Acupuncture and Herbal Medicine

Chemical nature of metabolic activation of natural products in traditional Chinese medicines possibly associated with toxicities

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Abstract

Ensuring the safety of traditional Chinese medicines (TCM) has perennially presented a universal challenge in the healthcare realm. Meticulous investigations into the toxicological intricacies of natural products are of paramount significance, particularly regarding the metabolic transformation of these substances and the subsequent generation of reactive intermediates. This biochemical process underlies the genesis of diverse toxic manifestations, including hepatotoxicity, nephrotoxicity, pulmonary toxicity, and genotoxicity. Compounds sorted within TCM, including pyrrolizidine alkaloids, anthraquinones, furanoterpenoids, alkenylbenzenes, bisbenzylisoquinoline alkaloids, flavonoids, and methylenedioxyphenyl derivatives, evince a spectrum of deleterious mechanisms upon metabolic activation. This review provides a comprehensive delineation of the pathways through which these compounds induce toxicity *via* metabolic activation. This review emphasizes the chemical mechanisms involved in the metabolic activation of natural products that may trigger a toxic cascade, rather than a superficial phenomenon. Furthermore, this study enriches the extant literature by delving into advancements in elucidating the mechanisms of toxicity engendered by metabolic activation. In conclusion, this review highlights the importance of scrutinizing the mechanisms of toxicity and provides insights into the judicious and safe use of TCM.

Keywords: Covalent binding, Metabolic activation, Natural products, Toxicological mechanisms, Traditional Chinese medicines

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Introduction

With its rich heritage of treating ailments in Asia, traditional Chinese medicines (TCM) has witnessed a burgeoning global reach in recent decades, extending its practice to Europe and America. However, ensuring safety remains a challenge in international medical and healthcare landscapes. Over the past few years, China has actively promoted TCM internationally to expand its global influence[1]. In 2019, the World Health Organization (WHO) recognized traditional medicines and included them in the "International Classification of Diseases (ICD-11)" for the first time[2]. However, this decision has been met with criticism due to lingering concerns regarding the clinical safety of TCM practices[3]. To foster the healthy advancement of TCM, it is imperative to prioritize in-depth research on the safety and toxicological mechanisms of the natural products employed in it[4].

Factors contributing to adverse reactions caused by TCM include misidentification of herbs, improper processing, overdose, prolonged administration, incorrect diagnosis, inappropriate herb combinations, and contamination by microorganisms or heavy metals[5]. One salient example is the infamous case of aristolochic acid (AA) in the 1990s, which caused severe kidney damage[6]. The culprit was the inadvertent substitution of *Stephania tetrandra S.Moore* (Fang Ji), a benign herb, with *Isotrema fangchi* (Gang Fang Ji), which harbors nephrotoxic AA[7,8]. Recently, researchers have focused on the toxicity of *Pleuropterus multiflorus* (Thunb.) Nakai owing to a spate of poisoning incidents[9]. Mounting evidence indicates that Polygoni Multiflori Radix Praeparata (Zhi Shou Wu) exhibits lower toxicity than Polygoni Multiflori Radix (Sheng Shou Wu)[10]. The complexity of the chemical composition of TCM is attributed to the lack of clarity regarding the underlying toxic substances and mechanisms involved. Metabolism

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is an important process in drug disposal. Therefore, it is crucial to clarify the metabolic processes of natural products in TCM to thoroughly elucidate their mechanisms of toxic action.

The metabolic activation of natural products, mediated by drug-metabolizing enzymes (P450s) or intestinal microbiota, yields reactive metabolites capable of reacting with glutathione (GSH) and macromolecules[11], forming the corresponding adducts, such as proteins, DNA, and RNA. Such adduction can result in various toxicities[12]. This review delves into the intricate mechanisms by which the metabolic activation of natural TCM products triggers adverse effects.

Toxic mechanisms through metabolic activation

Some chemical substances exhibited no or low toxicity. However, subsequent biotransformation *in vivo* lead to the formation of metabolites that surpass the toxicity of the parent compounds, often manifesting carcinogenic, mutagenic, and teratogenic effects. This phenomenon is known as metabolic activation[13]. Natural products present in TCM typically demonstrate chemical inertness; however, they give rise to reactive intermediates or metabolites through metabolic activation, instigating hepatotoxicity induced by pre-toxic natural products[14]. These reactive intermediates or metabolites may encompass electrophilic centers and free radicals, which can be quenched by endogenous protective nucleophiles, such as GSH. Simultaneously, these reactive species assail biological macromolecules, including proteins and DNA, in nucleophilic groups[15]. Covalent binding to cellular proteins or DNA ultimately induces a cascade of toxic effects, including protein chemical adduction, mechanism-based inactivation of enzymes, abnormal DNA adduction or crosslinking, formation of immunogenic species, cell death, oncogene activation, and organ injury[16]. Consequently, the reactive intermediates and metabolites produced by the metabolic activation of natural products in TCM represent pivotal initiating factors responsible for toxicity[17] (Figure 1).

Metabolic activation of natural products in TCM

This review focuses primarily on the metabolic activation of natural products found in TCM and the metabolic events that possibly trigger the development of toxicities. The structures of natural products in TCM are diverse and complex. This review focuses on several typical natural products, as their structures can be classically metabolized into specific electrophilic intermediates, leading to toxicity.

Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PAs) are ubiquitous in hundreds of plant species across diverse botanical families, and are distributed across various geographical regions worldwide[18,19]. It has been documented that PAs have been detected in approximately 3% of the world's flowering plant species with over 6,000 plants harboring these compounds[20]. Human exposure to PAs can occur through inadvertent consumption of natural products or food items containing PAs[21]. Furthermore, PAs are commonly used in the clinical practice of TCM, particularly in herbal medicines derived from *Boraginaceae* (all genera), *Compositae* (tribes Senecionae and Eupatoriae), and *Leguminosae* (genus *Crotalaria*) families[11].

PAs present in TCM have the potential to induce various toxic effects, including hepatotoxicity, tumorigenesis, pneumotoxicity, developmental toxicity, genotoxicity, phototoxicity, and photogenotoxicity[22–28]. *Gynura japonica* (Thunb.) Juel (Tu San Qi) harbors four hepatotoxic

