Acupuncture and Herbal Medicine

Integrated toxicology: an effective solution for explaining the "Toxicity" of traditional Chinese medicine

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Conceptualization of "Toxicity" in traditional Chinese medicine (TCM)

In this evolving era, there is an increasing demand for superior-grade TCM products. However, a spate of safety incidents related to Chinese herbal medicines in recent years has garnered considerable scrutiny and concern[1], both nationally and globally. These safety concerns[2] have emerged as critical impediments in the advancement of the TCM industry, markedly constraining the modernization and globalization efforts of TCM practices. A reassessment and delicate understanding of the concept of "toxicity" related to TCM coupled with advancement of contemporary TCM toxicology are now recognized as imperative strategies to effectively address these challenges[3,4].

The concept of "toxicity" in TCM conveys a double meaning. Over 2,000 years ago, the ancient text *Origin of Chinese Characters* (说文解字) described "toxicity" as "thick," portraying it as a neutral adjective. Gradually, TCM practitioners developed a unified understanding: as one of the intrinsic properties of Chinese herbs, "toxicity" represents a biased terminology. The fundamental principle of TCM using "bias to counteract bias" consists of this understanding, as highlighted in *Jingyue Quanshu* (景岳全书): if a disease is present and a corresponding herb is used, the disease is affected; however, if there is no disease and a herb is used, the vital energy is affected. This ancient theory has been substantiated by modern medical evidence[5,6]. Presently, the perception of "toxicity" related to TCM is often categorized in broad and narrow terms. In broader terms, it refers to the biased nature of a medicinal property, whereas in narrower terms, it is understood to have harmful effects on the body. Based on the degree of harmfulness, the current version of the *Chinese Pharmacopoeia* classifies the toxicity of Chinese herbs into three categories: highly toxic (10 types), toxic (42 types), and slightly toxic (31 types), listing a total of 83 types.

Numerous factors influence the toxicity of Chinese herbs, and their compatibility related to clinical applications is a key aspect of the rational TCM usage. Compatibility is the primary method used in TCM prescriptions, in which drugs are combined according to specific rules and in certain proportional ratios to create appropriate prescriptions[3]. Prescriptions represent the evolution of drug compatibility and also represent the most advanced applications. In the field of TCM toxicology, research related to attenuation of toxicity through compatibility is crucial. It also serves as an effective approach to scientifically illustrating the rational clinical application of "toxic" Chinese medicines (Figure 1).

Characteristics and critical scientific problems in research on safety of TCM

Due to its inherent properties, research on safety aspects of TCM exhibits the characteristics that are distinct from those of modern toxicology (Western toxicology). These include the latency of toxicity manifestations, uncertainty of the material basis, ambiguity of safe dosage, complexity of the toxicity-efficacy mechanism, and differences in the specificity of individuals. Consequently, this leads to critical scientific problems in research on the safety aspects of TCM; for example, it is necessary to understand how do "toxic" Chinese medicines herbs produce toxicity after interacting with

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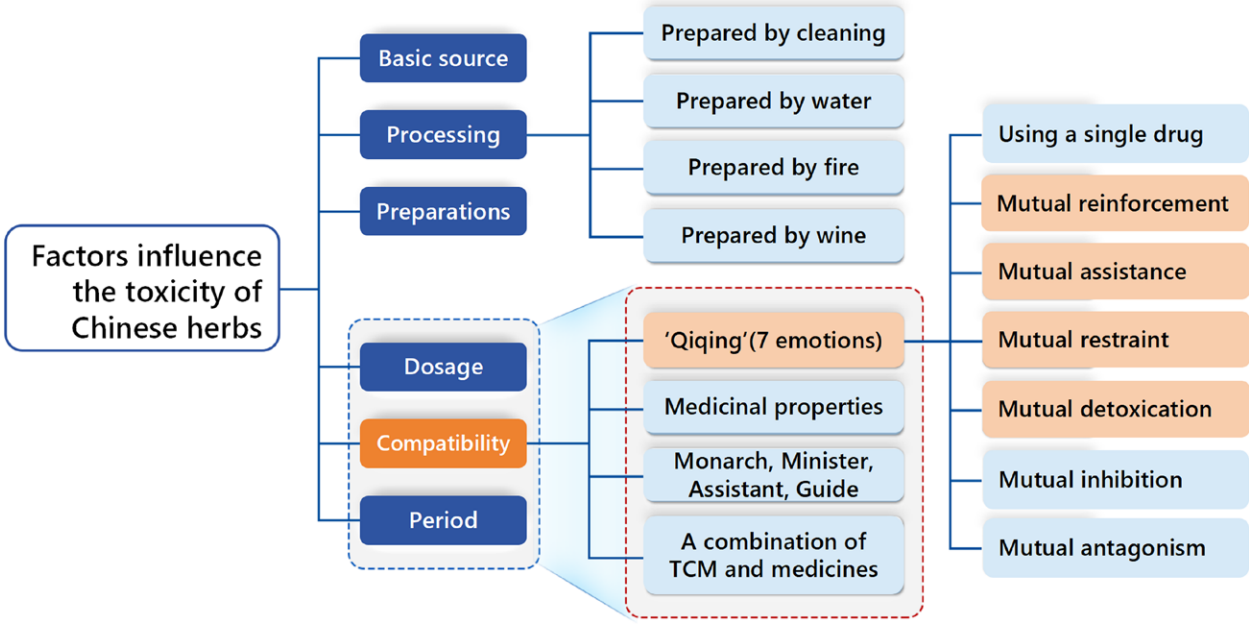

