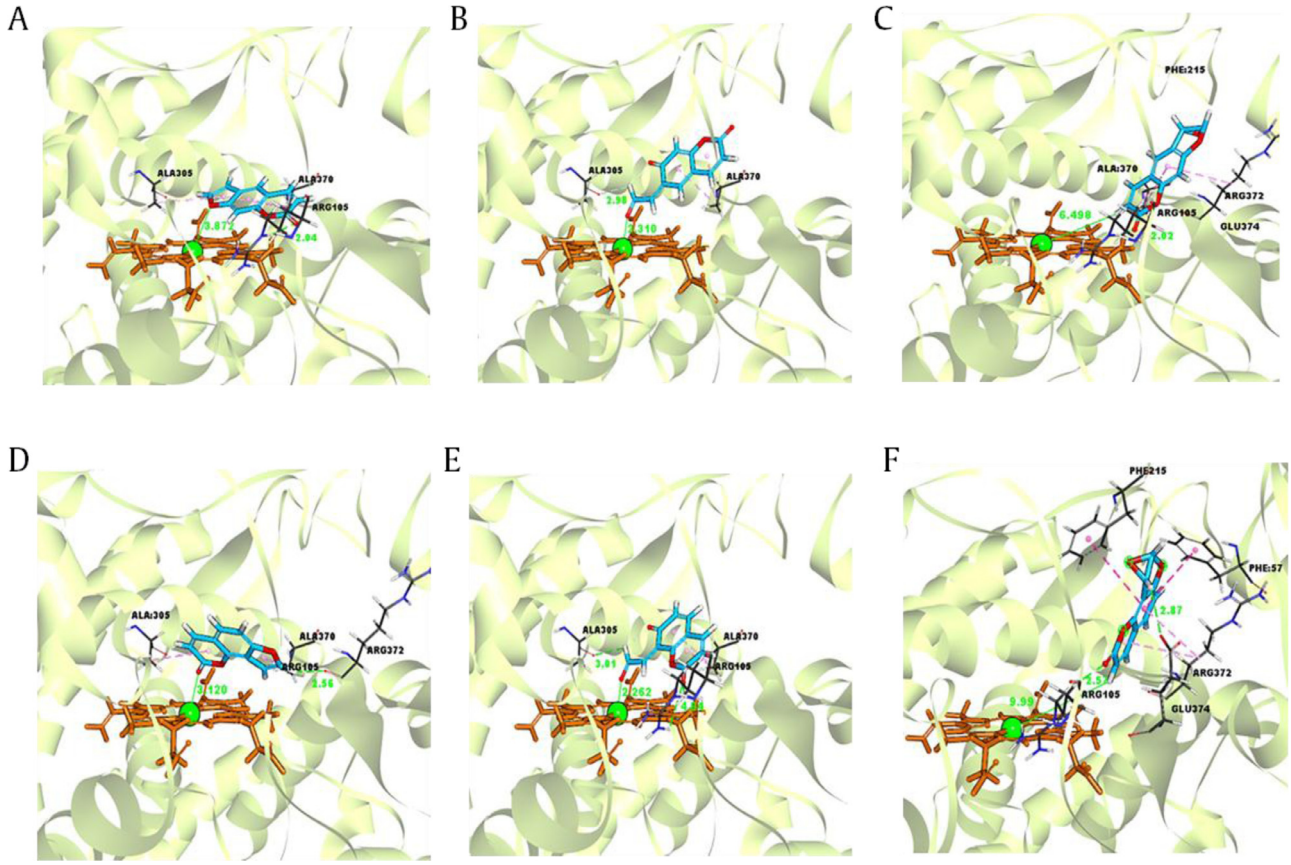


Fig. 15. Docking simulation of Ligand into CYP3A4. (A) Psoralen–CYP3A4; (B) γ-ketoenal intermediate of psoralen–CYP3A4; (C), Furanoepoxide intermediate of psoralen–CYP3A4; (D) Isopsoralen–CY3A4; (E) γ-ketoenal intermediate of isopsoralen–CYP3A4; (F) Furanoepoxide intermediates of isopsoralen–CYP3A4. The heme and iron atoms are colored with brown and green, respectively.