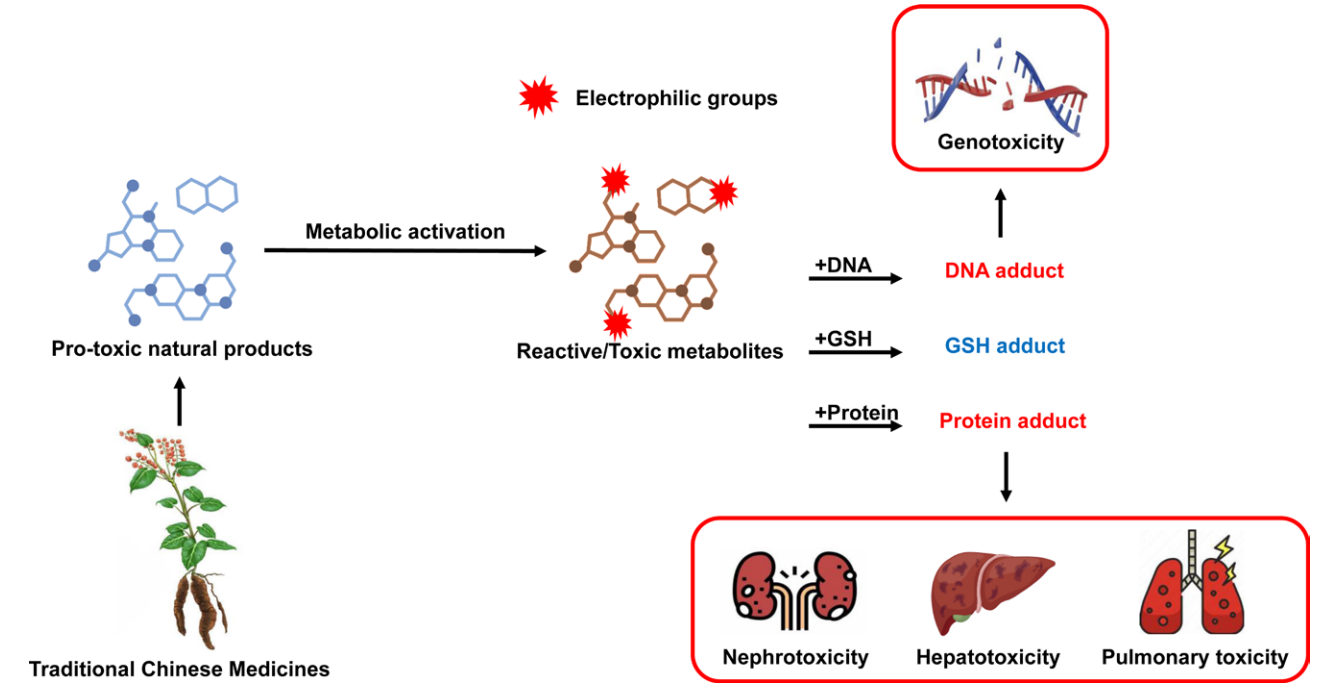

Figure 1. Proposed metabolic activation pathways for the toxicity of natural products.

PAs, namely, seneciphylline, senecionine, seneciphylline *N*-oxide, and senecionine *N*-oxide. The misuse of Tu San Qi, which is often confused with non-PA-producing herbs (San Qi), can result in symptoms such as abdominal distension, liver pain, ascites, jaundice, and hepatomegaly[29]. The toxicity associated with PAs has emerged as a global concern in terms of both TCM and food safety, posing a significant threat to public health.

Based on the necine bases inherent in their chemical structures, PAs are categorized into three primary types: retronecine (inclusive of its 7-α enantiomer, heliotridine-type), otonecine, and platyphylline[30]. The unsaturated necine base harboring the C1-C2 double bond functions as a toxic moiety in PAs. Retronecine- and otonecine-type PAs are metabolically activated by P450 enzymes, leading to the generation of reactive pyrrolic intermediates (pyrrolic esters)[31]. CYP3A and CYP2B are the principal enzymes responsible for PA metabolic activation[32]. These pyrrolic intermediates display significant chemical reactivity to nucleophilic biomolecules such as cellular DNA and proteins, resulting in pyrrole-DNA adduction, pyrrole-protein adduction, and crosslinking adduction[33] (Figure 2). Moreover, PAs can be converted into PA *N*-oxides, which are catalyzed by P450s. PA *N*-oxides can be enzymatically reduced to their parent PAs. Subsequently, reduced PAs undergo the same metabolic activation pathways, leading to toxicity[34]. Recently, Li et al.[35] identified that the metabolic activation of retrorsine induces RNA adduction in cultured primary hepatocytes and animals, potentially contributing to epigenetic toxicity in the liver.

Anthraquinones

Anthraquinones (ATQs) are found in terrestrial and marine environments. The most well-characterized ATQs, emodin, aloe-emodin, physcion, chrysophanol, and rhein, are widely distributed in herbs of the *Polygonaceae* family, including *Pleuropterus multiflorus* Thunb. Nakai (He Shou Wu), *Reynoutria japonica* Houtt. (Hu Zhang), *Rheum palmatum* L. (Da Huang), Aloe vera (L.) Burm. f. (Lu Hui), *Cassia obtusifolia* L. (Jue Ming Zi)[36,37]. There is a potential risk of hepatotoxicity associated with the administration of ATQ-containing TCM. ATQs may generate reactive metabolites during metabolic activation (Figure 3).

Emodin, a prevalent ATQ constituent widely distributed in herbs of the *Polygonaceae* family, has been shown to induce hepatotoxicity[38–40]. Emodin and three hydroxylated metabolites of emodin produced by P450s have been identified as electrophilic species that are reactive to *N*-acetyl cysteine (NAC) and GSH[41]. The electrophilicity of emodin enables it to react with cysteine residues in murine liver proteins. Aberrant hepatic protein adduction by emodin leads to hepatotoxicity[42]. Furthermore, 5-hydroxyemodin, generated by emodin metabolic activation of CYP1A1, is found to induce oxidative stress in cells[43].

Emodin-8-glucoside serves as a pre-toxin and can undergo metabolic activation to form emodin, which contributes to hepatotoxic events. The reduction in EG content resulting from processing is a crucial mechanistic factor that initiates the detoxification of He Shou Wu[44]. Other ATQs, including aloe-emodin, physcion, chrysophanol, and rhein, along with their oxidative metabolites have also been shown to conjugate with GSH and NAC following metabolic activation[45–49]. The phase II metabolism of aloe-emodin involves sulfation, and the resultant sulfate derivative of aloe-emodin exhibits chemical reactivity towards thiols, potentially contributing to aloe-emodin-induced cytotoxicity[45].

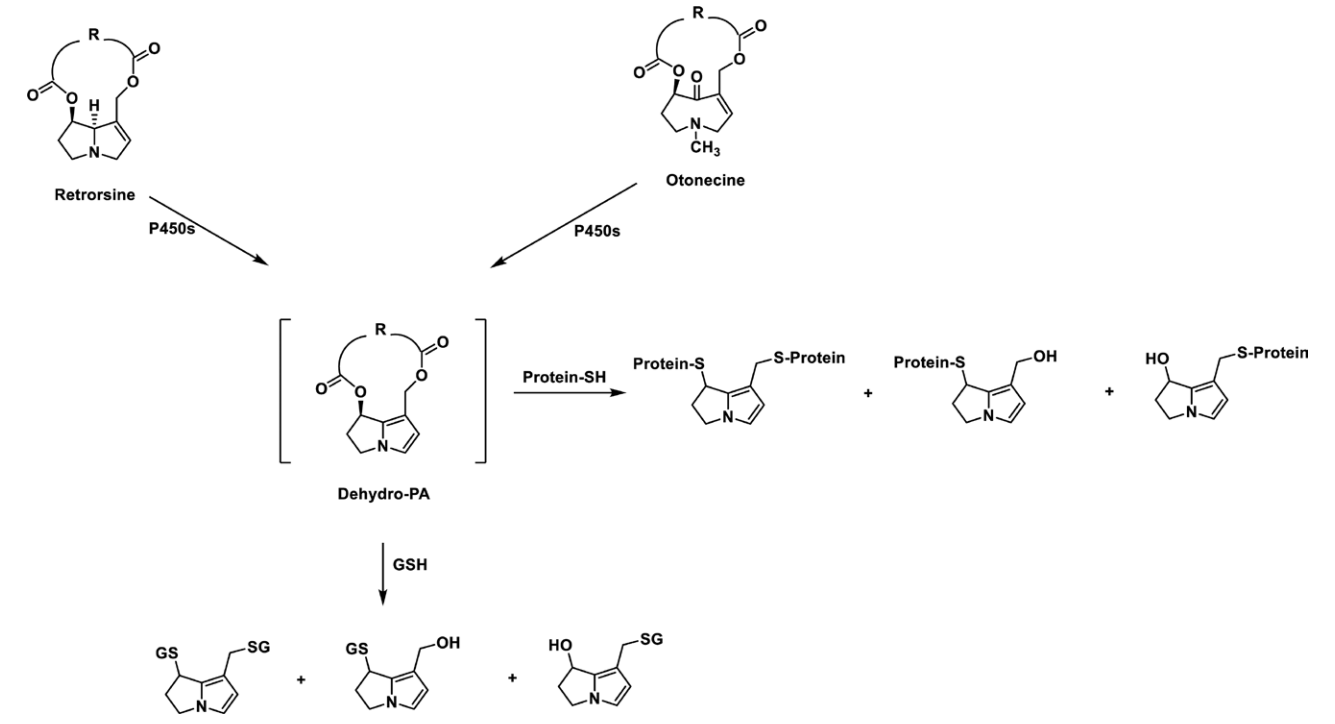

Figure 2. Proposed mechanism for the formation of reactive pyrrolic intermediates derived from retronecine-type and otonecine-type PAs. PAs: Pyrrolizidine alkaloids.

