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Study on the Possibility of Aflatoxin Contamination in Fermentation Starter

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ABSTRACT

This study explored the possibility of Aspergillus flavus contamination and toxin production during the fermentation of starter. Aspergillus flavus isolated from natural fermentation was selected as the research object, and mixed with Transocephalia racemosa and Mucor circinelloides to establish starter. Toxin production and toxin level of Aspergillus flavus in starter were analyzed by PCR, HPLC, competitive ELISAand mouse experiment. The results showed that the isolated Aspergillus flavus strain contained aflR, omt-1 and ver-1 gene fragments; HPLC chromatogram showed that this strain mainly produced AFB1 and the concentration was 16.8μg/kg; Aspergillus flavus grew well on the drug block under construction, completely covered the drug block surface after 36h, and had strong toxin production ability. When mixed with Transverse Rhizopus racemosus and Mucor circinellus to establish starter, the growth and toxin production ability of Aspergillus flavus were inhibited. Afteroral administration oftwo kinds of AFB1-containing extractsto mice, the liver tissue was obviously damaged, and Jianqu did not show inhibitory effect on the toxicity of AFB1. This study provides a reference for strengthening quality control and preventing pollution in the fermentation process of Jianqu.

KEYWORDS

Jianqu; Aspergillus flavus; AFB1; HPLC; Liver index

1. INTRODUCTION

Jianqu, also known as Jianshenqu and Fanzhiqu, is made from a variety of Chinese herbal medicines such as Liushenqu, wheat flour and Artemisia annua after careful mixing and fermentation. It can improve intestinal function, promote digestion, relieve headaches, colds, and loss of appetite, and It can improve abdominal pain, diarrhea and other symptoms caused by weakness of the spleen and stomach. Mostof theexisting starter is produced bynatural fermentation technology. In the open natural fermentationprocess, the bacteria involved are uncontrollable andthe environment is complex, resulting in the quality of the starter can not be effectively controlled. The investigation found that the starter of natural fermentation was often contaminated by miscellaneous bacteria, which led to high product lossand also brought greater safety risks to the product. Contaminated bacteria also includeAspergillus flavus, a widely distributed harmful fungus, of which 30 to 60 percent produce aflatoxins. Aflatoxins are mainly divided into aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2 (AFB1, AFB2, AFG1, AFG2). Aflatoxin B1 is the most toxic and carcinogenic chemical found so far, and has been identified as a Class I carcinogen by the United Nations. Therefore, it is very important to analyze the toxin producing ability and toxicity level of Aspergillus flavus in the starter. Aspergillus flavus, the most dangerous fungus, was selected from several common toxin-producing fungi in the koji for analysis. PCR and HPLC techniques were used to identify the toxin production

ability and toxin production level of Aspergillus flavus, and the growth characteristics and toxin production level of Aspergillus flavus in the established starter were investigated. Meanwhile, the toxicity inhibition ability of Aspergillus flavus in the established starter was investigated through animal experiments. The results provide evidence for the safety of natural fermentation starter.

2. MATERIAL

Reagent: Aflatoxin immunoaffinity column, aflatoxin mixed standard (G1, B1: 1μg/ml; G2, B2: 0.3μg/ml) and AFB1 ELISA detection kit: Qingdao Bangpuri; dimethyl sulfoxide (DMSO): Sigma; fungal RNA extraction kit, 2×Taq Master Mix: Novizam; DNA marker: Shanghai Shenggong Biological Engineering Co., Ltd.; PCR primer: BGI.

Instrument: nucleic acid electrophoresis instrument: DYY-6C Puyang Science Research Institute; PCR instrument: GeneAmp-9700 ABI; liquid chromatography-fluorescence detector: 2998PDA Detector Waters; microplate reader: 800TS American Prot Company; ultraviolet gel imaging analysis system: 2500B Tanon.

Animals: SPF male mice of 7 weeks old (purchased from Southwest Medical University) weighing (37±3) g were used in the experiment. Mice were housed in a controlled environment at 26℃ with a 12-hour light/dark cycle and free access to sterile granular food and water. After one week of acclimatization, the experiment began.

3. METHOD

3.1. PCR Identification of Key Genes of Toxin Production

Aspergillus flavus DNA was extracted according to the kit method, and the extracted genomic DNA was used as template to amplify the target genes or sequence fragments of ver-1, omt-1, aflR and ITS respectively with primers. See Table 1 for primers.

