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Review

Network toxicology and LC-MS-based metabolomics: New approaches for mechanism of action of toxic components in traditional Chinese medicines

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ABSTRACT

Network toxicology combined with metabonomics is of great significance for the study of the toxic mechanism and prediction of toxicity of traditional Chinese medicines (TCMs). In this study, we reviewed the application of network toxicology based on LC-MS metabolomics, mainly in the study of toxic components and the toxicity mechanism of TCMs, which provides new ideas and methods for the further study of the toxicity mechanism of TCMs.

Contents

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1. Introduction

Traditional Chinese medicine (TCM) is the rarity of the Chinese nation, and the research on the toxicity of Chinese medicine cannot be ignored. In general, toxic herbs have potent pharmacological activities (Liu et al., 2017). During the development of Chinese medicine for thousands of years, toxic herbs have always playing an important role. Consequently, the prediction of the TCMs toxicity has become a part of the research of the modernization of TCMs. In recent years, network toxicology technology, the metabonomics technology and the LC-MS based omics technology have been used to predict the toxicity of TCMs.

The concept of network toxicology was first put forward by academician Chang–xiao Liu in 2011. It is an important method for biopharmaceutical research developed by network pharmacology (Fan, Zhao, Jin, Chen, & Liu, 2011). Network toxicology of Chinese herbal medicines (CHMs) is designed to describe network toxicology, and it refers to the study of the toxicological characteristics of a network model and analyzes the interaction and regulation of poison materials in biological systems by the established network model, which plays an important role in predicting the toxic components of TCMs (Fan et al., 2011; Liu et al., 2015; Liu, Fan, Li, & Xiao, 2016; Zhang et al., 2015). Metabolomics is a crucial part of systems biology (Shi et al., 2016). It can measure a large number of metabolic pathways of endogenous metabolites with low molecular weight, reflecting the basic metabolic state. Recently, dozens of metabonomics research has been committed to study the toxic components of TCMs, explore its mechanism, identify potential biomarkers to visit action goals, and achieve fruitful results. In recent decades, LC-MS and other technology methods are applied in many researches of TCMs, such as the identification of chemical constituents and metabolites, serum pharmaceutical chemistry, pharmacokinetics, metabonomics, fingerprint and adulterants. In a paper by Li et al. (2011), a sensitive, rapid and simple liquid chromatography-mass spectrometry (LC-MS) method was used to determine the salidroside content in rat plasma and the pharmacokinetics of Erzhi Pills and *Ligustri Lucidi Fructus* oral solution in rats was investigated.

Network toxicology technology plays an important role in studying the toxicity mechanism and predicting the toxic components of TCMs. Here we reviewed the application of network toxicology based on LC-MS metabolomics, mainly in the study of toxic components and the toxicity mechanism of TCMs, which provides new ideas and methods for further study of the toxicity mechanism of TCMs.

2. Application of network toxicology

Network toxicology refers to the description of the toxicological characteristics of drugs by constructing a specific network model to analyze and predict the toxicity of the drug, thereby elucidating the toxic side effects of the drug on the human body and predicting the toxic components of TCMs (Fan et al., 2011; Liu et al., 2015).

Using network pharmacology techniques to analyze the interaction between "toxicity-gene-targeting-drugs", we can infer and judge the toxicity and side effects of complex Chinese medicine ingredients, so as to find out the toxicity of drugs. It provides theoretical basis and technical support for drug safety evaluation. The research ideas of network toxicology generally include four aspects (Fan et al., 2011): (i) Single Chinese medicine or toxic ingredients prediction and analysis; (ii) the toxic mechanism exposition of TCMs; (iii) the scientific connotation of compatibility taboo theory in Chinese Medicine interpretation; (iv) the reciprocity between TCM and western medicine explanation.

