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# Safety Research in Traditional Chinese Medicine: Methods, Applications, and Outlook

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ABSTRACT

Traditional Chinese medicine (TCM) is a medical system that has collected and summarized abundant clinical experience over its long history of more than 2000 years. However, the frequent occurrence of TCM-induced adverse reactions has hindered the modernization and internationalization of TCM, while attracting increasing attention from around the world. Unlike chemical drugs and biological agents, the difficulties involved in research on the toxicity and safety of TCM mainly include the complexity of its components and the unpredictability of drug–body interactions. Much of TCM, which has overall therapeutic effects, has the typical mechanisms of multiple components, multiple pathways, and multiple targets. While considering the gradualness and unpredictability of TCM toxicity, the ambiguity of toxicants and safe dosage, and individual differences during long-term TCM administration, we have systematically established key techniques for the toxicity assessment of TCM. These techniques mainly include TCM toxicity discovery in an early phase, based on a combination of drug toxicology genomics and metabolomics; methods to identify dose–toxicity relationships in TCM; and integrated techniques for the exploration of TCM interactions, such as fast-screening tests based on drug-metabolizing enzymes and receptor pathways. In particular, we have developed a new technical system for TCM safety evaluation using molecular toxicology, which has been validated well in research on TCM compatibility contraindication, quality control, and allergen discovery. The application of this key technical platform is introduced here in detail. This application includes model organisms, toxicant biomarkers, a magnetic suspension technique, and the application of network toxicology and computational toxicology in research on the toxicity of *Fructus toosendan, Semen cassiae, Polygonum multiflorum*, and *Fructus psoraleae*.



## 1. Introduction

It has been more than 2000 years since the first clinical application of traditional Chinese medicine (TCM). Over this period, rich experience in medication has been accumulated. In fact, the geo-herbalism of TCM species, normativity of TCM processing methods, feasibility of dosage forms, normativity of decocting and administration, rationality for safe dosage, and specificity in different body constitutions—along with the principles of "four properties and five tastes," "ascending, descending, effusing, and gathering," "channel tropism," and "combination contraindication"—form a complete theory and standard of TCM [1,2] that have been proven to be effective and scientific through practice over thousands of years. Nevertheless, accidents in TCM safety frequently occur, causing TCM safety to become a bottleneck for TCM modernization and internationalization—a situation that is of concern both within China and around the world [3].

In order to guide the safety evaluation of new TCMs, the Bureau of Medicine Policy and Administration of the Ministry of Health of the People's Republic of China (PRC) (now rebranded and restructured as the Department of Medicine Policy and Basic Pharmaceuticals System of the National Health Commission of PRC since 2018) organized a team of experts to compile the *Research Guide for New Drug for Chinese Materia Medica—Pharmacy, Pharmacology, and Toxicology* in 1993. In 2005, 2007, and 2008, the State Food and Drug Administration (rebranded and restructured as the China

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Food and Drug Administration (CFDA) in 2013, and now rebranded and restructured as the National Medical Products Administration of the State Administration for Market Regulation of PRC since 2018) published many guidelines on TCM research and applications. In 2014, the CFDA revised its TCM-related research instructions. With the establishment and perfection of these instructions, TCM safety research is becoming more standardized and is gradually aligning with international standards.

Unlike the safety evaluation of chemical drugs and biological agents, difficulties with TCM toxicity and safety research mainly come from the complexity of TCMs and the unpredictability of drug–body interactions. TCMs are composed of complicated ingredients that function through multiple pathways and on multiple targets. The active ingredients and toxicants in TCMs interact with and limit each other. This complicated interaction system is nonlinear between the body and the multiple ingredients. Thus the wholeness and complexity of the medicine determine the pivotal links in TCM toxicity research [4–6].