50μL of PCR reaction system was as follows: 0.5μL of primer (20pmol/μL), 0.5μL of template DNA (100ng/μL) 10ng and 25μL of 2×Taq Master Mix, making up ddH2O to 50μL. PCR amplification procedure was as follows: pre-denaturation at 94℃ for 2min, then cycling, denaturation at 94℃ for 30 s, annealing at 59℃ for 30 s, extension at 72℃ for 90s, 35 cycles in total, extension at 72℃ for 7min, incubation at 4℃. PCR products were verified by 1.5% agarose gel electrophoresis, and finally observed and analyzed by gel imaging system.

Table 1. qPCR primer sequence list

Primer name	upstream primer	downstream primer
ITS	TCCGTAGGTGAACCTGCGG	TCCTCCGCTTATTGATATGC
aflR	CGAAAGCTCCGGGATAGCTGTACG	CCGTCAGACAGCCACTGGACACGG
Omt-1	GTGGACGGACCTAGTCCGACATCAC	GTCGGCGCCACGCACTGGGTTGGGG
Ver-1	GCCGCAGGCCGCGGAGAAAGGTGGT	CCGCAGTCAATGGCCATGCAGCG

3.2. Determination of Aspergillus Flavus Toxin Production Level by HPLC

Aspergillus flavus was inoculated in the culture medium under construction, cultured for 2 days, then placed in an oven at 80℃, dried to constant weight, and used as the test sample.

Liquid chromatography-pre-column derivatization:

The samples were processed according to the method for determination of mycotoxins in the general chapter of China Pharmacopoeia 2020 Edition, purified by immunoaffinity column, and the content of AFT species was quantified by high performance liquid chromatography (HPLC).

Chromatographic conditions: HPLC： waterse2695 series, binary pump, fluorescence detector 1046A, C-18 column (150×4.6mm;5μm); mobile phase: water (60%): acetonitrile (10%): methanol (30%); flow rate: 1mL/min; retention time: G2: 15min, G1: 18min, B2: 20min, B1: 25min; column temperature: 30℃; injection volume: 10μL; excitation wavelength: 360nm; emission wavelength: 440nm.

Calculation: AFB1, AFG1:5ng/ml, AFB2, AFG2: 1.5 ng/ml;AFB1, AFG 1:10ng/ml, AFB 2, AFG2:3ng/ml; AFB 1, AFG1:20ng/ml, AFB 2, AFG2:6ng/ml;AFB1, AFG1:30ng/ml, AFB2, AFG2:9ng/ml;AFB1, AFG1:40ng/ml, AFB2, AFG2:12ng/ml) were injected respectively to determine aflatoxin at different concentrations. Linear regression equation between peak area and aflatoxin content was obtained by liquid chromatography. 5 g of starter material was mixed with 10μL aflatoxin (AFB1, AFG1: 1μg/ml, AFB2, AFG2: 0.3μg/ml), and the recovery rate was calculated as an internal standard through pretreatment steps such as immunoaffinity column.

3.3. Determination of Growth Characteristics and Toxin-producing Ability of Aspergillus flavus in Fermentation Process

(1) Preparation of fungal spore suspension: Inoculate Transoceum racemosum, Mucor circinatum and Aspergillus flavus in PDA medium respectively, and culture in constant temperature incubator at 28℃. Fungal spores were collected by washing with sterile water containing Tween 80 (0.2%, v/v) to prepare spore suspension, adjusted to 2×108 spores/mL by hemocytometer, and stored at 4℃ for future use.

(2) Fermentation process of Jianqu: dry heat sterilize Jianqu raw material powder at 120℃ for 60min, transfer to sterile beaker, add spore suspension of 8% of dry powder mass, and make up sterile water to equal weight of dry powder. Mix well and press into lumps, then ferment at 28℃, 30g each. The samples were numbered JQH and JQM respectively. The JQH group was inoculated with Aspergillus flavus only, while the JQM group was mixed strains containing Transverse Rhizopus racemosus, Mucor circinatus and Aspergillus flavus. Aspergillus flavus contamination during fermentation was simulated. The starter was fermented for 72 h, and samples were collected every 12h. All samples were photographedand recorded, and then put into sterile bags, and marked with time points respectively, and then aflatoxin B1 levels in samples of each fermentation group were detected respectively.