The process of studying toxicity mechanism of TCMs by network toxicology technology is as follows: (i) Access to literature, search database, extract drugs, genes, proteins, toxicity and other information; (ii) use drugs, genes and toxicity as the nodes of the network and construct a network model of "genes-targets-drugs" using relevant software; (iii) scientifically analyze the model, study the toxicological characteristics of drugs, and explore the toxic mechanism of TCMs (Fig. 1).

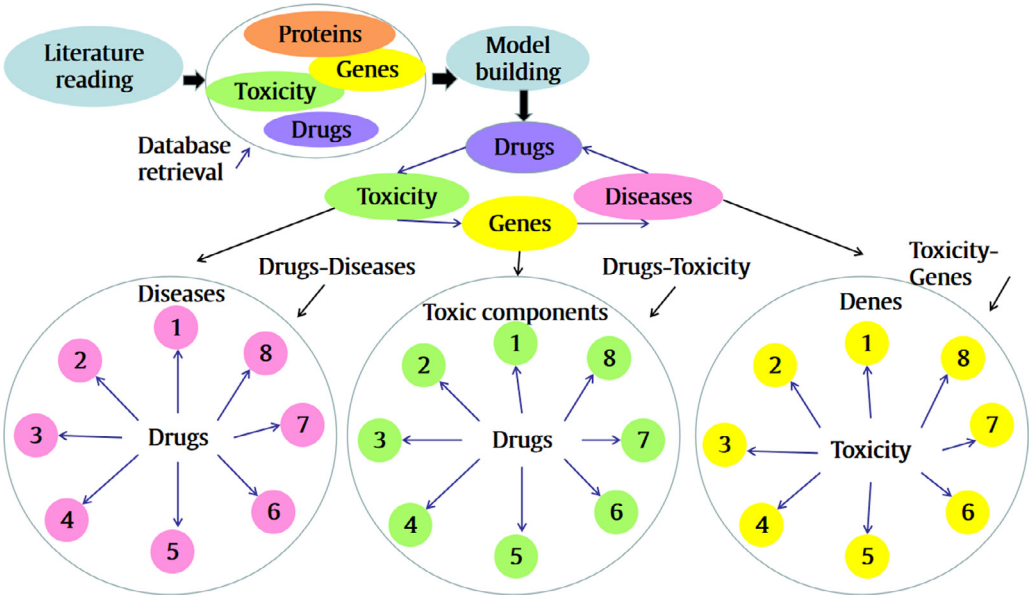

Fig. 1. Network toxicology research model.
Firstly, we consult the literature and search the database to find the information of "drugs, proteins, genes, toxicity and so on", and use the relevant software to construct a "drugs-toxicity-genes-diseases" network model. Then, according to the needs, specific network models such as "drugs-diseases", "drugs-toxicity" and "toxicity-genes" will be constructed to analyze and predict.

2.1. Prediction of toxic components of TCMs

Network toxicology is mainly used to study the toxicological characteristics of the constructed network models, analyze the interaction and regulation of toxic substances and their biological systems, explore the toxic effects of drugs and drugs on the body, and elucidate the toxicity mechanism of TCMs.

In the research of TCMs, network toxicology can be used to identify active toxic ingredients, clarify the mechanism of toxicity, and predict potential adverse reactions in humans and incompatibility of TCMs (Yu et al., 2015). In a paper by Dong et al. (2015), chemical toxic components of experimental methods by metabolomics related to TCMs of *Aconiti Radix* were analyzed and predicted, including 5-hydroxy-6-methoxy indole glucuronide, 4,6-dihydroxyquinoline, glycine 17 related biomarkers, which have determined the toxicity of *Aconiti Radix*.

2.2. Elucidation of toxicity mechanism of TCMs

Combining genomics, proteomics and other technologies, network toxicology can clarify the toxic mechanism of toxic Chinese medicines under the support of traditional toxicity theory of Chinese medicines (Li et al., 2018). Firstly, according to the important toxic target organs, information on TCMs, proteins, genes and toxic reaction is compiled from databases and literatures. Then, the network model of "poisonous TCMs-target" is established by using relevant software, and the network is analyzed systematically to clarify the possible poisoning mechanism of poisonous TCMs.