With a focus on the gradualness and unpredictability of toxicity occurrence in long-term TCM usage, the uncertainty of toxic substances in TCM, the ambiguity of safe dosages, the complexity of toxicity mechanisms, and the specificity of different body constitutions, we were the first to establish a systematically matched key technical platform for TCM safety research. In addition, a TCM early-stage toxicity discovery technique based on a combination of drug toxicology genomics and metabolomics was established [7–9]. Our techniques also included a toxicity evaluation method for TCM dose–toxicity research, and TCM interaction research based on the fast screening of drug-metabolizing enzymes and receptor pathways [10,11]. These integrated techniques—and particularly the new technique of molecular toxicology, which is compatible with TCM incompatibility, quality control, and allergen discovery—constitute a new system of techniques for TCM safety evaluation. The techniques systematically reveal the substance basis, metabolic features, incompatibility, and mechanism of toxicity genesis in common TCMs with adverse reactions (i.e., TCM injections, aristolochic acids, pyrrolizidine alkaloids, heavy metals, toxicants for external use, and the "18 incompatible medicaments"). As a result of this work, five toxic TCMs have been adopted by the *Chinese Pharmacopoeia* (2010 edition) for their function in external use, and the anaphylactoid features of 10 kinds of TCM injections have been discovered. These achievements have greatly promoted the development of TCM molecular toxicology.

## 2. Establishment of a key technical platform for TCM safety research

There was an urgent need to establish a safety evaluation platform that is compatible with TCM toxicity features, in order to address specific TCM toxicities and safety evaluations. The established platform contains multiple methods that are suitable for many aspects of the study of TCM toxicity; it includes methods for TCM toxicity discovery, methods that are suitable for research on TCM toxicology, methods for research on TCM interactions, TCM toxicity-reduction techniques, and more. The platform supports the use of cutting-edge techniques including genomics, metabolomics, fast screening methods based on drug-metabolizing enzymes and receptor pathways, computational toxicology, and molecular toxicology.

### *2.1. A TCM early-stage toxicity discovery technique based on drug toxicology genomics and metabolomics*

New "omics" technologies such as toxicology genomics, macro-genome sequencing, mass cytometry (i.e., cytometry by time-of-flight, CyTOF), exosomes, model organisms, and metabolomics were brought together and combined with high-throughput sequencing, high-content screening, network pharmacology, and other technologies. An early-stage toxicity detection platform was established based on a combination of drug toxicology genomics and metabolomics; this platform was then applied in research on TCM early-stage safety prediction. Based on a systematic investigation of the platform-transferring abilities of a variety of commercial gene chips, we developed a toxicology genomic data integration analysis approach for the platform. This approach includes an improved decision forest (IDF) method with an accuracy of up to 92%. Furthermore, a gene expression spectrum chip-detection method was established on this platform based on certain common TCM biological toxicants such as aflatoxin $B_1$, which is more sensitive than the traditional serum biochemical indicator and the histopathology examination method [7–9].

### *2.2. An acute toxicity evaluation method for TCM dose–toxicity research*

An acute toxicity evaluation method for the TCM dose–toxicity relationship was established for the first time using the relevant methods of common toxicology and bioinformatics. Current common toxicity evaluation methods are inappropriate for research on the TCM dose–toxicity relationship. The "18 incompatible medicaments" (a rule explaining which two single drugs cannot be in the same prescription at the same time, due to the safety issue; there are 18 pairs of single drugs, so it is called "18 incompatible medicaments") were taken as research subjects, and the death rate of mice was used as the index. Acute toxicity experiments were performed for 1:1 compatibility, $2^7$ uniform design, and a dosage fixed median lethal dose ($LD_{50}$), respectively. The results revealed that the 1:1 compatibility experiment provided too little practical data; in comparison, the $2^7$ uniform design experiment yielded sufficient compatible toxicity data, but the data were large, disperse, and unfit for analysis and summary. The fixed $LD_{50}$ dosage experiment under different compatibilities was determined to be the most suitable for TCM dose–toxicity research, as it not only acquired the same data as the 1:1 compatibility and $2^7$ uniform design experiments, but also provided a compatibility ratio that can be used to increase or reduce toxicity [12,13]. Thus, this experiment contributed most to the toxicity evaluation methods for TCM compatibility.