(3) AFB1 determination by competitive ELISA

Samples collected during Aspergillus flavus growth were dried at 80℃ to constant weight. Crushing and sieving with 20 mesh sieve, fully mixing, as test sample.

Detection steps: Weigh 5.0 g of sample, add 25 ml of 60% methanol solution, shake and mix for 5min; take 200μL of supernatant, add 300μL of sample diluent, and prepare the solution to be tested. Add 50 μL of standard solution or sample solution to be tested to 6-well plate, add 50μL of enzyme standard solution to 6-well plate, add 50μL of antibody reagent to 6-well plate, make 3 wells of each sample parallel, mix well, cover plate membrane, and react for 30min at room temperature. Repeat washing three to four times by adding 300μL washing working solution/well, and pat dry with absorbent paper for the last time. Washing process is very important, add 100μL color solution/well, cover the plate membrane, and place it at room temperature in a dark environment to react for 15 minutes. Stop the reaction by adding stop solution 50μL/well. Read with microplate reader at 450nm.

b) Calculation: Determine AFB1 standard at 5 different concentrations (0mg/ml, 0.2 mg/ml, 0.4 mg/ml, 1mg/ml, 2.5 mg/ml) and draw standard curve. 5 g of starter material was added to 10μL of

AFB1 (1μg/ml) standard, and the recovery was calculated as an internal standard through the pretreatment step.

3.4. Aflatoxin Toxicity Test

(1) Preparation of AFB1 stock solution

AFB1 is recognized as the most toxic aflatoxin, therefore, we only considered AFB1 effects on mouse liver when evaluating aflatoxin toxicity levels.

100g of Aspergillus flavus naturally fermented starter contaminated by Aspergillus flavus and Aspergillus flavus naturally grown on PDA medium were taken together with the culture medium, 200ml of 60% methanol solution was added, crushed and mixed evenly, centrifuged at 4000r/min for 5 minutes, supernatant was extracted, and then filtered by glass fiber filter paper to obtain aflatoxin extract. AFB1 extract was concentrated with nitrogen sparger and its organic solvent was removed. After concentration, add another 5ml of DMSO solution. AFB1 concentrations were determined by ELISA, and the concentrated extracts were prepared into 1mg/ml AFB1 stock solutions using DMSO solution.

(2) Determination of aflatoxin B1 by competitive ELISA method, see 3.3 for method.

(3) Grouping and treatment of test animals

28 healthy male Kunming mice (25±5g) were randomly divided into 4 groups, 7 mice in each group. AFB1 was extracted from PDA plate. Two kinds of AFB1 extracts (2mg/kg body weight) were orally administered to mice in two groups, respectively. Mice in blank control group and solvent control group were orally administered with the same volume of physiological saline and DMSO solution (2mL/kg body weight), respectively. The experimental animals were fed standard diet, ad libitum food and water, and the experimental period was 2 weeks.

(4) Determination of indicators

Clinical symptoms of mice in each group were observed during the experiment. After the experiment, all mice were fasted for 12 h, and then killed by neck dislocation method to observe the changes of liver and collect liver rapidly. The weight of liver was weighed by electronic balance and liver index was calculated. Liver index = (liver net weight/antemortem weight) ×100.

3.5. Statistical Analysis

The experimental data were analyzed by SPSS 17.0 software, and the results were expressed as "mean ± standard deviation". One-way ANOVA was used to analyze the significance of differences, and LSD method was used to test the differences between groups. P 0.05, P 0.01 means statistically significant difference.

4. RESULTS

4.1. Identification of Toxin-producing Aspergillus Flavus Gene

PCR detection method was adopted to detect key genes of Aspergillus flavus toxin production to judge toxin production ability of target strains. PCR amplification products of the sample were about 979bp after electrophoresis (atlR gene fragment), about 600bp (ITS gene fragment), about 797bp (omt-1 gene fragment) and about 452bp (ver-1 gene fragment), while the positive control amplification products after electrophoresis of the target fragments about 979bp (aflR gene fragment), about 600bp (ITS gene fragment), about 797bp (omt-l gene fragment) and about 452bp (ver-1 gene fragment), negative control only appeared about 600bp (ITS gene fragment), the determination results are positive.