2.3. Rational design of compatibility of TCMs

Using TCM network pharmacology method, Li and Zhang (2013) predicted the active components and pharmacological action of Chinese herbal compounds, and revealed the association between "drug-gene-diseases", which explained the compatibility rules and network regulation of Chinese herbal compounds. The results confirmed the discovery of bioactive compounds of TCMs based on the method of network pharmacology, and clarified the mechanism of the action of compounds in Chinese medicines, such as the Six-Ingredient Rehmannia Pills. The method of network pharmacology of TCMs provides a new research paradigm for the transformation of TCMs from empirical medicine to practical medicine.

2.4. Evaluation of curative effect of Chinese medicine compounds

In order to reveal the effect of Huangqi Danshen Decoction [Astragalus Salvia Compound (ASC)] on pregnancy-induced hypertension syndrome (PIH), Zeng, Yang, and Ge (2017) built a network of PIH, composite target network of ASC, ASC-PIH network, composite target-PIH target-other human proteins in the PPI network by combing network pharmacology with clinical practice based on the basic theory of TCMs and collecting active ingredients of ASC by traditional Chinese Medicine databases. Results showed that the ASC can regulate endothelial cells of several biological processes and gene activation and injury, and hypertension in the placenta and trophoblast cell ischemia model in pregnancy-induced hypertension. Results showed that the ASC can regulate endothelial cells of several biological processes and gene activation and injury, and hypertension in the placenta and trophoblast cell ischemia model in pregnancy-induced hypertension. Zhou, Cheng, and Zhang (2016) analyzed the pharmacological effects of Six-Ingredient Rehmannia Pills by network pharmacology, especially for learning and memory, immune regulation, neuroendocrine immune interactions, including the effects of Liuwei Dihuang Decoction (LW) on the central nervous system, endocrine system, and immune system. Experimental results showed that LW has extensive pharmacological actions through various pathological factors of interference in the neuroendocrine immunomodulation (NIM) network adjustment and recovery. The Chinese and English literatures on network toxicology and network pharmacology in PubMed and CNKI databases are yearly increasing.

In summary, network toxicology has the advantages of improving drug efficacy, reducing side effects, increasing the success rate of clinical trials, and reducing the cost of drug discovery. Network toxicology provides a new way of thinking for the toxicological research of TCMs. It provides a new method for screening toxic components of toxic Chinese medicine, provides a theoretical basis for the development of new drugs, and provides new technical support for improving the safety of traditional Chinese medicine. It will also further advance the process of modernization of traditional Chinese medicine. Traditional toxicity analysis methods are complicated in steps, difficult to operate, and low in accuracy.

Network toxicology technology overcomes the shortcomings of traditional methods, and has the advantages of simple operation, accurate results, and wide application.

3. Application of metabonomics in TCMs

Metabonomics is a significant part of systems biology focusing on small molecule metabolites (Shou et al., 2018). We can study the changes of metabolites in blood, urine and tissues by this method and identify early biomarkers in animal biological samples. Metabonomics has been widely applied in nutrition, toxicology and clinical diagnosis (Miao et al., 2016).

3.1. Determination of toxicity mechanisms of TCMs

Huang et al. (2018) studied the toxic components and toxicity mechanisms of *Aconiti Lateralis Radix Praeparata* (ALRP) extracts based on metabolomics. The toxic chemical constituents of ALRPA water and ethanol extracts were different. Reversed-phase chromatography and ultra-high performance liquid chromatography (UPLC) were used to detect the expression of small molecule metabolites of different extracts in rats. Qualitative and quantitative analysis of small molecule metabolites confirmed that the ALRPA extracts were cardiotoxic, and the toxicity of the ethanol extract was greater than that of the water extract. Based on the results of metabonomics, the toxicity mechanisms of aconite extract were studied. ALRPA produced cardiotoxicity by activating PI3K/Akt/mTOR signaling pathway and regulating TNF-β signaling pathway.