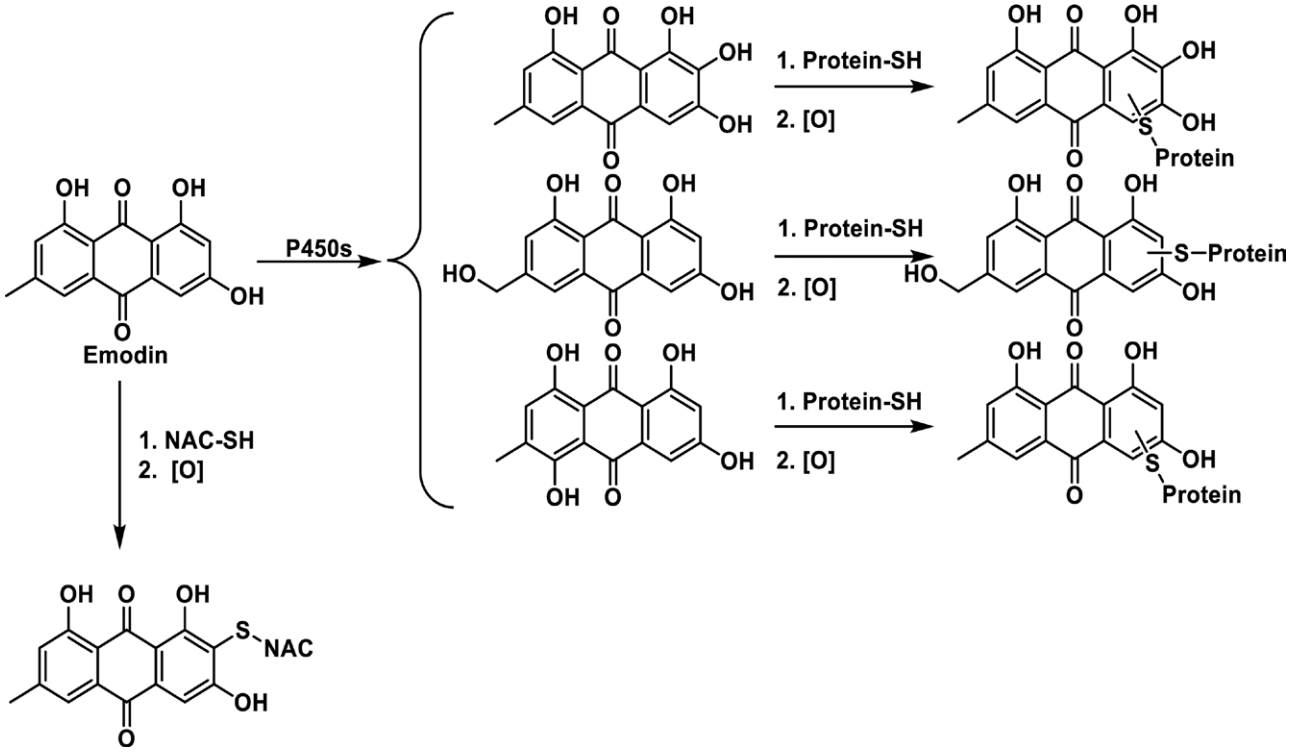

Figure 3. Proposed pathways for the formation of hydroxylation emodin metabolites mediated by P450s.

The soft electrophilicity inherent in ATQs and their hydroxylated metabolites enables their chemical reactivity with thiols, leading to the formation of ATQ-derived GSH and NAC conjugates. Depletion of GSH results in the accumulation of these electrophilic substances, which are more prone to react with proteins or DNA, generating corresponding adducts and altering the normal structure of biomolecules, thereby exacerbating ATQ-induced hepatotoxicity. Currently, direct evidence regarding the mechanisms underlying ATQ-induced hepatotoxicity and nephrotoxicity induced by ATQs necessitates further investigation.

Furanoterpenoids

Furanoterpenoids are found in numerous fruits, herbs, foods, and beverages. Human exposure to widespread furanoterpenoids has garnered significant attention because of their high risk of toxicity. Furanoterpenoids, which are abundant in herbs, induce acute liver injury. The utilization of *Dioscorea bulbifera* L. (Huang Yao Zi), *Dictamnus dasycarpus* Turcz. (Bai Xian Pi), *Aristolochia debilis* Siebold & Zucc. (Ma Dou Ling), and *Tetradium ruticarpum* (A.Juss.) T.G. Hartley (Wu Zhu Yu) often results in liver injury, with the metabolic activation of furan compounds serving as the direct cause of liver injury induced by these TCM[50–53].

Diosbulbin B (DSB) is the principal diterpenoid lactone constituent containing a furan group isolated from *Dioscorea bulbifera* L. Previous investigations have demonstrated that DSB can induce severe liver injury, with the mechanism of DSB-induced hepatotoxicity closely associated with CYP3A4-mediated metabolic oxidation of the furan moiety to the corresponding *cis*-enedial metabolite[54–56]. This metabolite is recognized as an electrophilic species reactive to various biomolecules such as GSH, DNA, and proteins, resulting in the generation of the corresponding pyrrole and pyrroline derivatives, which are responsible for DSB-induced hepatotoxicity[54–58] (Figure 4).

8-Epidiosbulbin E acetate (EEA), a furanoterpenoid, is another component of *Dioscorea bulbifera* L. EEA can be metabolized by CYP3A to form the corresponding *cis*-enedial electrophilic intermediate, which then undergoes condensation reactions with GSH and/or *N*-acetyllysine (NAL) to yield cyclic GSH/NAL conjugates[59,60]. Additionally, the corresponding *cis*-enedial intermediate can alkylate the lysine, asparagine, and glutamine residues of proteins to form pyrroline derivatives[61,62]. Furthermore, EEA-derived biological amine adducts were detected in mouse liver microsomal incubations, cultured mouse primary hepatocytes, and mice treated with EEA[63]. Reactive oxygen species (ROS) resulting from EEA metabolic activation, possibly in conjunction with DNA alkylation caused by EEA-derived reactive intermediates, contribute to the DNA damage observed in cultured primary hepatocytes and the liver of mice[64,65].