The results of nucleic acid electrophoresis showed that Aspergillus flavus isolated from natural fermentation culture contained omt-1 gene fragment, atlR gene fragment and ver-1 gene fragment, while Aspergillus oryzae, which was similar to Aspergillus flavus colony morphology, only amplified ITS gene fragment successfully. Therefore, we can confirm that Aspergillus flavus isolated from natural fermentation starter is a toxin producing Aspergillus flavus strain.

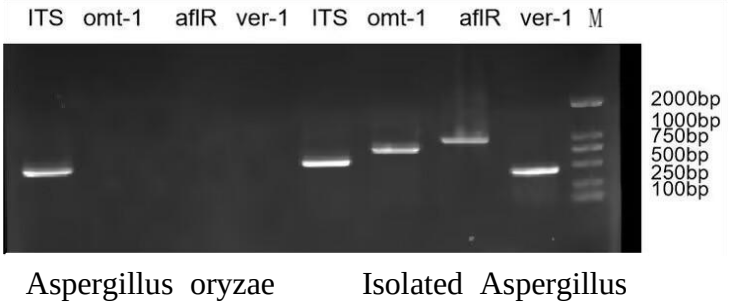

Figure 1. Results of agarose gel electrophoresis

4.2. Determination of Toxin-producing Ability by HPLC

According to the liquid chromatograms of aflatoxin B1, B2, G1 and G2 standards (Figure 2), determine the peak emergence time of different toxins and determine the peak emergence order. The peak emergence time of AFG2, AFG1, AFB2 and AFB1 is 15min, 18min, 20min and 25min respectively. By determining aflatoxin at different concentrations, the linear regression equation between peak area and aflatoxin concentration was obtained by liquid chromatography, AFB1: y = 326454x + 298174, R2= 0.9997; AFB2: y = 861901x+217873,R2= 0.9997; AFG1: y = 139953x +93302,R2= 0.9998; AFG2: y = 421157x + 69759,R2 = 0.9997;(Figure 3). The recovery calculated by internal standard method was 92%. AFB1 was mainly produced by this strain. The AFB1 concentration was calculated to be 16.8μg/kg.