Figure 1. Factors that influence toxicity of Chinese herbs. TCM: Traditional Chinese medicine.

the body, or how does the compatibility attenuate toxicity (Figure 2).

Integrative toxicology: an effective solution to explaining the "Toxicity" of TCM

To address the aforementioned key scientific questions and based on years of practice in the field of TCM safety, we propose the concept of integrative toxicology for research on TCM. Integrative toxicology is an interdisciplinary field that combines TCM, alternative toxicology, pharmacology, computational science, chemical medicine, and molecular biology. In this field, interactions between complex systems and organisms, and rules regarding attenuation of toxicity through compatibility are studied. Integrative toxicology aims to overcome the ambiguous assessment of TCM toxicity, leading to a unique toxicological evaluation system and characteristics of research methodology related to TCM.

Application of integrative toxicology in toxicity attenuation compatibility research: the case of Realgar-Indigo Naturalis formula (RIF)

The RIF[7] is the only globally marketed oral arsenic drug proven to be effective for acute promyelocytic leukemia (APL)[8,9]. Oral administration of arsenic initiated a new treatment protocol for M3 leukemia and

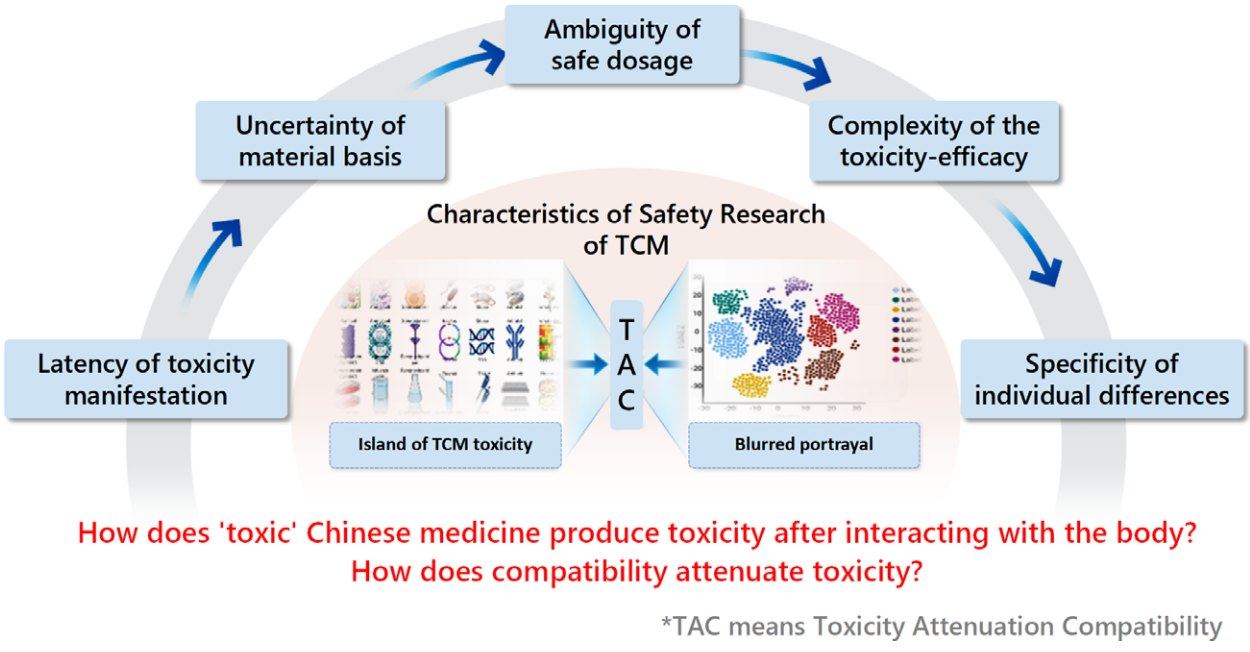

Figure 2. Characteristics and critical scientific problems related to research on safety aspects of traditional Chinese medicine. TCM: Traditional Chinese medicine.

