Research progress in simultaneous detection of mycotoxins in traditional Chinese medicine

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Abstract: Mycotoxins, as secondary metabolites of fungi, have become key risk factors in Chinese medicinal materials. There are risks of fungal contamination and mycotoxin induced in the process of natural growth or artificial planting of Chinese medicinal materials to clinical use. The common mycotoxins in traditional Chinese medicine and the synchronous detection methods of various mycotoxins in modern Chinese medicine were reviewed in order to further understand the relevant standards of mycotoxins in traditional Chinese medicine, so as to improve the quality of Chinese medicinal materials and safeguard human safety and health.

1. INTRODUCTION

Chinese herbs are susceptible to mildew under good temperature and humidity conditions, producing the highly toxic secondary metabolite fungal toxin. Studies have shown that mycotoxins can affect the human endocrine and exocrine systems and suppress immune function, thus causing cancer, teratogenicity and mutagenicity. Although fungal toxins in Chinese herbal medicines are usually measured in μg/kg as trace levels, Chinese herbal medicines are mainly used to treat chronic diseases and are usually taken for a long period of time, and prolonged ingestion of Chinese herbal medicines with contaminated toxins may lead to potential dangers[1].

In the 2020 edition of Chinese Pharmacopoeia (Part 2351 Mycotoxin Assay), compared with the 2015 edition, more methods were added for the determination of zearalenone, ochratoxin A, deoxyniniolenol, penicillitoxin and a variety of mycotoxins. In the 2020 edition of Chinese pharmacopoeia, there are 19 kinds of medicinal materials applicable to xanthomycins (cypress seed, jujube, leech, earthdragon, nutmeg, scorpion, cassia seed, Radix polygonae, tangerine peel, Junzi, fat sea, lotus seed, peach kernel, centipede, areca nut, sour jujube seed, Coix seed, rigidworm and malt). The 2020 edition of the pharmacopoeia on the basis of the original new additions of rhizoma corydalis, tereocarpus, nine fragrant worm, honeycomb, strychnos. In addition, the new detection of zearalenone in Coix seed can be seen that the traditional Chinese fungal toxin has aroused people's attention.

2. COMMON MYCOTOXINS IN TRADITIONAL CHINESE MEDICINE

2.1. Aflatoxin

Aflatoxins (Afs) are mainly produced by Aspergillus flavus and Asp.parasiticus, which are derivatives of difuranocoumarin. They mainly include aflatoxin B_1(AFB), B_2(AFB_2), G1(AFG_1) and G_2(AFG_2), among which AFB_1 is the most toxic and carcinogenic in the whole AF family, and its carcinogenic mechanism has been widely concerned by medical organizations. AFB_1 was classified as a Class I carcinogen by the International Agency for Research on Cancer (IARC) in 1993. Studies have shown that injection of high doses of AF in animal experiments can cause acute death, medium doses (AF) can cause chronic poisoning, and long-term injection of low doses (AF) can cause liver bleeding and bile duct hyperplasia and a series of diseases, eventually leading to liver cancer[2].

Aflatoxins are associated with human and animal populations (toxicity and carcinogenicity). Diseases caused by the consumption of aflatoxins are loosely referred to as aflatoxicosis. Acute aflatoxin poisoning leads to death; chronic aflatoxin poisoning leads to cancer, immunosuppression and other "slow" pathological conditions. The liver is the primary target organ, and liver injury occurs when aflatoxin B is fed to poultry, fish, rodents, and nonhuman primates. There are substantial differences in species susceptibility. In addition, within a given species, the degree of response is influenced by age, sex, body weight, diet, exposure to infectious agents, and the presence of other mycotoxins and pharmacologically active substances.

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2.2. Ochratoxin

Ochratoxin (OT) is a kind of toxin produced by Aspergillus ochratoxin and several penicillin fungi. OT includes ochratoxin A(OTA), B(OTB), C(OTC) and D(OTD), among which OTA is the most toxic, second only to aflatoxin. Otas also have strong nephrotoxicity, carcinogenicity and teratogenicity. In 1993, the International Agency for Research on Cancer (IARC) classified OTAs as Class 2B carcinogens. Relevant studies have shown that when infants eat OTA contaminated breast milk, their own immune system is damaged, unable to degrade toxins, and they may suffer from kidney disease and various types of cancer [3].