### *2.3. Fast-screening methods for TCM interaction research based on drug-metabolizing enzymes and receptor pathways*

Fast-screening methods for drug interactions based on the pregnane X receptor (PXR)-CYP3A4, constitutive androstane receptor (CAR)3-CYP2B6, and hydrocarbon receptor (AhR)-CYP1A1 pathways were established using relevant molecular biology methods. These methods mainly involved the following: construction of the secreted luciferase reporter gene, which is linearly cascaded by double distal enhancers and proximal promoters; construction of *in vitro* screened cell lines; a reliability test for the reporter gene technique; and the application of the latter in the fast screening of TCM chemical ingredient interactions. Using these *in vitro* screening techniques, many common TCM components such as ginsenosides and aconitines were screened, and targeted compounds with potential CYP450 inhibition/induction abilities were acquired. These methods can offer an important means for TCM compatibility toxicity research and for the metabolite analysis of TCM compatibility toxicants [14].

### 2.4. TCM toxicity-mechanism research techniques that combine computational and molecular toxicology

Techniques for research into TCM toxicity mechanisms that combine computational toxicology and molecular toxicology were established using relevant bioinformatics methods. Based on databases such as SuperCYP and HIT, a computational toxicology method based on the laws of drug compatibility was established. Since the probe drug cocktail method can spontaneously offer probe drugs for multiple sub-enzymes, the characterization of multiple metabolizing enzymes can be acquired simultaneously by measuring the metabolic rate of every probe metabolite in a biological sample. A method to measure the activity of cytochrome P450 sub-enzymes in rats and a drug interaction platform were established based on the reverse transcriptase polymerase chain reaction (RT-PCR) and the western blot assay [15–18].

### 2.5. An optimized toxicity-reduction technique involving the compatibility of multiple components

An optimized toxicity-reduction technique involving the compatibility of multiple components based on a knowledge model and network regulation was established using relevant network toxicology methods. A common drug-toxicity prediction (quantitative structure–toxicity relationship, QSTR) method based on molecular structure was systematically analyzed for its unsatisfactory predicting ability. The problems with the QSTR were classified into three categories: known toxicity mechanism; unknown toxicity mechanism, but known acting pattern; and unknown toxicity mechanism with unknown acting pattern. Systematic modeling predictions based on the toxicity mechanism, toxicity acting pattern, and statistic model were proposed, which improved the accuracy of the drug-toxicity prediction. Integrated toxicity-predicting methods based on support vector machine (SVM), *k*-nearest neighbors algorithm (*k*-NN), and nearest centroids were studied, and software was developed that could rapidly calculate and analyze TCM toxicants. In this way, an optimized toxicity-reduction technique was established [19].

### 2.6. Novel methods for the discovery of allergens in TCM injections

An anaphylactoid reaction evaluation system for TCM injections was established based on the relevant clinical symptoms, pathological mechanisms, and complex cell biology mechanisms. Based on the increase in vascular permeability, which is the main pathological mechanism of anaphylactoid reactions, rat and mice cell models and test models were established that could analyze TCM injection-induced anaphylactoid reaction rapidly, objectively, and quantitatively. Allergenicity evaluation methods using a beagle dog model were established by simulating certain clinical symptoms of anaphylactoid reactions such as skin changes, blood pressure drop, and breathing difficulty. The sensitivity and reliability of these methods were validated by a known negative control and positive control, as well as by drugs that had a risk of inducing clinical anaphylactoid reactions. These methods formed an integrated evaluation system and a breakthrough of the methodology for TCM injection anaphylactoid reaction. A new mechanism for penicillin anaphylactoid reaction was discovered and confirmed in our experiment for the first time; this finding overturned the conventional idea that penicillin allergy is a type I hypersensitive reaction. A new view was proposed that past penicillin allergic history was not the absolute contraindication [20].

### 2.7. Methods of biomarker screening, verification, and optimization for early target-organ toxicity prediction

Rats were taken as the research object for these methods. After dosing with toxic drugs targeting different organs, the endogenous biomarkers for early target-organ toxicity evaluation were screened when no obvious abnormality of the pathological and biochemical indexes was observed. Multivariate statistical methods including an SVM and the receiver operating characteristic (ROC) curve were used to verify, optimize, and identify the optimum predictive biomarkers. Ten specific biomarkers of cardiotoxicity (e.g., *L*-carnitine and lysophosphatidylcholine (LPC) (14:0)) were identified [21]; five biomarkers of early nephrotoxicity (i.e., thymidine, LPC (16:1), LPC (18:4), LPC (20:5), and LPC (22:5)) were identified, with the prediction rate of the SVM model reaching as high as 95.8% [22]; and 10 early biomarkers of hepatotoxicity (e.g., lysophosphatidylethanolamine (LPE) (16:1), LPC (14:0), LPE (18:2), and LPC (16:1)) were identified, with the prediction rate of the SVM model reaching as high as 94.9% [23].