3.2. Discovery of toxic biomarkers

Drug-induced nephrotoxicity is a major problem because many pharmacological compounds are filtered or excreted into the urine by the kidneys. There are many readily measurable metabolites in urine and serum that provide reliable evidence of drug toxicity, kidney function and kidney damage. Metabolomics experiments are designed to discover valuable metabolites that are potential biomarkers for early detection of drug nephrotoxicity. The primary metabolic pathway can be monitored by modern metabolomics techniques through specific surrogate biomarkers in urine and blood (Zhang et al., 2012a).

Lu et al. (2013) used a urinary metabolomics technique based on high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) to investigate the characteristic potential biomarkers of *Xanthii Fructus* in rats. Ten

metabolites were found to be potential toxic biomarkers. These results suggested that metabolomics analysis can be used to predict toxicity. In a paper by Liu et al. (2016b), potential biomarkers were studied based on UPLC/TOF-MS-based metabolomics techniques. Plasma metabolic profiles and metabolic biomarkers were identified by multivariate data analysis. They identified 28 biomarkers in MCAO rats. The identified biomarkers are mainly related to amino acid metabolic disorders, energy metabolism, lipid metabolism, and monoamine neurotransmitter metabolism. This study provides an effective way to explore the mechanism of MCAO-induced cerebral ischemia and evaluate the efficacy of Buchang Naoxintong Capsules (BNC).

3.3. Evaluation of toxicity of TCMs

In recent years, there have been numerous articles on the use of metabolomics to study the toxicity of TCMs. From 2012 to 2018, the number of articles on network pharmacology, network toxicology technology and the number of articles using metabolomics techniques to study the toxicity of traditional Chinese medicine from CNKI and PubMed were shown in Fig. 2.

Pinelliae Rhizoma (PR) is a common Chinese medicinal herb, but its toxicity is often reported (Ye et al., 2016). Su et al. (2016) discussed the mechanism of PR-induced toxicity and the reduction effect of processing toxicity. The toxicity of PR was evaluated by biochemical and histopathological methods. LC-TOF-MS was used to analyze rat serum metabolites. Metabolomics technology is original. The results showed that raw material PR can cause cardiotoxicity. The data revealed the mechanism of primitive PR-induced cardiotoxicity. This experiment provides a scientific basis for the convention processing theory of PR. It also helps in the in-depth study of metabolomics techniques and applies metabolomics techniques to the toxicology of Chinese medicine.

As a valuable TCMs, PR has been traditionally used to treat cough, infection, vomiting and inflammation. However, it also has many side effects and toxicity. Zhang et al. (2013) proposed a UPLCQ-TOF/MS metabonomics method to elucidate the toxicity of oral PR in various organs such as kidney, liver, and heart in rats. Metabonomics methodsblood, biochemistry, histopathological and examination were used to confirm that oral crude PR had no significant hepatic and renal toxicity in SD rats. PR may have certain cardiotoxicity to SD rats. Metabolic changes indicated that metabolomics methods are promising tools for the study and diagnosis of TCM induced toxicity.

4. Application of liquid chromatography-mass spectrometry (LC-MS) in TCMs

LC-MS is a commonly used method to study metabolomics and it is a formidable technique for the analysis of complex botanical extracts. MS provides rich information for the structural analysis of compounds (Wang et al., 2014). The method has the characteristics of high sensitivity, high resolution and convenient sample preparation. LC-based metabonomics technology is suitable for metabolism of exogenous substances (Fang et al, 2014). Metabonomics study based on LC-MS was illustrated in Fig. 3.