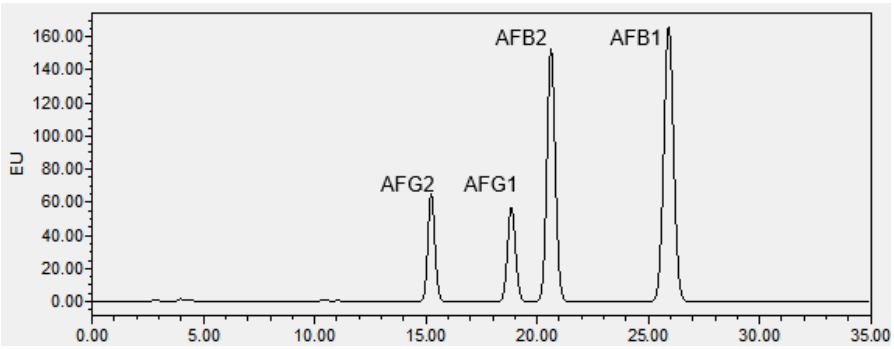

Figure 2. Liquidchromatograms ofaflatoxin B1, B2, G1 and G2 standard

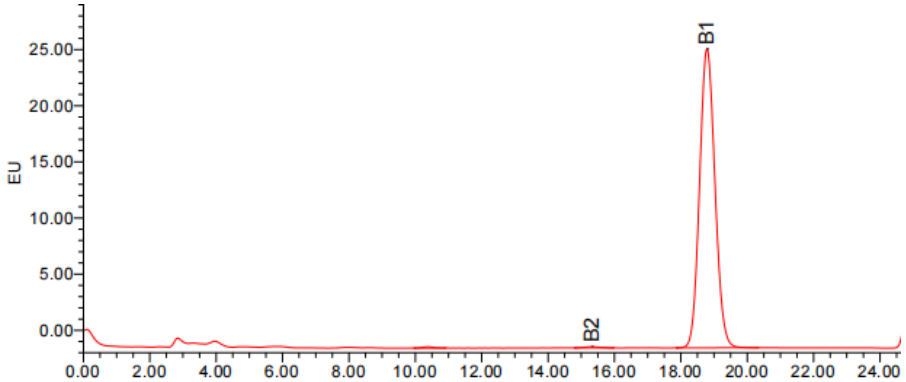

Figure 3. Liquid Chromatogram of Sample

4.3. Determination of Growth Characteristics and Toxin-producing Ability of Aspergillus flavus in Fermentation Process

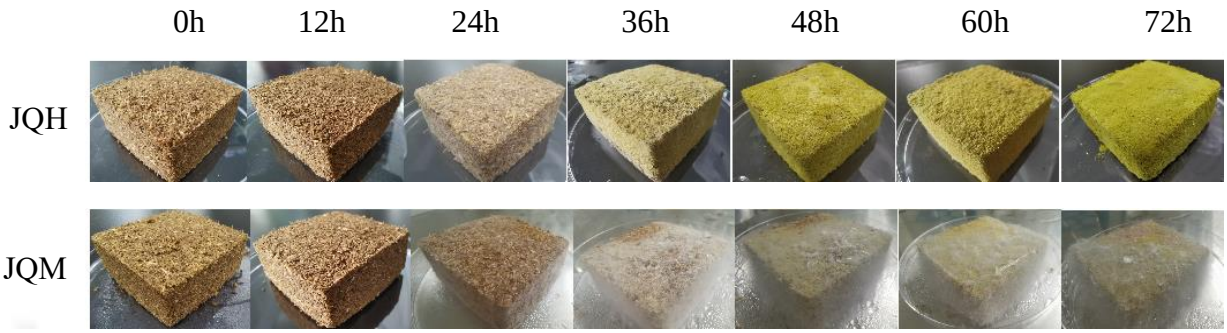

Figure 4. Appearance characteristics of two groups of Aspergillus flavus fermentation at different stages

Note: JQH) Aspergillus flavus inoculation group; JQM) Aspergillus flavus contamination simulation group;

After 24h fermentation of starter blocks, a small amount of white hyphae were foundin JQH group, and a small amount of yeastand white hyphae werefound in JQM group; at 36h, the surface of starter blocks in JQH group was covered by yellow mold, and weak yellow mold growth was found in JQM group; from 48h to 72h,starter blocks in JQH group deepened yellow-green, hyphae were thickened and dense continuously, and the color did not change significantly;starter blocks inJQM group were covered by yellowmold, and white hyphae were gradually aged (Figure-4).

The results showed that Aspergillus flavus grew rapidly and completely covered the starter block in 36h. Compared with the simulated Aspergillus flavus pollution group, the growth of Aspergillus flavus was obviously inhibited under the condition of competing with Transocephalia racemosa and Mucor circinelloides. The analysis may be due to the competition of multiple strains for development, and the nutrients provided by the starter are always limited, resulting in the growth of Aspergillus flavus strains being restricted.

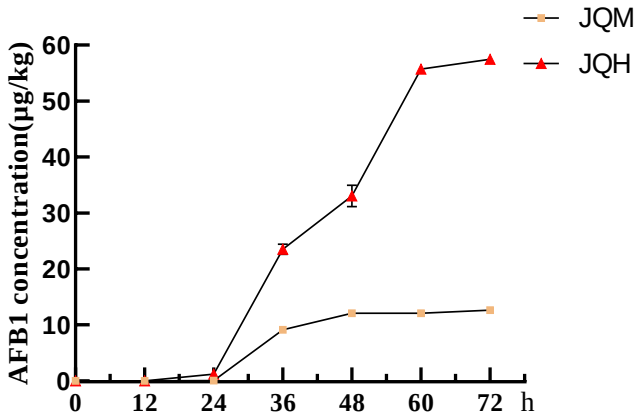

Figure 5. AFB1 concentration of two groups of Aspergillus flavus in different fermentation periods

Note: JQH) Aspergillus flavus inoculation group; JQM) Aspergillus flavus contamination simulation group;

Aflatoxin antigen is pre-coated on microwells of an ELISAplate by an indirect competitive ELISA method, aflatoxin in a sample competes with the antigen for aflatoxin antibody, aflatoxin antibody is combined with an enzyme-labeled secondary antibody at the same time, color is developed by a color developing solution, the absorbance value of the sample is negatively correlated with the amount of aflatoxin contained in the sample, and aflatoxin content in the sample can be obtained by comparing

with a standard curve and multiplying by a corresponding dilution multiple. Quantitative analysis by measuring different concentrations of AFB1 at wavelength 450nm. The recovery was 89%. A standard curve was drawn and the linear regression equation slope was-0.7166; intercept was-0.2509; R^2=0.9936.