2.3. Zearalenone

Zearalenone (ZEN), also known as F-2 toxin, is a non-steroidal estrogen mycotoxin produced by Fusarium fungi. Postmenopausal women in their mid-40s who take ginseng or American ginseng have been reported to have some estrogen-related illnesses, such as vaginal bleeding and changes in the uterine vaginal epithelium. Gray[4] found that such symptoms in women were probably caused by zezeolenone, a secondary fungal toxin produced in ginseng or American ginseng.

In conclusion, the medical community is cautiously closely monitoring the potential for ochratoxin toxicity in patients with signs of renal pathology. Although the role of ochratoxin A in human disease remains speculative, its acute nephrotoxicity, immunosuppressive effects, and teratogenic effects in animal models, coupled with its ability to be carried through the food chain, are of concern.

2.4. Vomitoxin

Deoxynivalenol (DON), also known as emetic toxin DON, enters the human body by oral administration and quickly passes through the small intestine barrier and is absorbed, and enters various peripheral organs of the human body through the blood circulatory system. Emetic toxin can even enter the central nervous system through the blood-brain barrier [5]. The reason why it is called emetic toxin is that studies have shown that taking DON can cause digestive tract diseases in animals. Animals will vomit, refuse to eat, resulting in malnutrition and even change the intestinal morphology and structure. The International Agency for Research on Cancer (IARC) identified it as a tertiary carcinogen [6]. Zealenone, a fungal secondary toxin produced in ginseng.

2.5. Fumonitoxin

Fumonisins are secondary metabolites produced by Fusarium moniliforme, Fusarium verticillioides and others under suitable temperature and humidity conditions. Fumonitoxin is composed of diester compound of polyhydroalcohol and tricarboxylic acid. Different types of fumonins can be divided into FA, FB, FC and FP because of the different substituents on the carbon skeleton, among which B_1 fumonins is the most widespread and toxic. FB1 inhibits ceramide and enzyme activities and exerts its toxic effects, which seriously endangers human health [7].

Unlike most known mycotoxins that are soluble in organic solvents, fumonisins are hydrophilic. This makes them difficult to study. Usually, they are extracted in aqueous methanol or aqueous acetonitrile solutions. High performance liquid chromatography with fluorescence detection is the most widely used analytical method. The story of fumonisins has drawn attention to the fact that there may be many other hidden but toxic fungal metabolites that have not yet been discovered because of their hydrophilicity.

3. SIMULTANEOUS DETECTION OF MULTIPLE MYCOTOXINS IN TRADITIONAL CHINESE MEDICINE

3.1. Liquid chromatography

High Performance Liquid Chromatography (HPLC) is one of the methods commonly used in qualitative and quantitative analysis of substances. It has the advantages of fast analysis speed, good repeatability, high resolution, high sensitivity, high quantitative accuracy and wide application range. The result has high reliability. The disadvantage is that the instrument is expensive and the testing cost is high. Although fixed phase extraction has high efficiency and less solvent usage, it does not have the specificity of immune affinity column, which is very likely to lead to subsequent testing due to incomplete sample purification.

The matrix matching standard curve method was used for quantitative analysis in multiple response monitoring (MRM) mode. The linear relationship between 12 mycotoxins was good (r> 0.9950), the recoveries were 66.1% to 109.8%, and the limits of detection (LOD) and quantitation (LOQ) were 0.10-5.00 μg/kg and 0.30-15.00 μg/kg, respectively.

The principle of Ultra Performance Liquid Chromatography (UPLC) is basically the same as that of HPLC. Cao, J [8] used UPLC method to detect OTA in ginger. Acetonitrile-aqueous solution was used for extraction, molecularly imprinted solid phase extraction column for purification, UPLC and FLD detection, and MS method was used to verify the recovery rate of the method was more than 80%. This method provides a scientific basis for the detection of OTA in ginger. Xing Y. [9] used UPLC method to detect AFB1, AFB2, AFG1 and AFG2 in traditional Chinese medicine orange peel. The HPLC was extracted with methanol PBS buffer solution, enriched and purified with immunomagnetic beads, and finally detected with UPLC-FLD method. The results showed that the chromatographic peak area had a good linear relationship with the corresponding concentration, and the recovery was more than 63.9%. This method does not need derivatization and has high sensitivity and accuracy.

3.2. Liquid-mass spectrometry technology

Liquid phase mass spectrometry (HPLC-MS) is a combination of liquid chromatography and mass spectrometry. Liquid chromatography is used to separate substances and mass spectrometry is used to analyze the mass charge ratio of charged particles. It has the characteristics of high precision, high sensitivity and strong specificity. It is applied to the separation, detection, qualitative and quantitative of each component of complex compounds. Combined with these advantages, this method has become the core method of mycotoxin detection.