Other furanoterpenoids, such as rutaevin, limonin, obacunone, and fraxinellone, have been found in *Dictamnus dasycarpus* Turcz. can also be metabolically activated by P450s to generate the corresponding reactive *cis*-enedial intermediates[66]. The cascade reaction triggered by the metabolic activation of these furanoterpenoids leads to hepatotoxicity, representing a significant mechanism of liver injury caused by TCM. Conversely, certain furanoterpenoids act as mechanism-based inactivators, producing reactive *cis*-enedial intermediates that can react with moieties in the active site of the enzyme, resulting in the irreversible inactivation of host P450s. For instance, xanthotoxin (methoxsalen, a biologically active

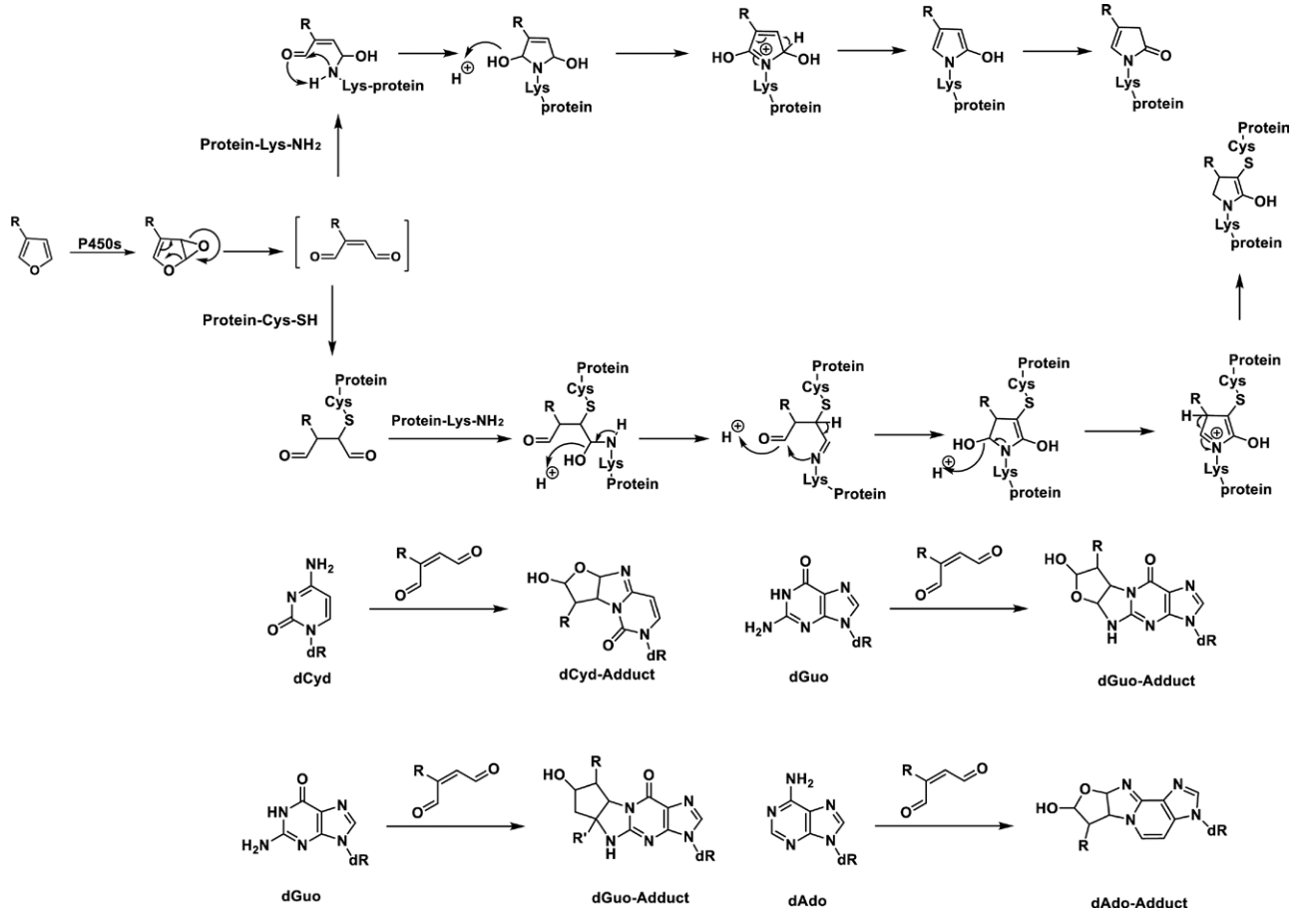

Figure 4. Proposed pathways of NAC/protein adduct formation resulting from the metabolic activation of furan-containing compounds. NAC: *N*-acetyl cysteine.

furanocoumarin widely present in foods and plants) and bergamottin (a major furanocoumarin constituent of grapefruit) can be characterized as mechanism-based inactivators of CYP1A2 and CYP2C9, respectively, acting *via* the formation of an epoxide and/or γ-ketoenal reactive intermediates[67,68].

Apart from hepatotoxicity, furan has been categorized as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC), prompting significant concern regarding the detection of this substance naturally occurring in food. Plasma protein covalent modification by reactive metabolites of DSB can be used as a biomarker for *Dioscorea bulbifera* L. exposure and hepatotoxicity in rats[69]. In addition, an analytical platform was developed to screen *cis*-enediones (*cis*-enedials or γ-ketoenals) resulting from the metabolic activation of potentially harmful furans by employing GSH and 4-bromobenzylamine (BBA) as trapping agents, yielding GSH/BBA-derived pyrroles in the incubation system. Inductively coupled plasma mass spectrometry (ICP-MS) was used to analyze the resulting bromine-labeled pyrroles without authentic standards. Crude extracts obtained from the TCM *Dioscorea bulbifera* L., known to contain furanoditerpenoids, were analyzed using this approach[70]. Similarly, *para*-bromobenzyl mercaptan (BBM) was used to capture furanoterpenoid-derived protein adduction in cells by monitoring lysine-furan-containing pollutants (FCP)-BBM using liquid chromatography (LC)-ICP-MS[71]. A chemoproteomic platform was developed to map furanoterpenoid-derived protein adducts in cultured primary hepatocytes treated with furanoterpenoids, whereby the *cis*-enedial intermediate can form a "clickable" pyrrole structure with propynethiol and protein-free lysine residue or with propargylamine and protein-free cysteine residue[72]. Furanoterpenoid exposure can also be detected *via* antibody immunization. This detection is applicable after protein adduction caused by metabolic activation of furanoterpenoids *in vivo*, and it is necessary to prepare the corresponding antibodies[73,74].

Alkenylbenzenes

Dietary alkenylbenzenes (AKBs) are a class of aromatic natural products prevalent in a variety of vegetables, spices, and medicinal herbs, including cinnamon, clove, nutmeg, pepper, fennel, anise, and basil. Common natural AKBs include estragole, safrole, methyleugenol (ME), elemicin, and myristicin. Individuals are frequently exposed to these compounds; for instance, human exposure to ME estimated to range between 14 and 217 μg/kg body weight per day, stemming from the consumption of foods containing ME[75]. Substantial evidence supports the genotoxicity and carcinogenicity of AKBs.

The carcinogenicity induced by AKBs stems from DNA adduction caused by AKB-derived carbocation-reactive metabolites. Safrole, myristicin, and ME have been observed to form DNA adducts *in vitro* and *in*

vivo following metabolic activation[76]. RNA adduction caused by safrole and ME has also been found[77,78]. Urinary ME-deoxyadenosine adducts are potential biomarkers of ME exposure[79]. The metabolic activation of AKBs into their ultimate carcinogens necessitates the key catalytic involvement of both P450s and sulfotransferases (SULTs). Initially, AKBs undergo transformation into 1'-hydroxyalkenylbenzenes through hydroxylation reactions at the alkene side chain 1'-C mediated by P450s. Subsequently, 1'-hydroxyalkenylbenzenes are sulfated into 1'-sulfooxyalkenylbenzenes through sulfation reactions at the hydroxyl moiety mediated by SULTs. The sulfate moiety in sulfated metabolites serves as a good leaving group, eventually yielding carbocations as the ultimate electrophilic metabolite following the departure of the sulfonate ion. Ultimately, the carbocations react with adenine or guanine bases to form DNA adducts (Figure 5). ME also leads to protein adduction through the aforementioned metabolic activation process[80,81].