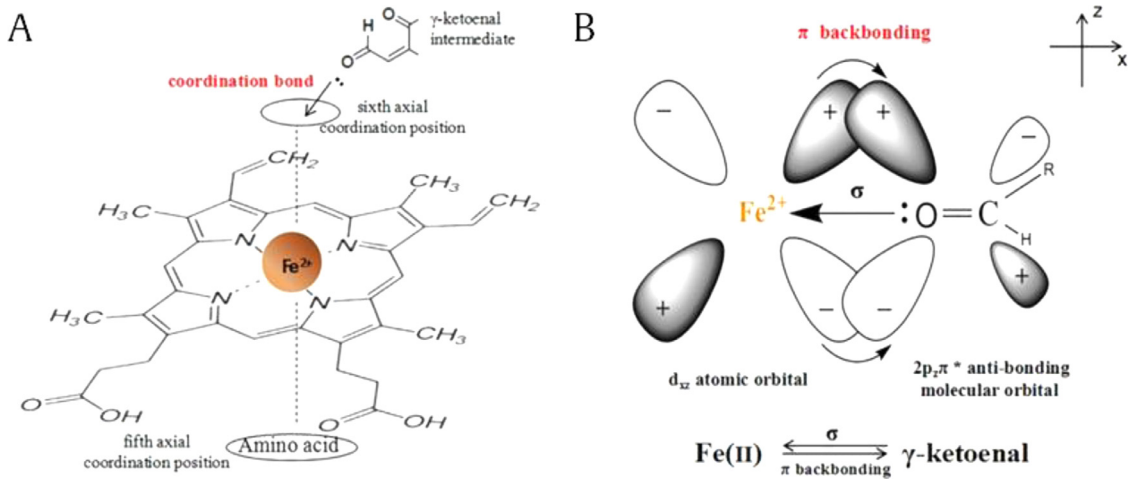


Fig. 16. Illustration of the γ-ketoenal intermediate of psoralen/isopsoralen–heme Fe(II) CYP3A4 coordination compound (A); Illustration of σ and π backbonding interaction between γ-ketoenal intermediates and heme Fe(II) of CYP3A4: mechanism of coordination binding between γ-ketoenal intermediates and heme Fe(II) of CYP3A4 (B).

medica researches will be helpful to explain the effects of Chinese materia medica in the treatment of complex diseases holistically and systematically. The recent progress in the applications of network pharmacology in Chinese materia medica studies has been reviewed, including prediction and identification of targets and core bioactive components, clarification of the mechanism of action, explanation of the prescription composition rules, development of new indications, discovery of new active compounds and the combined application of network pharmacology and omics technologies, so as to accelerate the extensive applications of this new technology. TCM chemical information is usually analyzed by high resolution mass spectrometry or searching online database of Chinese chemical composition, such as TCMID (Traditional Chinese Medicines Integrated Database) database (Xue et al., 2013), Herb BioMap database (Li et al., 2014), etc. After obtaining chemical information, we use computer virtual screening to predict protein targets of different components. Many mature network analysis platforms have been formed by using different computing methods. For example, drug CIPHER network model was constructed by integrating structural similarity and protein interaction, AlzPlatform platform based on molecular docking technology (Liu et al., 2014), TCMID database based on text mining method and so on. In addition, there are commonly used target prediction methods, such as reverse pharmacophore identification and molecular dynamics simulation (Li et al., 2012).

6. Discussion

6.1. A potential hepatotoxic component information database based on active functional groups might provide an early information for basic research of hepatotoxic substances in TCMs

CMM are a complex synthesis with mass chemical components. The efficiency and toxicity are also a combination of components. Many kinds of components can cause liver toxicity without certain structural regularity. Therefore, the basic research of hepatotoxic substances in CMM is facing great challenges. However, structures determine its function. Even if the ingredients of CMM are complex, there must be a close connection between efficacy or toxicity and material basis of the TCMs.

As mentioned above, these herbs with hepatotoxicity do not have structural similarity, but they can produce RMs under the action of drug metabolizing enzymes to induce liver injury depending on their active functional groups. This provides a good chance for the early discovery of toxic ingredients in CMM. Since there is a correlation between the generation of RMs and the active functional groups, the early prediction of potential hepatotoxic components based on RMs will have certain clinical significance. We can build a potential hepatotoxic component information database based on active functional groups, so did in *Dioscoreaceae, T. wilfordii, Fallopia multiflora* and *Tetradium ruticarpum*. This provides a standard and accurate information for the basic research of hepatotoxic substances in TCMs, and is a great significance for the safe use of TCMs.

6.2. Potential idiosyncratic liver toxicity might be predicted by testing RMs-CYP450 protein antibody in plasma

Herbal medicines often contain multiple active substances which undergo complicated disposition *in vivo*. The identification of the disposition pathways and metabolites may provide new insights into the mechanisms for herbal toxicity.

With the rapid development of modern science and technology, a variety of new evaluation techniques and methods continue to deepen through China and beyond for the hepatotoxicity of CMM research. Hepatotoxicity biomarker techniques have made some progress. RMs can combine with the proteins in the liver (such as CYP450s) to form a complete antigen, which eventually leads to an antigen-specific immune response. We can set up RMs-CYP450 protein complete antigen by Elisa methods, and test RMs-CYP450 protein antibody in mice plasma to predict the potential idiosyncratic liver toxicity

Metabolic activation of herbal and dietary constituents to generate reactive intermediates appears a critical step for the toxicity induction. During development of new herbal formulations, early discovery of herbal components that undergo bioactivation and bind covalently to DNA and important cellular proteins can help minimize or avoid toxicity.

Given that there is an increased consumption of herbal plants globally by various ethnic groups, it is necessary to address this important safety issue. The formation of intermediate reactive drug metabolites is a double-edged sword, as it is a necessary step for many drugs to achieve the desired pharmaceutical effect, but can also lead to cellular damage if not controlled properly.

While conventional parameters used to detect hepatotoxicity in drug safety assessment studies are generally informative, the need remains for parameters that can detect the potential for hepatotoxicity at lower doses and/or at earlier time points. New discoveries and technologies often offer new parameters that may complement those conventionally used in existing testing

strategies. Ideally such new parameters fill gaps in evaluating the multiple events leading to toxicity, particularly in the ability to monitor early events in the sequence leading up to overt toxicity. Such parameters would be expected to provide signals at lower doses and/or earlier time points than provided by conventional parameters (that may be monitoring events later in the sequence). One promising new technology that offers new parameters is that of metabolite profiling, the measurement in biological systems of the full complement of endogenous low-molecular-weight metabolites and their RMs. This can offer a global view of the comprehensive metabolic response of a biological system to genetic or environmental modification. If such metabolic responses are occurring early in the sequence of events leading up to overt toxicity, they could be used to detect the adverse potential of herbal ingredients at an early stage in their development.

Conflict of interest

The authors declare that there is not any conflict of interest.

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