### 2.8. An in vitro multi-parameter cell-based imaging method for TCM preparation safety evaluations

Strategies to reduce animal testing by implementing human cell-based models for early-stage safety screening were recommended by the US Food and Drug Administration (FDA). An *in vitro* multi-parameter cell-based imaging method that featured high throughput and limited cost was considered as an alternative to animal testing, in order to enable a mechanistic understanding of TCM preparation-induced adverse effects. Post-marketing hepatotoxicity re-evaluation of TCM injections (Chuanhuning Injection, Chuanxinlian Injection, Xiangdan Injection, Danhong Injection, Matrine Injection, etc.) were analyzed by means of high-content analysis (Operetta CLS; Perkin Elmer, Waltham, MA, US) on the HepG2 and L02 cell lines [24]. The mitochondrial membrane potential, cell membrane integrity, intracellular and extracellular ion flux intensity, and cell membrane permeability were determined, along with cell viability. This method held promise as an ideal model for the *in vitro* hepatotoxicity testing of TCM injections with higher sensitivity. In addition, aloe emodin, emodin, rhein, and gallic acid from *Radix Polygoni multiflori* were found to cause a significant decrease in cell viability at a concentration of 100 $mol \cdot L^{-1}$. The hepatotoxicity of *Radix Polygoni multiflori* may be related to mitochondrial-mediated apoptosis, due to the significant enhancement of mitochondrial mass and mitochondrial membrane potential [25].

## 3. Application of the key technical platform for TCM safety research

### 3.1. Analysis of the material basis of TCM toxicity

Levels of TCM toxicants are often too low to be detected; furthermore, their metabolisms are complicated and they are affected by many factors such as decoction method and time. For the first time, the chemical fingerprint, toxicant differential fingerprint, related mass-spectrum database, and retrieval methods for each pair of contradictory drugs in the TCM "18 incompatible medicaments" were established. A comparison of different decoction methods (i.e., single decoction, combination after a single decoction, combined decoction, decoction after combination) and times (i.e., 30, 60, 90, and 120 min) was performed. Based on the findings, it was proposed that the combined decoction and the decoction after combination were closely related to toxicant crystallization. Take ginseng/hellebore as an example: A combined decoction soap,

a combined soap after a single decoction, and a decoction soap after combination were found to differ in their chemical ingredients—the quantities of veratrine, jervine, deoxojervine, rubijervine, red jervine, and 3-angeloylaygadenine varied significantly. The combined decoction soap had more jervine, rubijervine, and red jervine than the combined soap after a single decoction; furthermore, the combined decoction soap had considerably more veratrine, slightly more jervine and protopanaxadiol, and less germidine and 3-angeloylaygadenine than the decoction soap after combination [26]. These results showed that the main reason for the toxicity increase in ginseng and hellebore compatibility was the increase concentration of hellebore steroid alkaloid toxicants, such as jervine, zygadenine, and veratridine, and the decrease concentration of active ingredients, such as ginsenosides. Therefore, it is suggested that toxicity can be reduced by changing the decoction methods and time.

Diester diterpenoid alkaloids such as aconitine, mesaconitine, hypaconitine, 10-OH-aconitine, 10-OH-mesaconitine, and deoxyaconitine significantly increased after monkshood was decocted and combined with *Rhizoma pinelliae*, *Fructus trichosanthis*, and hyacinth bletilla, respectively. Hypaconitine increased after the combined decoction of monkshood and fritillary (*Fritillaria cirrhosa* and *Fritillaria verticillata*). The compatibility of monkshood and *Fritillaria cirrhosa* had less toxicity, and the active ingredients showed no obvious changes. However, the compatibility of monkshood and *Fritillaria verticillata* showed a remarkable increase in toxicity and decrease in effect. The material basis indicated the possibility that monkshood is not contradictory to *Fritillaria cirrhosa*; this possibility was further confirmed clinically. At the same time, it was revealed that the fritillary that is contradictory to monkshood is *Fritillaria verticillata*. The reason for the toxicity increase in the compatibility of *Rhizoma pinelliae*, *Fructus trichosanthis*, *Fritillaria cirrhosa*, *Ampelopsis radix*, and hyacinth bletilla with monkshood is the increase in the solutes and inhibition of the hydrolysis of diester alkaloid toxicants, such as aconitine, mesaconitine, hypaconitine, 10-OH-aconitine, 10-OH-mesaconitine, and deoxyaconitine [27]. This research thus explains the material basis of the toxicity increase (and effect decrease) of compatibility from the perspective of chemical changes. It also offers important references for clinical drug use.