Bisbenzylisoquinoline alkaloids

Bisbenzylisoquinoline alkaloids (BBIQs) are one of the largest groups of plant alkaloids and typically comprise two benzylisoquinoline moieties linked through diphenyl ether, benzyl phenyl ether, or biphenyl bonds[82]. BBIQs are widely distributed in herbal medicines belonging to the *Annonaceae*, *Menispermaceae*, *Berberidaceae*, *Lauraceae*, and *Ranunculaceae* families and are predominantly found in tropical and subtropical regions[83]. Numerous TCM and dietary components used in East Asia have been reported to contain these types of alkaloids, such as *Menispermum dauricum* DC. (Bei Dou Gen), *Menispermum japonicum* Thunb. (Qian Jin Teng), *Stephania tetrandra S. Moore* (Fang Ji) and *Coptis chinensis* Franch. (Huang Lian), and others[84]. BBIQs exhibit various pharmacological activities including antiarrhythmic, antihypertensive, anti-tumor, anti-inflammatory, and neuroprotective effects[85,86]. However, previous studies indicated that BBIQs can induce cytotoxicity and pulmonary toxicity[87,88].

Dauricine, the major bioactive BBIQ isolated from the roots of *Menispermum dauricum* D.C., has shown promising pharmacological activity and great potential for clinical use. Tetrandrine, another BBIQ found in the roots of *Stephania tetrandra S.Moore*, has been extensively used in China. These two alkaloids have been found to induce pulmonary injury in CD-1 mice and elicit apoptotic effects in cultured pulmonary cells through metabolic activation mediated by CYP3A[87,88]. Corresponding quinone methides derived from dauricine and tetrandrine *via* oxidation have been identified both *in vitro* and *in vivo*[89–91]. The reactive quinone methide intermediate of dauricine can covalently bind to proteins, and protein adducts were detected based on the alkaline permethylation of proteins, followed by LC-MS/MS analysis[92]. Additionally, neferine isolated from the seed embryos of *Nelumbo nucifera* Gaertn (Lian Zi Xin) was shown to induce cytotoxicity, significantly enhanced by metabolic activation through CYP3A4 and GSH depletion[93]. Berbamine, a well-known BBIQ, is the major bioactive component isolated from herbal medicines such as *Berberis amurensis* Rupr. (Huang Lu Mu). A previous study provided clear evidence for the generation of a quinone methide intermediate from berbamine, catalyzed by CYP3A4, raising concerns regarding the safety of berbamine for consumption[94] (Figure 6).

Flavonoids

Flavonoids are secondary metabolites present in various herbs, fruits, and vegetables and feature a parent nucleus structure comprising 2-phenylchromone[95]. These compounds are recognized as crucial for promoting health and alleviating the symptoms of various diseases[96–98]. Quercetin, a natural flavonoid found in numerous plants, has garnered considerable attention because of its various health-promoting effects, making it an attractive candidate for studying its therapeutic and preventive effects on a wide range of diseases[99]. Early studies indicated that the metabolic activation of quercetin primarily involves initial enzymatic or chemical oxidation, leading to the formation of quercetin *ortho*-quinone. Subsequently, the *ortho*-quinone undergoes isomerization to form a quinone methide. These quinone methides have been identified as DNA-reactive intermediates capable of inducing the mutagenicity associated with quercetin[100]. Further investigations revealed that quercetin is metabolized by catechol-*O*-methyltransferase (COMT), leading to bioactivation of the parent compound. The 3'-*O*-methylation of quercetin results in the generation of geraldol and isorhamnetin, respectively. These metabolites demonstrated significant antiproliferative effects on HepG2 cells[101].

Metabolism-mediated cytotoxicity of kaempferol and quercetin was demonstrated by incubation with S9. Metabolites resulting from methylation, hydroxylation, and glucuronidation of free flavonoids (aglycones) have been identified in human urine and feces. These metabolic transformations occur predominantly in liver and intestinal microbiota[102]. Icaritin is a flavonoid aglycone present in *Epimedium brevicornu* Maxim. (Yin Yang Huo), has been implicated in the induction of liver injury. According to these findings, icaritin undergoes a metabolic transformation mediated by CYPs3A5 and 3A4, resulting in the formation of a quinone methide intermediate. This intermediate has been proposed as a probable cause of the adverse effects associated with epimedium consumption[103]. Silymarins are a class of flavonoid lignans found in the fruits and seeds of *Silybum marianum* (L.) Gaertn (Shui Fei Ji). Chen et al.[104] demonstrated that *O*-demethylenation and hydroxylation of silymarin resulted in the formation of an electrophilic intermediate *ortho*-quinone. CYP3A4 was identified as the primary enzyme responsible for the bioactivation of silymarin (Figure 7).

Methylenedioxyphenyl derivatives

The methylenedioxyphenyl (MDP) derivatives represent a common structural motif found in various TCM, particularly in the form of MDP-lignans, MDP-alkaloids, and MDP-flavonoids[105–107]. Numerous natural MDP products have demonstrated a potent mechanism-based inactivation of P450s[108]. Mechanism-based inactivation