AFB1 levels were measured by indirect competitive ELISA. The results showed that AFB1 was not detected in JQM group and JQH group at 0h-12h, and no growth of Aspergillus flavus was observed at this time. AFB1 was detected at 1.2μg/kg in JQH group at 24h, but no growth of Aspergillus flavus was observed on the drug block surface. Toxin levels in both groups increased significantly from 24h to 36h, and then tended to stabilize at about 12μg/kg in JQM group; AFB1 concentration in JQH group continued to increase significantly from 36h to 60h, and finally stabilized at about 55μg/kg (Figure 5). Toxin levels in both groups were consistent with the growth of Aspergillus flavus. We found that AFB1 concentrations were still high, posing a high safety risk to the quality of the starter, although the toxin levels of naturally fermented starter contaminated with Aspergillus flavus were much lower than those of directly inoculated Aspergillus flavus.

4.4. Analysis of the Toxicity of Jianqu to Aspergillus Flavus

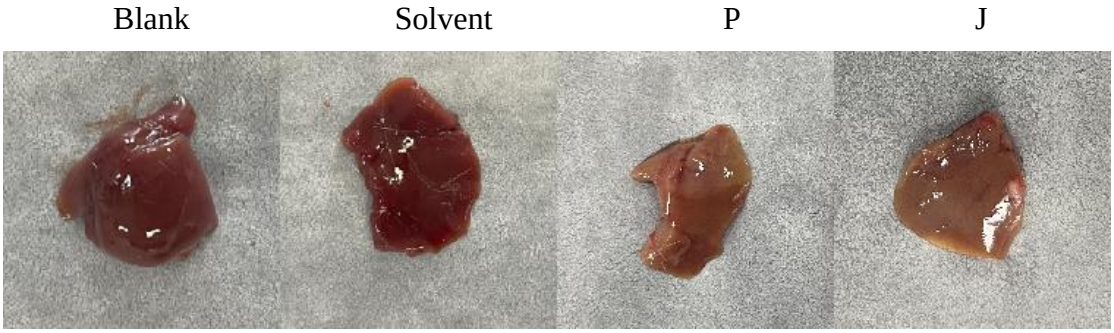

Figure 6. Effect of AFB1 on liver morphology in mice

Note: P: AFB1 group produced by Aspergillus flavus in PDA solid culture medium by gavage; J: AFB1 group produced by Aspergillus flavus in starter material by gavage

AFB1 solution containing Jianqu extract was given to mice by gavage to investigate whether Jianqu inhibited the toxicity of AFB1. The experimental results showed that the liver tissue surface of blank control group and solvent group presented normal dark red color, and the surface was normal; the liver tissue of aflatoxin group in both groups was yellow with obvious pathological changes (Figure 6).

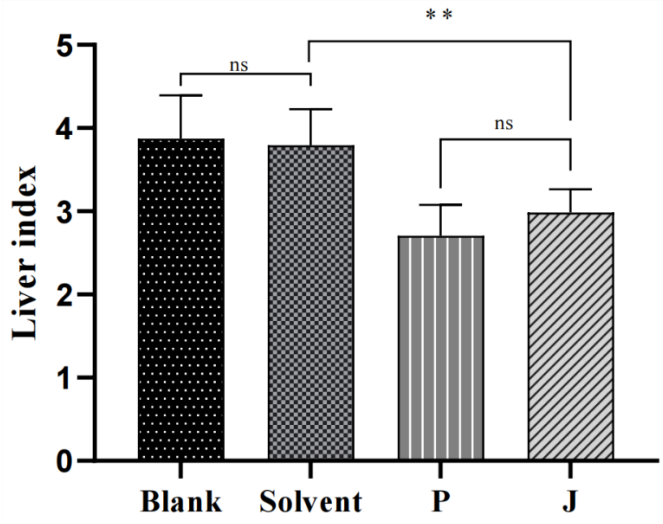

Figure 7. Effects of AFB1 action on liver morphology in mice

Note: ** indicates significant difference at P 0.05 level; ns indicates nosignificant differenceat P>0.05 level; P: AFB1 group produced by Aspergillus flavus in PDA solid medium by gavage; J: AFB1 group produced by Aspergillus flavus in starter material by gavage

Weigh the mice and the corresponding intact liver weight respectively. After calculation, the liver index of mice in each group is shown in Figure7. Compared with the blank control group, there was no significant difference between the solvent group and the blank control group, and the solvent had no effect on the liver index of mice (p>0.05). Compared with the blank control group, the liver index of the positive control group and the experimental group decreased significantly (p> 0.05), and the liver index of the experimental group increased compared with the positive group. The extract of Aspergillus flavus may have some improvement on the liver index, but the effect was not significant (p>0.05).