Emmanuel Njumbe Ediage[10] determined the content of 25 mycotoxins in samples by HPLC-MS and extracted them with methanol/ethyl acetate/water mixture (70:20:10,v/v/v). The limits of quantification (LOQ) of the method were 0.3~ 106μg/kg. Most mycotoxins have good precision and linear relationship.

Ultra Performance Liquid chromatography-Tandem Mass Spectrometry (UPLC-MS/MS), Ultra-high Performance Liquid Chromatography/ms spectrometry combined with mass spectrometry, It can be used for both qualitative and quantitative analysis. It has the advantages of low detection limit and high sensitivity. The results can be more efficient and accurate in the detection of mycotoxin in traditional Chinese medicine.

Fan Miaoxuan [11] established an ultra-high performance liquid chromatography-tandem mass spectrometry quantitative analysis method for simultaneous determination of 16 fungomycin species in rhizoma mongolicum. The rhizoma mongolicum was extracted by 1% acetate-acetonitrile ultrasonic extraction, and pre-treated by QuEChERS(Quick, Easy, Cheap, Effective, Rugged, Safe). Gradient elution was carried out with methanol and aqueous solution containing 0.01% formic acid as mobile phase, and electrospray was used as ion source in positive ion mode for multi-reaction detection. The results showed that the linear relationships of the 16 mycotoxins were good in the linear range, R was 0.9962~1.000, limits of quantitation were 0.03 ~ 186.68 μg/kg, recoveries were 60. 28%~129. 2%, RSDS were 0.29% ~11%. This method has good purification effect, high sensitivity and good repeatability, and is suitable for the quantitative detection of various mycotoxins in the root of A. mongolicum.

Gas Chromatography (GC) was first used in the detection of mycotoxins in the 1970s. It has the advantages of high sensitivity, high accuracy and high accuracy. Gc is often used to detect mycotoxins without chromophobe or with weak fluorescence. All gas chromatography became a common method for the detection of penicillin, ZON, and streptosporin. However, gas chromatography has the disadvantages of relatively slow speed and high cost.Yue et al. [12] used methanol-water extraction, immunoaffinity column purification of samples, and GC-ECD method to detect T-2 toxins in Chinese medicinal materials. Results The recoveries of T-2 toxin were 82.2% to 98.6%, RSDS were less than 7.5%, and the detection limit was 2.5μg/kg.

3.3. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a method based on immune, enzyme and biochemical techniques. The principle is the specific binding of antigen to antibody, which opens up a new field of rapid mycotoxin analysis. The antigen or antibody is adsorbed on the surface of the stationary phase carrier, and forms an enzyme conjugate with the enzyme through covalent bonding. The enzyme reaction substrate is added, and the substrate is catalyzed by the enzyme to produce color. Qualitative or quantitative analysis is carried out according to the color depth. Although ELISA is convenient, quick, specific, and does not require high purification of toxins in samples, it can simultaneously detect the qualitative and quantitative detection of mycotoxins in a variety of samples, but its results have poor reproducibility, poor enzyme stability, and easy to produce false positive results[13]. With the increasingly strict international standards for the limited detection of mycotoxins, ELISA has been difficult to meet the needs of current social detection. ELISA is currently used as a qualitative test for a variety of mycotoxins.

4. CONCLUSION

Nowadays, fungal toxin contamination in traditional Chinese medicine occurs one after another, which has become an unavoidable problem. Mycotoxin pollution in traditional Chinese medicine has aroused people's high attention, but there are still two outstanding problems that have not been solved[14-19]:

The existing standard system of mycotoxin at home and abroad is still not detailed, lack of uniformity, and many mycotoxins that have been proved to have safety risks to human animals have not been included in the safety system.

The detection methods of mycotoxins in traditional Chinese medicine are still not perfect. Many rapid detection methods can only detect one mycotoxin, and sometimes false positive phenomenon may occur. It is not possible to detect multiple mycotoxins quickly and synchronously.

At present, there are relatively clear standards for mycotoxins in food and feed, but the structure of Chinese medicinal materials is complex and the system is numerous. We can enrich the mycotoxin detection methods of traditional Chinese medicine by referring to the mycotoxin detection methods of food and feed, etc., and find the rapid detection methods for a variety of mycotoxins. To realize the rapid, precise and diversified mycotoxins of traditional Chinese medicine. The rapid and synchronous detection of mycotoxins will become the research focus and direction in the future.

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