The analysis of toxic compounds in plasma, serum and urine is an important part of toxicology. High selectivity, accuracy, sensitivity and ease of use are needed in toxicology. LC-MS has the advantages of high selectivity and sensitivity, and plays an important role in toxicological analysis (Véronique, Denis, & Marc, 2012).

4.1. Analysis of toxicity components of TCMs

LC-MS technology has the advantages of high sensitivity and high selectivity (Discenza, Obermeier, Westhouse, Olah, & D'Arienzo, 2012), and is of great significance for routine, high-throughput and special toxicological studies (Couchman Lewis et al, 2011).

Hyoscyami Semen, dried seeds of *Hyoscyamus niger* L., is highly toxic. The main active ingredients of *Hyoscyami Semen* are hyoscyamine, scopolamine, hyoscine, and atropine. Qi et al. (2012) used LC-MS combined with multi-reaction detection scanning method to determine the active alkaloids in the samples, and qualitatively and quantitatively analyzed three batches of different origins. The content of total saponin (in terms of atropine) is in the range of 0.02%–0.05%. Based on the toxicology of *Hyoscyami Semen*, a qualitative and quantitative analysis method for toxic pharmacodynamic components based on LC-MS was established for the existing quality standard verification method. Wu et al. (2015) studied the selective cytotoxicity of aspartic acid *in vitro* and *in vivo*, and studied the pharmacokinetics of aspartic acid-modified doxorubicin using HPLC-MS. The results indicated that aspartic acid-modified doxorubicin increased LAT1 excess drug accumulation at its *N*-terminus and inhibited the growth of tumors.

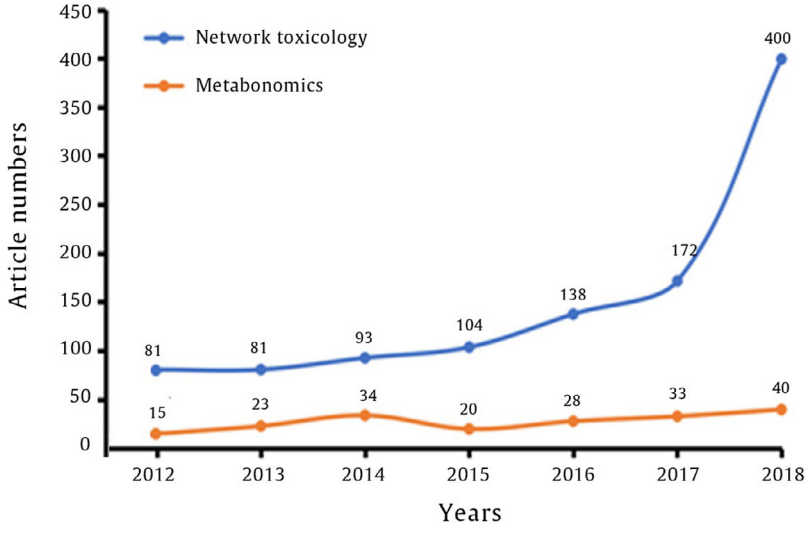

Fig. 2. Number of articles on network toxicology and metabonomics from PubMed and CNKI databases (2012–2018).