Safety evaluation methods for TCMs such as realgar and cinnabar, which contain heavy metals, were established. The distribution of total visceral arsenic, inorganic arsenic ($iAs^{III}$ and $iAs^{V}$), and organic arsenic (arsenocholine, arsenobetaine, monomethylarsine, and dimethylarsine), along with the accumulation characteristics of total arsenic and their relation to its toxicity, were studied. It was demonstrated that realgar arsenic mainly distributes and accumulates in blood, which may be one of the mechanisms of its application in leukemia therapy. It was discovered that toxic inorganic arsenic ($iAs^{III}$) in realgar can rapidly be converted into dimethylarsine, which has a lower toxicity. This result offered some experimental foundation for the objective understanding of the safety of realgar. The quantity–time–toxicity relationship was studied further. The distribution and accumulation characteristics of soluble mercury and their relation to its toxicity were also elucidated [28]. This work offers scientific references for realgar administration in a safe dose and over a safe period of time.

### 3.2. Systematic revelation of the biological mechanism of toxic TCMs

#### 3.2.1. Aristolochic acid

Aristolochic acid (AA) nephropathy was first reported internationally, which led to negative consequences for the internationalization of TCM. Using the established TCM safety platform, a mechanism for reducing AA toxicity by inducing CYP1A2 was discovered. An evaluation platform for acute kidney injury, chronic renal interstitial fibrosis, mutagenesis, and oncogenesis was established. Acute nephrotoxicity, chronic nephrotoxicity, oncogenicity of the AA monomer *Caulis aristolochiae manshuriensis*, and the Longdan Xiegan Pills were studied. The findings revealed that acute kidney injury, chronic kidney injury, and kidney oncogenesis were induced by TCM containing AA. Our study showed that the cytochrome P450 enzyme was crucial in the toxicity genesis and development of TCMs containing AA. Different cytochrome P450 enzymes had different influences on AA, which was the main reason for individual differences in AA toxicity. For the first time, it was discovered that the combination of the CYP3A4 inducer and TCM containing AA can enhance toxicity, and that the combination of the CYP1A2 inducer and TCM containing AA can reduce toxicity [29–32]. This finding suggested that the adverse drug reaction of a TCM containing AA can be avoided or alleviated through combination with the CYP1A2 inducer. This study thus contributes to the rational clinical administration of TCM containing AA. It also contributes to the elimination of internationally identified negative consequences of TCM.

#### 3.2.2. Hepatotoxic pyrrolizidine alkaloids

Hepatotoxic pyrrolizidine alkaloids (HPAs) have a widespread existence in hundreds of herbs. These are the most toxic botanic hepatotoxic substance. An analysis and evaluation system for drug hepatotoxicity was established that elucidates the newly discovered toxic mechanism of HPAs, thus proving for the first time that HPAs are a compounded hepatotoxic substance. HPAs induce liver injury through direct oxidative stress and indirect cholestasis. A new mechanism through which HPAs induce hepatotoxicity through the regulation of mitochondria-dependent apoptosis was discovered. This mechanism elucidates the age and gender differences that have been observed with pyrrolizidine alkaloid (PA)-induced hepatotoxicity. It also reveals that the liver metabolizing enzyme system, hepatocyte mitochondria apoptosis pathway, glutathione anti-oxidization system, and bile acid regulation system have important regulatory effects on the toxicity of HPAs. This report was the first to discover that toxicity can be produced without the metabolism activation of otonecine-type HPAs; thus, this finding re-addressed the international idea that HPA toxicity must be metabolism activated [33–37].