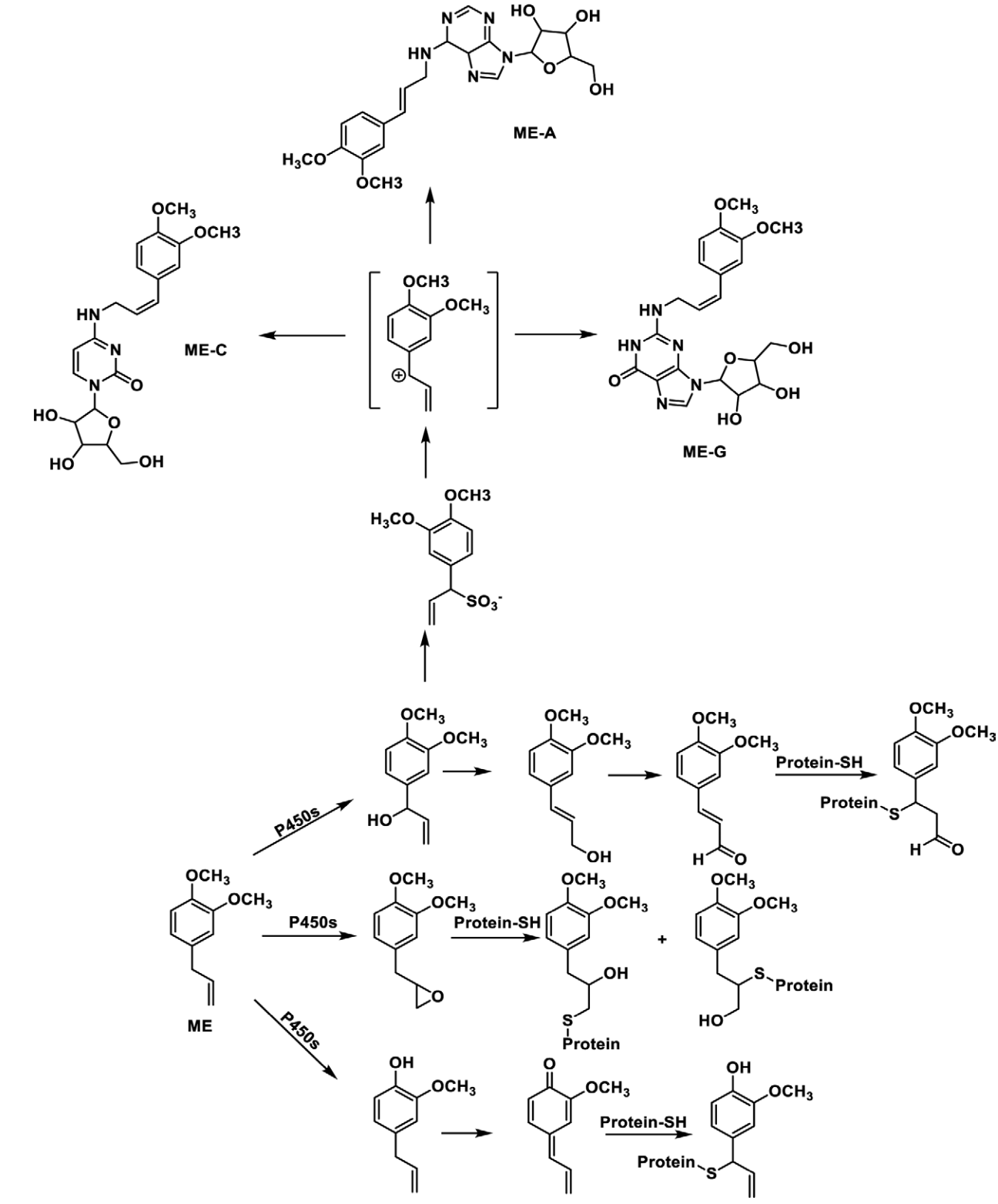

Figure 5. Proposed pathways for the bioactivation of methyleugenol covalent binding with proteins or nucleic acids.

is a process by which a xenobiotic undergoes metabolic bioactivation by host enzymes, leading to the formation of a highly reactive intermediate. This intermediate subsequently reacts with the enzyme, resulting in quasi-irreversible or irreversible enzyme inhibition[109]. Inhibition of P450s is widely recognized as the primary factor contributing to drug-drug interactions (DDIs)[110]. For MDP natural products, the metabolic oxidation of MDP groups results in the generation of carbene intermediates, which can complex with heme iron in P450s[108]. In addition, MDP-derived *ortho*-quinone intermediates participate in irreversible enzyme inactivation. Specifically, the bioactivation process commences with *O*-dealkylation, wherein the 1,3-benzodioxole group is transformed into the corresponding catechol, followed by the sequential oxidation of the catechol to produce an *ortho*-quinone[111] (Figure 8). Moreover, numerous studies have demonstrated that the toxicity

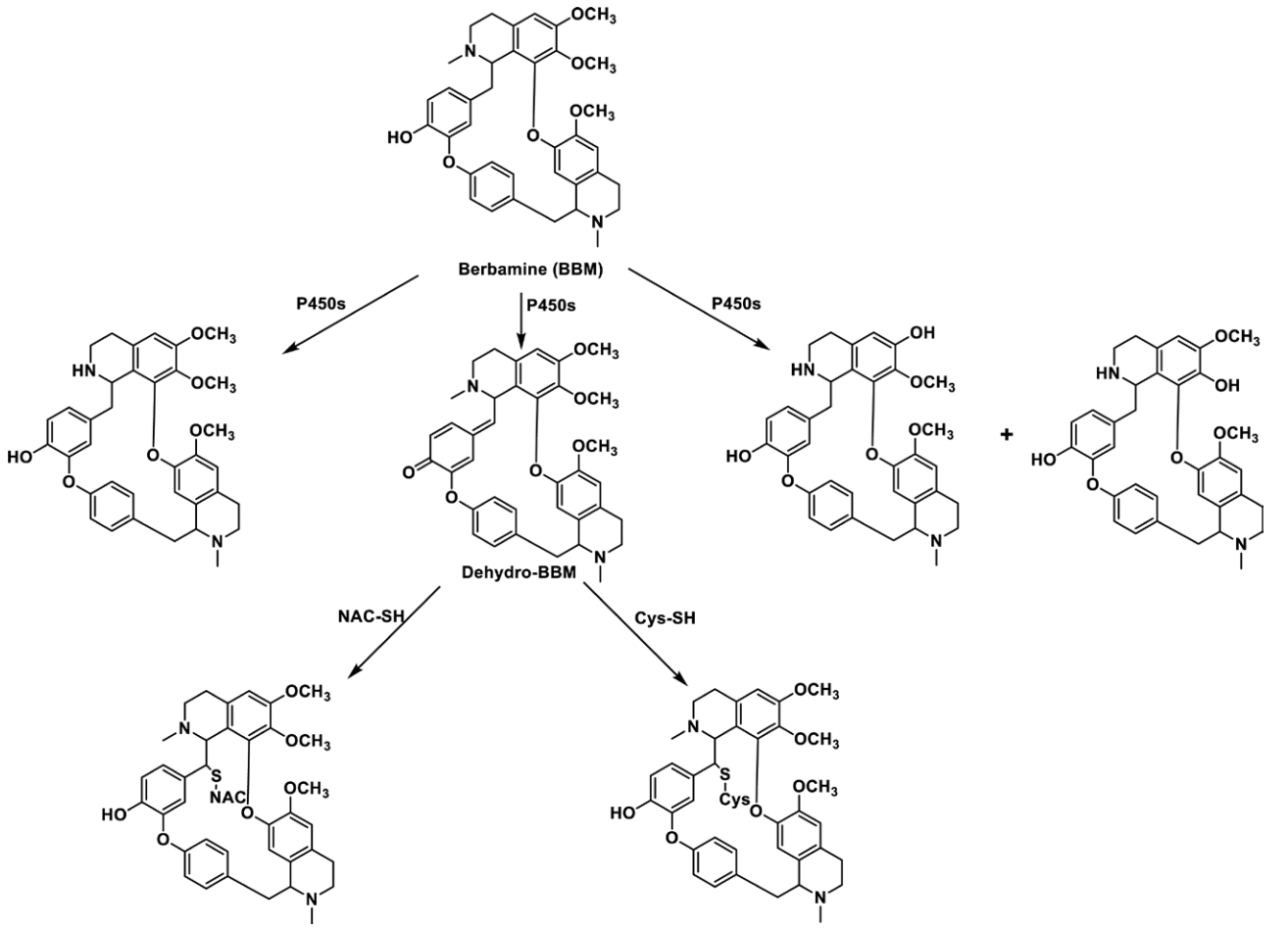

Figure 6. Proposed metabolic activation pathway of berbamine mediated by P450s.