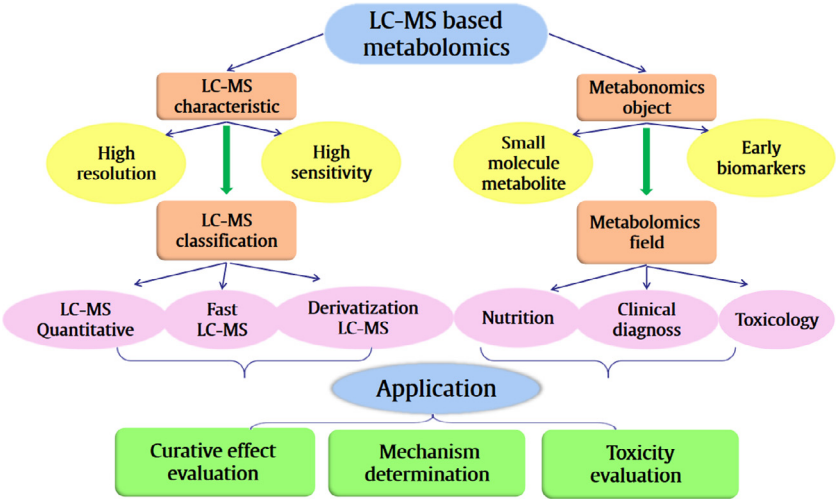

Fig. 3. Metabonomics study based on LC-MS showing characteristics of LC-MS: High sensitivity and high resolution.
Classification of LC-MS: LC-MS quantitative, fast LC-MS, derivatization LC-MS. Metabolomics research: small molecule metabolites and early biomarkers. Research areas of metabolomics: nutrition, toxicology and clinical diagnosis. LC-MS metabonomics can be used for efficacy evaluation, mechanism determination and toxicity evaluation.

In summary, the LC-MS method overcomes the cumbersome shortcomings of the traditional method, which has the advantages of simple experimental steps, high accuracy, and high sensitivity. LC-MS has high resolution in the determination of pharmacokinetics, tissue distribution and selective cytotoxicity *in vitro* and *in vivo* of the active ingredients of single Chinese medicine.

4.2. Application of LC-MS in toxicity study of TCMs prescriptions

The compatibility of TCMs may affect the therapeutic effect of the active ingredient or the toxicity of the drug (Wang et al., 2016). Drug-plant interactions affect the pharmacokinetic characteristics of active ingredients in the body (Hu et al., 2016). Liu et al. (2016c) suggested that the increase in Corynoline exposure after oral administration of Shuanghua Baihe Tablets (SBT) was less than that of Corynoline and berberine. This result may be due to interactions between drugs and drugs in SBT, and other compounds in SBT may also affect the pharmacokinetic characteristics of Corynoline and berberine hydrochloride. It can be explained by the compatibility of TCMs, which proves that the synergy between Chinese herbs can affect the therapeutic effect and pharmacological activities of drugs (Zhang et al., 2012b). Peng, Wang, and Jin (2014) used the LC-ESI-MS/MS technique to evaluate the PK characteristics of eight bioactive components after oral administration of Huanglian Jiedu Decoction. They performed pharmacokinetic experiments and used LC/MS/MS to detect the metabolites of Tongmai Granules in rats' plasma (Liu et al., 2013).

The composition of compounds in TCMs is complex, and the interaction between drugs will affect the toxicity and pharmacological activity of the prescriptions. LC-MS method has high accuracy and resolution. It can accurately detect the active ingredients and pharmacokinetic characteristics of compound Chinese medicines and accurately locate the toxic ingredients in compound Chinese medicines, providing basis for safe medication of TCMs prescriptions.

5. Toxicity mechanisms of TCMs based on LC-MS metabolomics

The toxic components of Chinese medicine can be detected by LC-MS technology, and then the toxic mechanism of Chinese medicine is further explored by the combination of metabonomics technology.

Asari Radix et *Rhizoma* is a common Chinese herb whose main component is aristolochic acid analogs (AAA_S). These compounds are nephrotoxic and carcinogenic. To date, little information has been reported on the phytochemistry and nephrotoxicity of *Asari Radix* et *Rhizoma* (Jing et al., 2017). A study using high-resolution LC-MS metabolomics method to analyze the chemical changes of medicinal *Asari Radix* et *Rhizoma* (Michl, Bello, Kite, MSJ, & Heinrich, 2017). Most samples contain potentially toxic AAAS, including 9-methoxy aristolochic acid (AL) IV, AL I and AL IV. These compounds exist not only in methanol but also in water extracts. However, other mechanisms associated with the development of aristolochic acid nephropathy (AAN) and cancer may occur, such as DNA adduct formation. The results of this study provided a model to analyze the toxicity of smaller known plants. LC-MS-based metabolomics is very promising in the research of drug toxicity.