The toxicity of *Senecio scandens* and of Qianbai Biyan Tablets, both of which contain PAs, was systematically studied. *Senecio scandens* was shown to have potential embryotoxicity, making it necessary to further investigate the toxicity of the total alkaloid content. Our studies showed for the first time that the water extract of *Senecio scandens*, its total alkaloid content and Qianbai Biyan Tablets, which contain *Senecio scandens*, all showed significant embryo toxicity, with the effect on bone deformities being extremely serious in all of our experimental results [38]. These findings indicate that exposure to *Senecio* spp. as well as Qianbai Biyan Tablets may carry potential risk of fetal development. Obvious embryotoxicity could be observed in five kinds of PA, including monocrotaline, senecionine, retrorsine, seneciphyllin, and senkirkin treatment groups in an *in vitro* whole-embryo culture system. The five kinds of PA induced fetus abnormality by affecting the morphological differentiation of embryo organs such as the caudal neural tube, audiovisual olfactory system, gnathism, and limb buds. Retrorsine, jacobine, and monocrotaline were discovered to be related to the genesis of embryo toxicity through a mechanism that involves induction of the expression of heat shock proteins (HSPs) HSP70 and HSP90 [39–42]. As a result of these findings, it has been suggested that *Senecio* should only be taken with great attention during pregnancy.

*3.2.3. Heavy metals*

There are many mineral drugs that contain heavy metals in TCM. These heavy metals, such as arsenic and mercury, are considered to be an important component of the prescriptions. Some mineral drugs have heavy metals within the principal component, such that the quantity exceeds the standard limits of heavy metal pollution. This heavy metal excess in some TCMs has attracted international attention.

The species transformation of cinnabar was studied in simulated gastric acid and in a simulated intestinal tract *in vitro*. It was determined via infrared spectrum analysis that the leachable of cinnabar in simulated gastric acid could be $Hg_3S_2Cl_2$. The leachables of cinnabar in the simulated intestinal tract were studied using many established spectroscopy methods (i.e., infrared spectrum, electrospray ionization mass spectrometry (ESI-MS), Raman spectrum, and X-ray absorption spectrum). It was determined that these leachables could be various types of complexes of mercury and sulfur, with a main composition of $HgS_2(OH)^-$. It was inferred that the active ingredient might be a complex of mercury polysulfide that was absorbed by the intestinal tract. There was little chance that the cinnabar was metabolized into severely toxic methyl mercury by means of intestinal bacteria. Most of the metabolites were mercury polysulfides. Using proteomic methods, a chemical probe (i.e., "bait") of HgS was built and then used to seek and "hook" the specific targeted protein in nerve cells. The aim of this experiment was to recognize the targeted protein that the main ingredient of cinnabar, HgS, exerted its pharmacological or toxicological effect upon [43].

*3.2.4.* Aconitum carmichaelii

Based on previous research, we found that the metabolism pathway of steroid hormones is closely related to drug-induced cardiotoxicity. Therefore, a fast, accurate, and sensitive new method for the quantification of the steroid hormones contained in batch samples was established by means of the semi-automatic high-throughput isotope label derivatization ultra-performance liquid chromatography/multiple-reaction monitoring (UPLC/MRM) technology platform [44–46].

*Aconitum carmichaelii*, an important medicine in TCM, often leads to arrhythmia. Based on the abovementioned high-throughput detection method, rat heart tissue was obtained after *Aconitum carmichaelii* administration and used for proteomics and metabonomics study. It was found that steroid hormones such as dehydroepiandrosterone sulfate (DHEA-S) are closely related to cardiotoxicity. Further functional analysis revealed that the cholesterol in the adrenocortical cells generates pregnenolone through 20,22-lyase, followed by catalyzation by 17,20-lyase to form dehydroepiandrosterone (DHEA); finally, it forms DHEA-S, catalyzed by adrenal sulfonase (Hss). A certain concentration of DHEA inhibits the expression of the messenger RNA (mRNA) of the oxidative stress vascular smooth muscle cell inflammatory factor called monocyte chemotactic protein-1 (MCP-1), which is induced by $H_2O_2$, and also inhibits the formation of the intracellular oxidation product malondialdehyde. These findings suggest that this inhibition effect via DHEA may be achieved by the direct antioxidant mechanism of DHEA.