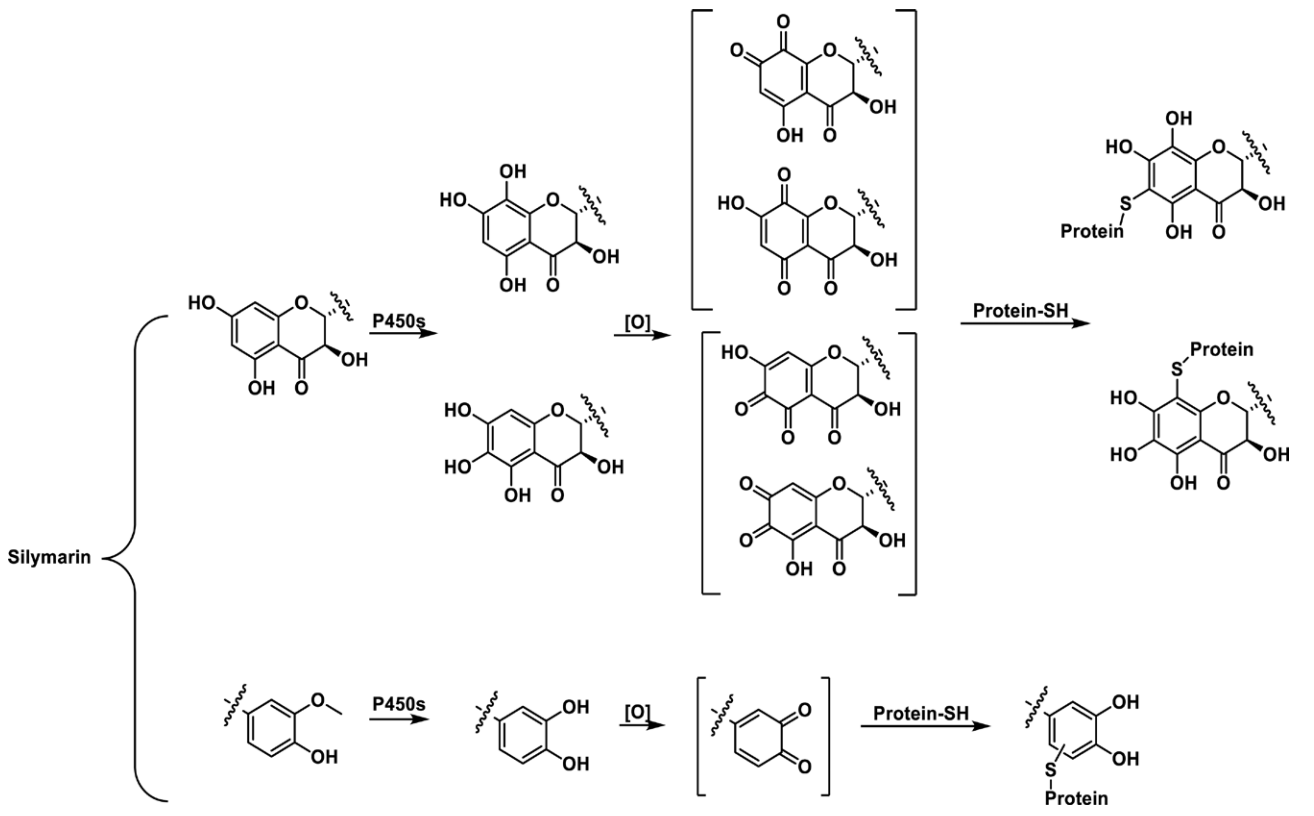

Figure 7. Proposed pathways for the formation of silymarin-drived *ortho*-quinones by P450-mediated oxidation.

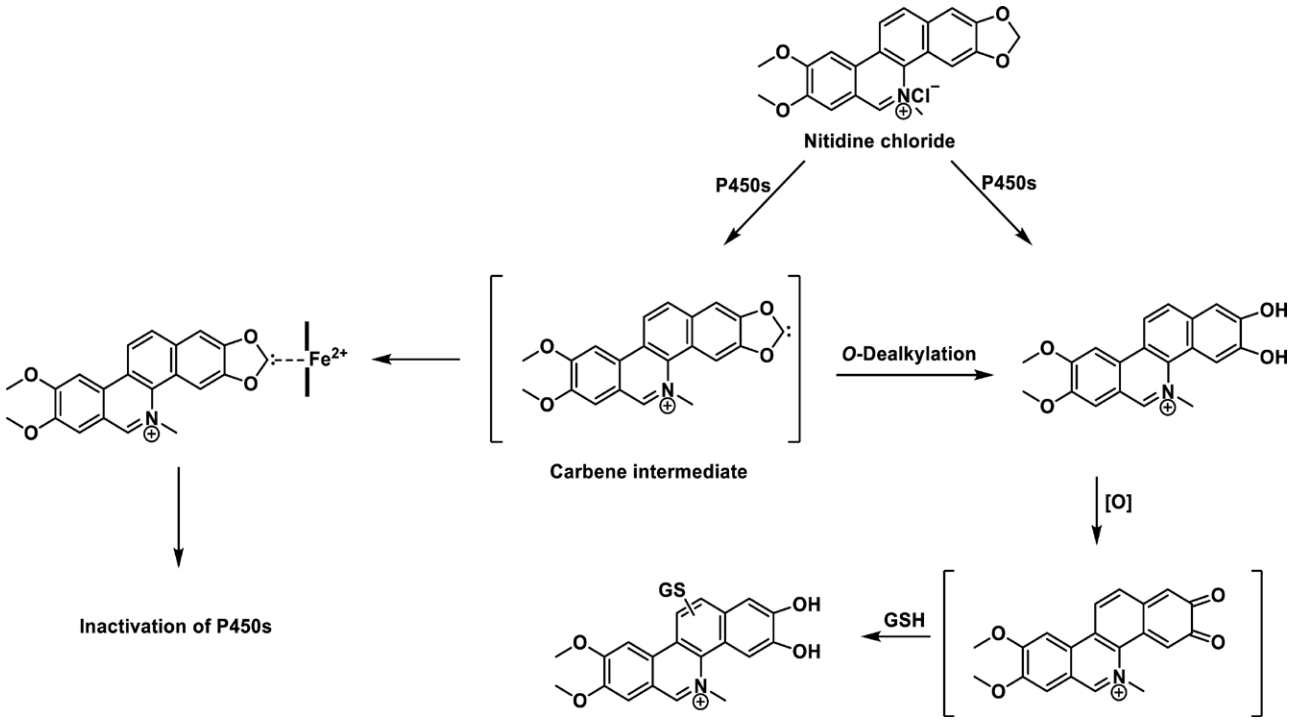

Figure 8. Proposed mechanism-based inactivation pathways induced by nitidine chloride.

induced by natural MDP products can be attributed to the formation of *ortho*-quinone metabolites.

Safrole is the principal active antifungal ingredient of Asarum volatile oil from Asari Radix et Rhizoma (Xi Xin). Previous studies have provided evidence supporting the bioactivation of safrole by CYP1A2, which converts safrole into *ortho*-quinone. This quinone intermediate formed *in situ* reacts with nucleophilic amino acid residue (s) at the active site of the host enzyme, resulting in its inactivation[112]. Further research has suggested that the overproduction of safrole-induced reactive intermediates initiates the depletion of GSH levels and leads to hepatocellular injury[113]. Nitidine chloride and chelidonine, containing MDP groups in their structures, were isolated and characterized from *Zanthoxylum nitidum* (Roxb.) DC (Liang Mian Zhen) and *Chelidonium majus* L. (Bai Qu Cai), respectively. These two MDP-alkaloids have been characterized as mechanism-based inactivators of CYP2D6. Their electrophilic intermediate carbenes may undergo covalent modification of the apoprotein or heme in the enzyme[114–116]. Berberrubine, a natural isoquinoline alkaloid containing an MDP moiety, is widely distributed in many TCM remedies, such as the Huanglian Jiedu decoction, Huanglian Shangqing pills, and Niuhuang Qingxin pills. Wang et al.[117] provided evidence for the formation of quinone metabolites from berberrubine, which can react with the cysteine residues of proteins to form protein adducts. Thus, protein adduction may play a significant role in berberrubine-induced hepatotoxicity.