was included in the "Iconic Scientific and Technological Achievements of Traditional Chinese Medicine" (2012–2022). A report by Chen Zhu's team, from a pharmacodynamic perspective, revealed the multi-component and multi-target mechanism of RIF in treating APL and the mechanism by which RIF extract promotes degradation of cancer proteins. This study elucidated the scientific essence of RIF treatment for APL based on the "monarch, minister, assistant, and guide compatibility principle" and the "fight poison with poison concept"[10]. Based on the concept of integrative toxicology, Gao Yue's team used an integrated toxicology approach, which included single-cell transcriptomics[11] and other multi-omics experimental schemes, coupled with high performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC-ICP-MS), molecular pharmacology, and toxicology techniques. They systematically conducted experiments on the effects of compatibility of RIF formula on acute toxicity, subacute toxicity[12], phase I[13], and II *in vivo* metabolism, *in vitro* biomimetic dissolution, intestinal flora[14], and hematopoietic stem cells. They discovered new evidence of *Indigo Naturalis* as a minister drug and *Salvia miltiorrhiza* as an assistant (counter-assistant) drug, offering new insights into the role of each herb in the RIF formula and scientifically interpreting the toxicity attenuation compatibility of the RIF from a novel toxicological perspective (Figure 3).

Application of integrative toxicology in discovering new mechanisms of toxicity: the case of Psoraleae Fructus

Psoraleae Fructus, a tonic Chinese herbal medicine, has a history of being used for clinical applications in China over thousand years. Traditional records include nearly a thousand formulae containing Psoraleae Fructus, and the pharmacopoeia lists over 40 TCM. In recent years, with increasing reports of adverse reactions, especially liver damage, caused by Psoraleae Fructus[15], this traditionally nontoxic herb has come under scrutiny. Based on an integrative toxicology research model, our research group first predicted the toxic substances in Psoraleae Fructus based on the structure alter method, and found that hepatotoxicity and skin sensitization were the main potential toxicity of Psoraleae Fructus, especially some flavonoids contributed to the risks of liver injury[16]. Subsequently, we further screen toxic substances based on the artificial intelligence (AI) profiling, by integrative analysis for biological reactions induced by chemical constituents in Psoraleae Fructus. A novel "molecular-cell-organ-holistic" four-in-one toxicity confirmation system was constructed and proved by a series of experiments, including cell, organoid, zebrafish[17], and other models. We found that bavachin in Psoraleae Fructus had a prolonged latency to overt drug-induced liver injury, initially only with subtle steatosis in hepatocytes. Single-cell transcriptomics indicated the obvious hepatotoxicity characteristic of enhanced expression of apolipoproteins, carboxylesterases, and stearoyl-CoA desaturase 1 (Scd1), and suggested that Scd1 could be a hub molecule for the prediction of hepatotoxicity at an early stage[18] (Figure 4).

Future perspective

The toxicity/effect of TCM is a result of interaction between multiple components of TCM and organisms. However, the characteristics of safety evaluation of TCM demonstrate a gap between "humane" and "disease syndrome status," and a lack of top-down "molecular-cell-organ-holistic" coherent design and evaluation. As a result, it is difficult to explain the "toxicity" of TCM; it is also difficult to fully reveal the scientific connotation of the changes in attenuation before and after the compatibility of "toxic" Chinese medicines. At this point, the need for integration of toxic effects, material basis, *in vivo* processes, mechanisms of action, and clinical verification has emerged. This can be applied not only to discover toxic substances and mechanisms but also to study the law of attenuation of toxicity through compatibility, which is an effective solution for explaining the "toxicity" to TCM.

Conflict of interest statement

Boli Zhang is the Editor-in-Chief of this journal. Yue Gao is editorial board members of this journal. None of the other authors declare any conflicts of interest.