*3.2.5.* Semen cassiae *and* Polygonum multiflorum

Previous research [47] has found that bile acid metabolism is closely related to drug-induced liver injury. An ethanol extract of the common TCM *Semen cassiae* was gavaged to mice for 14 d. This did not cause significant changes in the liver biochemical markers or morphological changes in the mice, although it did significantly inhibit the expression of liver bile acid efflux pump (BSEP) and increase bile acid concentration. However, in a mouse model of intrahepatic cholestasis induced by α-naphthyl cyanide, the anthraquinones of absorbed *Semen cassiae* components in blood were found to inhibit BSEP and significantly aggravate cholestasis liver injury; this finding suggested that anthraquinones are the main material basis for cholestasis. In the clinic, the compatibility of drugs with *Semen cassiae* with the propensity to cause cholestasis should be avoided [48]. In addition, studies have shown that the extract of *Polygonum multiflorum* can affect the bile acid transporter, further accelerate the intestinal circulation of bile acids in mice, significantly increase the cholestasis of mice with abnormal bile acid metabolism, and increase liver damage; these findings provide a basis for rational clinical medication.

*3.2.6. Others*

The molecular biological mechanism by which licorice reconciles various drugs was revealed, and licorice and glycyrrhizic acid were found to be regulated by the protein kinase C (PKC) pathway. Licorice and glycyrrhizic acid directly activate the PXR, induce the expression of the *CYP3A4* gene and protein, and regulate the synthesis of lithocholic acid and key metabolism genes: *SHP*, *CYP7A1*, and *CYP3A11*. Toxicants in TCM could be metabolized through this process. A reporter gene and an electrophoretic mobility shift assay (EMSA) further confirmed this result. It was a crucial link that licorice accelerated the metabolism of the relevant ingredients of toxic TCM through the inducing effect of the PXR on CYP3A. This drug interaction is mainly demonstrated by licorice reducing the potency of toxic TCM by means of the inducing effect of CYP3A, which results in a contradictory compatibility [49]. This finding reveals the scientific basis behind the use of licorice to reconcile various drugs over thousands of years.

*3.3. Solid evidence for the classic theory of TCM compatibility*

The "18 incompatible medicaments" of TCM is the most representative classic theory of TCM incompatibility. The scientific nature of the "18 incompatible medicaments" of TCM incompatibility was studied for the first time, based on cytochrome P450 enzyme activity, the expression level of mRNA and protein, and the variation of toxicants. A study on the antagonism of monkshood with *Rhizoma pinelliae*, fritillary, *Ampelopsis radix*, *Fructus trichosanthis*, and hyacinth bletilla showed that when monkshood antagonized all of these plants except *Ampelopsis radix*, it exerted an inhibitory effect on CYP3A and CYP1A2. This effect was particularly notable in *Fructus trichosanthis* and *Rhizoma pinelliae*, whose protein and enzyme activities were affected accordingly. However, monkshood is mainly metabolized through CYP3A and CYP1A2; thus, the inhibition of CYP3A and CYP1A2 reduced the removal of aconitine and enhanced its exposure and toxicity *in vivo*, resulting in incompatibility. Therefore, it was proposed that in order to avoid incompatibility based on the drug-metabolizing enzyme, TCMs whose toxicants were substrates of CYP3A and CYP1A2 should be taken with care. The inhibitory effect of the cytochrome P450 enzyme aligned with the material basis for the activity of *Fructus trichosanthis*, *Rhizoma pinelliae*, and hyacinth bletilla within the body. Here, the mechanism of incompatibility might involve metabolism inhibition and the increase in toxic alkaloid leachables. Aconitine and fritillary differed only in their metabolism of enzymes, and aconitine and *Ampelopsis radix* differed only in their material basis.