Triptolide with anti-inflammatory effects is the main component of Tripterygium glycosides (TG) (Chen et al., 2017). The mechanism of reproductive toxicity of triptolide remains to be studied. In a paper by Ma et al. (2015), they used a GC–MS-based metabolomics technique to study the mechanism of reproductive toxicity induced by triptolide, which provides new biomarkers for early detection of spermatogenesis dysfunction. The results indicated that triptolide exerts its effect by inhibiting spermatogenesis and the testosterone level of the marker enzyme, lowering sperm count and gonad index. In summary, this study provides the first comprehensive metabolomic analysis method for GC–MS analysis of testicular toxicity caused by triptolide. Metabonomics based on GC–MS plays an important role in the study of reproductive toxicity.

6. Toxic mechanisms of TCMs based on metabonomics and network toxicology

The method based on metabonomics and network toxicology to research the toxicity mechanisms of TCMs are more accurate and effective. Network toxicology combines structural ontology, statistics, analysis and mathematical methods were used to make the mechanism of drug toxicity prediction more precise and sensitive (Bai et al, 2013). The network includes not only correlated genomics, transcriptomics and metabolomics data, but also organ and physiological function data describing drug toxicity information.

Research methods offers potential for comprehensive evaluation of drug safety based on metabonomics and network toxicology.

For this reason, Li et al. (2016) used drug-induced hepatotoxicity as a model, a systematic strategy for screening specific biomarkers was established and these biomarkers were used to evaluate potential hepatotoxicity of the drug. Fifteen biomarkers were comprehensively analyzed using multivariate statistical analysis and metabolomics data. Then, specific biomarker candidate data were extracted from hepatotoxic drugs and non-hepatotoxic drugs and established a Support Vector Machine (SVM) model. The results demonstrated that those ten biomarkers are specific. Therefore, this SVM model can be used to distinguish hepatotoxicity or non-hepatotoxicity of medicine. This method provides a new strategy for screening specific biomarkers and also provides a new model for assessing hepatotoxicity, and will be a very useful technique for drug toxicity assessment and disease diagnosis.

7. Discussion

The network toxicology research method provides scientific technology and theory for toxicity prediction of TCMs and is widely used in the field of TCMs. The toxicity of TCMs is an important part of the modernization of TCMs. Network toxicology provides a simple, accurate and reliable screening tool for toxic substances, which overcomes the shortcomings of traditional toxicity prediction methods that require a lot of energy, material resources and complicated steps. The holistic advantages of network toxicology provide ideas for the toxicity study of complex Chinese medicine systems.

However, network toxicology still faces many challenges in predicting the toxicity of TCMs. Firstly, dynamic multi-scale models can be constructed based on drugs, diseases and genes. When using network toxicology technology, we need to consider the dynamics of the model and the characteristics of multi-scale, and consider the interference between the interactions of the model on multiple scales (Guo et al., 2017). Analysis of relevant toxic components from multiple perspectives and multiple levels is an important challenge for network toxicology. In addition, the chemical composition of TCMs has a large structural diversity, the complexity of molecular mechanisms is large, and the applicability of network toxicology methods also has certain limitations (Zhu et al, 2011). Therefore, combining the network toxicology prediction model with the dynamic characteristics of the drug-disease multi-scale model and the chemical structure characteristics of TCM components, establishing a suitable Chinese medicine toxicity prediction system is the focus of further research.

In recent years, tremendous advances in biotechnology, bioinformatics, and nanotechnology have provided opportunities and scientific frameworks for biomedical development (Liu, Constantinides, & Li, 2014). These new technologies combined with network toxicology will provide new ideas for drug toxicity mechanisms, new drug development, and drug innovation.

Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

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