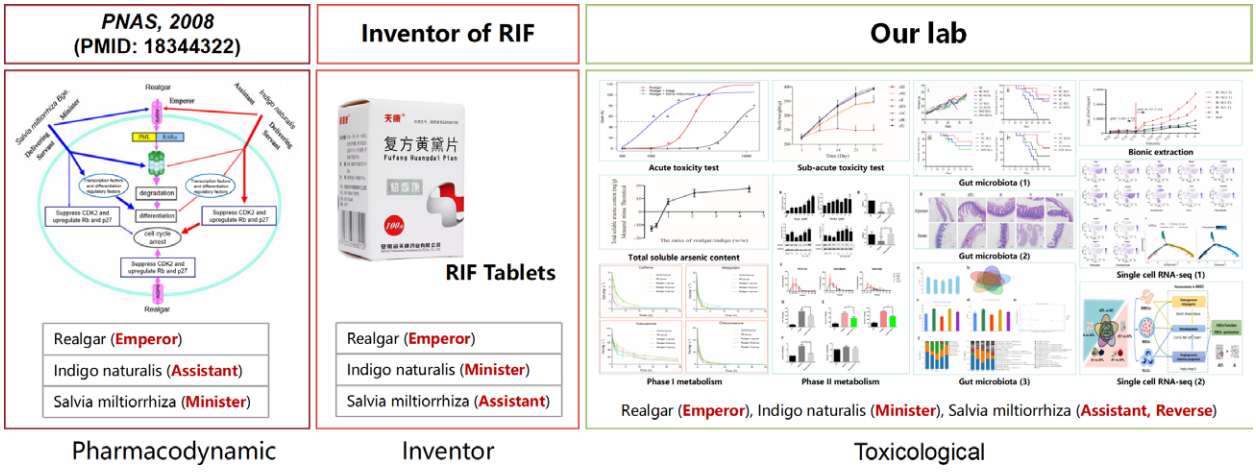

Figure 3. Integrative toxicology reveals the scientific essence of toxicity attenuation compatibility of RIF. RIF: Realgar-Indigo Naturalis formula.

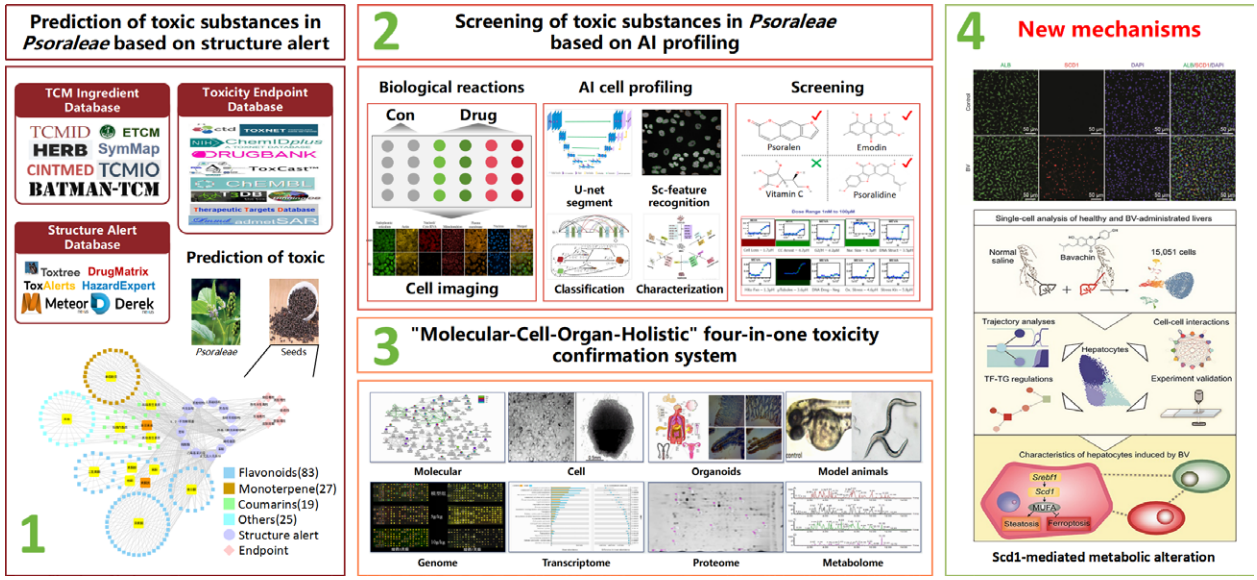

Figure 4. Integrative toxicology reveals a new mechanism of hepatotoxicity caused by Psoraleae Fructus. BV: Bavachin; TF: Transcription factor; TG: Targetgenes.

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Author contributions

Huanhua Xu drafted the manuscript. Wei Zhou and Jianfeng Yi revised the manuscript. Boli Zhang and Yue Gao revised the important intellectual content of this manuscript. All the authors have reviewed and approved the final manuscript.

Ethical approval of studies and informed consent

Not applicable.

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None.

Data availability

All relevant data are within the manuscript.

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