When hellebore was combined with *Radix Salviae miltiorrhizae*, *Radix Sophorae flavescentis*, or ginseng, the quantity of cytochrome P450 enzyme and the activity of CYP3A and CYP2E1 were inhibited to varying degrees. CYP1A enzyme activity was induced when ginseng and hellebore were combined, which aligned with the mRNA level. The cytochrome P450 enzyme aligned with the material basis when ginseng and hellebore were combined. The increase in toxic alkaloid leachables was the main reason for incompatibility. *Radix Salviae miltiorrhizae* and *Radix Sophorae flavescentis* only

inhibited CYP3A on the enzyme activity level when combined with hellebore. *Asarum sieboldi* Miq, *Radix Adenophorae*, and *Radix Scrophulariae* were partially unaligned with hellebore on the material basis level.

When licorice was synergized with alga, *Radix Euphorbiae pekinensis*, and *Flos Genkwa*, the expression and activity of CYP3A and mRNA were induced, especially for alga and *Radix Euphorbiae pekinensis*. The inducing effect of licorice on CYP3A accelerated the metabolism of related TCM toxicants. The contradictory effect was mainly reflected in the decrease in TCM potency that resulted from the inducing effect. This was the first time that the mechanism of *Radix Euphorbiae kansui* and licorice compatibility was revealed. The activity and quantity of CYP1A2 and CYP2E1 increased after compatibility; the CYP1A2 and CYP2E1 mainly activated pro-carcinogens and pre-toxicants, which are closely related to many cancers. Therefore, this compatibility increases the possibility of cancer. This study thus offers important theoretical evidence for clinical application.

The scientific nature and toxicity-reducing and effect-increasing mechanism of the cold-and-hot compatibility of the Zuojin Pills were revealed for the first time based on the fact that the adrenal medulla secretes catecholamine. The classic cold-and-hot compatibility of the Zuojin Pills was taken as a subject of study. *Coptis* root inhibited the secretion of acetylcholine, veratridine, and high levels of potassium-stimulated catecholamine by inhibiting calcium ion concentration within the cell. *Fructus evodiae* induced the secretion of catecholamine by increasing the calcium ion concentration within the cell. When *Coptis* root antagonized *Fructus evodiae* (6:1), the *Coptis* root strongly antagonized the catecholamine secretion of the bovine adrenal medulla cell and the calcium ion concentration within the cell. Thus, this study offers scientific evidence for the clinical application of cold-and-hot compatibility.

The MaTox Pre software was developed independently, based on metabonomics data on target-organ toxicity [1–3,50–52]. The current version, 1.0 (software registration number: 2016SR154181), supports early prediction of hepatic, cardiac, and nephritic toxicity. Taking *Pinellia ternata*, *Fructus trichosanthis*, fritillary, and *Bletilla striata* antagonizeaconitum from "18 incompatible medicaments" as the object of study, the MaTox Pre software was used to find the cardiac toxicity of *Aconiti Lateralis Radix Praeparata*; no cardiac toxicity were found in other single drugs. Furthermore, the combination of *Aconiti Lateralis Radix Praeparata* and other single drugs showed increased cardiac toxicity [53,54].

### 3.4. Promotion of innovative drug research and development

Based on the established key technical platform, innovative drug development was performed. Effective drug sites and the composition of various kinds of TCMs and compound prescriptions were screened and studied. In this way, 11 compounds representing TCM early toxicity were discovered, including ginsenoside F2, deoxyschizandrin, and indigo. Each of these compounds could be activated by the PXR. This discovery reduced the risk of new drugs being eliminated due to safety concerns. Based on the common evaluation of toxicity and the key technical platform, 103 safety evaluations were performed on new drugs, thus promoting the development process of innovative drugs.

## 4. A future outlook on TCM safety research

TCM safety evaluation is not an easy task, and still has a long way to go. It is necessary to establish a scientific system that emphasizes TCM safety evaluation. In particular, reducing toxicity through TCM compatibility and processing requires deeper study. More discussion is needed on the rules for compound changes in TCM compatibility and processing, as well as on the relation between TCM compatibility, processing, and toxic effects. Relevant national departments should develop an overall plan for TCM safety research. Innovative, well-designed, and systematic safety research on frequently used TCMs and other compounds should be carried out in a stepwise fashion. Finally, a system for controlling TCM toxicity and a technical system to guide clinical drug use and new drug development should be established.

## Compliance with ethics guidelines

Yue Gao, Aihua Liang, Xiaohui Fan, Limin Hu, Feiran Hao, and Yubo Li declare that they have no conflict of interest or financial conflicts to disclose.

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