Other types of natural products

Bakuchiol belongs to the monoterpene phenol family and is a constituent of *Cullen corylifolium* (Linnaeus) and Medikus (Bu Gu Zhi). Terpenoid derivatives have diverse pharmacological properties. Nonetheless, animal studies have indicated that oral administration of bakuchiol can induce renal toxicity. CYP1A2 mediates bakuchiol metabolism and generates reactive catechol and epoxide intermediates. Subsequently, the catechol was further oxidized to yield *ortho*-quinone. Both quinones and epoxides have demonstrated reactivity towards GSH *in vitro* and *in vivo*[118] (Figure 9). These findings enhance our understanding of the mechanisms underlying bakuchiol-induced nephrotoxicity.

Colchicine, an alkaloid found in *Colchicum autumnale* L., has been extensively used to treat gout and familial Mediterranean fever. This compound also exhibits utility as an anti-tumor and anti-ventilation agent. However, colchicine has also been linked to the onset of severe adverse effects, including hepatotoxicity, acute renal failure, and hematological toxicity. The quinone metabolite of colchicine is produced *via* two consecutive *O*-demethylation reactions[119].

Conclusion

The mechanisms underlying the toxicity induced by TCM remain insufficiently explored, necessitating further investigation. This review summarizes the toxicity mechanisms elicited by metabolic activation of natural products in TCM, as documented in the existing literature. We focused on the correlation between the metabolic pathways of natural products in TCM and their toxicities. This study aimed to elucidate the mechanisms through which natural products in TCM induce toxicity through various metabolic activation processes.

Many toxic natural products within TCM are metabolized by P450s or other enzymes to yield electrophilic

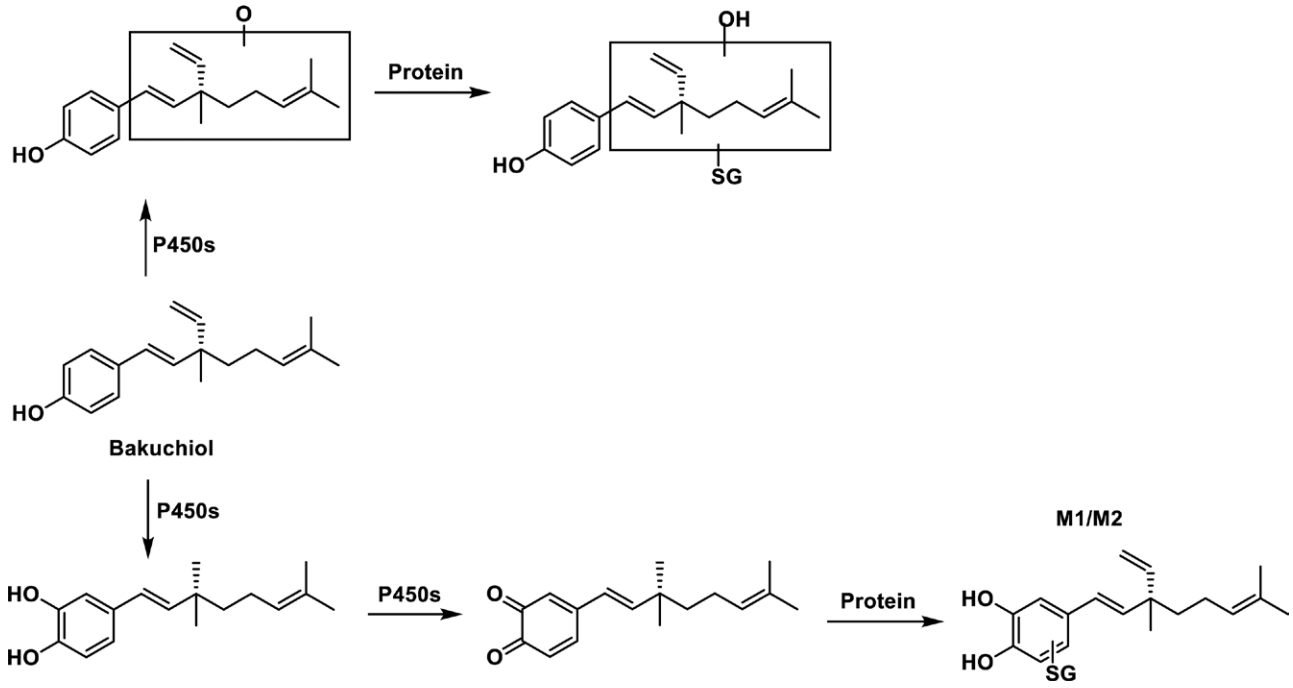

Figure 9. Proposed metabolic pathways of bakuchiol in hepatic microsomes supplemented with NADPH and glutathione. NADPH: Nicotinamide adenine dinucleotide phosphate, reduced form.

intermediates. These electrophilic intermediates induce cellular damage by covalent binding at nucleophilic sites of biomolecules, such as the sulfhydryl group of cysteine, the ε-amino group of lysine, or the N^2-nitrogen of deoxyguanosine[120]. According to hard and soft theory, covalent reactions between nucleophilic and electrophilic species are characterized by high selectivity and exhibit a significant degree of specificity in their interactions. Soft electrophilic groups, including quinones, epoxides, and α,β-unsaturated carbonyl groups, tend to form covalent bonds with soft thiol groups in cysteine, GSH and proteins. Conversely, hard electrophilic reagents such as aldehydes and ketones tend to bind to hard amino groups on lysine, DNA, or RNA. Covalent binding with specific nucleophilic sites can irreversibly impair the functions of enzymes, DNA, RNA, and other biological macromolecules, thereby leading to the toxicity or irreversible inactivation of enzymes.

Liver microsomal incubation systems are simple and rapid methods for studying metabolic activation. Reactive metabolites are unstable and cannot be detected directly using analytical approaches. These metabolites must be trapped by capture reagents fortified in the incubation system to form the corresponding conjugates for detection. GSH is a nucleophilic agent commonly used for capture, whereas other capture reagents, such as NAC, NAL, BBA, and BBM, are also utilized, depending on the purpose of the experiment and adherence to the HSAB principle. In most cases, GSH and NAC conjugates produced during incubation can respectively be found in the bile and urine of animals treated with protoxins. To verify the structure of the conjugates, they were chemically synthesized and subjected to liquid chromatography-mass spectrometry (HPLC-MS/MS) and nuclear magnetic resonance (NMR) analysis. The synthetic products should exhibit chromatographic and mass spectral identities similar to those of products generated in microsomal incubations and animals. NMR technology effectively confirms the structures of metabolic products further. With the increasing utilization of herbal medicinal remedies and dietary supplements, concerns regarding the toxicity induced by plants and herbs have been escalating, particularly in the context of TCM usage[121,122]. The National Medical Products Administration (NMPA) in China has committed to enhancing the regulatory capacity through the implementation of efficient measures aimed at improving the safety of TCM[123,124]. The mechanisms of toxic action mediated by the metabolic activation of various natural products containing warning structures are extensively reviewed in this article. These perspectives offer significant references for the safe and rational use of TCM in clinical practice.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Yuyang Liu and Xu Wang conducted the literature search and wrote the manuscript. Minglu Liu and Xialing Hao created the figures and revised the manuscript. Jiang Zheng and Ying Peng conceived and designed the study. All authors have read and approved the final version of the manuscript.

Ethical approval of studies and informed consent

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Data availability

All data generated or analyzed during this study are included in this published article.

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