5. DISCUSSION

Aflatoxin is one of the most potent carcinogens known to man. At present, aflatoxin in traditional Chinese medicine is often reported to exceed the standard. Jianqu is easily contaminated by Aspergillus flavus during natural fermentation, which leads to excessive aflatoxin in Jianqu. Aspergillus flavus was isolated from natural fermentation starter in early experiment. The colony characteristics of Aspergillus flavus and Aspergillus oryzae were basically the same, and the difference was aflatoxin producing gene. For this reason, we detected the genes controlling the toxin production of Aspergillus flavus and analyzed this strain of Aspergillus flavus. Aspergillus flavus DNA was extracted, and three key genes in aflatoxin biochemical synthesis, aflR, omt-1 and ver-1, were amplified by specific primers. According to the amplification results, Aspergillus flavus isolated from the natural fermentation starter contained the three gene fragments, while Aspergillus oryzae, which was similar to Aspergillus flavus colony morphology as negative control, only successfully amplified ITS gene fragments. Therefore, we can confirm that Aspergillus flavus isolated from natural fermentation starter is a toxin producing Aspergillus flavus strain. According to the biochemical synthesis process of aflatoxin, aflatoxin cannot be synthesized without one of the three key genes mentioned above, so the isolated aflatoxin can produce toxin.

AFB1, AFB2, AFG1 and AFG2 were tested respectively, AFB1 was the highest. This strain is AFB1 with yield, which threatens the quality and safety of established starter.

When aflatoxin content is detected, HPLC fluorescence detection method with photochemical derivatization after solid phase purification column, HPLC fluorescence detection method with photochemical derivatization after immunoaffinity column and enzyme-linked immunosorbent assay are often used. However, due to the complexity of the components in the starter, the two liquid chromatography methods could not accurately detect the toxin content. After calculation, the recovery rate of photochemical derivatization HPLC fluorescence detection method after immunoaffinity column was as low as about 60%, often false positive, and weak anti-matrix background interference ability. And liquid chromatography detection method, very complex, detection costs are high. Therefore, in this test, the AFB1 content in the starter was accurately determined by ELISA. It was found that the toxin level of the naturally fermented starter contaminated by Aspergillus flavus was much lower than that of the directly inoculated Aspergillus flavus, but the AFB1 concentration was still high, 12μg/kg. Therefore Aspergillus flavus is a big hidden danger to the production safety of Jianqu fermentation. This method is simple, efficient and low cost, and can be used as the main method for aflatoxin detection in Jianqu.

Aspergillus flavus was inoculated into the starter, and it was found that Aspergillus flavus grew rapidly in the starter. At 36h of inoculation, Aspergillus flavus completely covered the starter block, and the AFB1 content was 24 μ g/kg. At the end of fermentation, the AFB1 content was 55 μ g/kg. Although Aspergillus flavus in the simulated Aspergillus flavus pollution group grew slowly and its

toxin production level decreased greatly, it could still reach about 12 μ g/kg. The analysis of the reasons for the decrease of toxin production suggested that the growth of Aspergillus flavus was inhibited due to the fixed nutrient content in the starter, and other molds competed with Aspergillus flavus at the same time, or some components in the starter inhibited the toxin production of Aspergillus flavus. MO et al found that some tea extracts can inhibit AFB1 production. SCARPARI et al found that culture filtrate of Coriolus versicolor could stimulate antioxidant system of Aspergillus mycelium and inhibit toxin synthesis. The specific reasons for the decrease of aflatoxin levels in Jianqu remain to be analyzed. It should be noted that the growth of Aspergillus flavus is often covered by white mold hyphae in the simulated natural fermentation, which is difficult to identify in the large-scale production process and seriously endangers the quality and safety of the starter.

Some studies have found that some traditional Chinese medicine extracts can reduce the toxicity level of AFB1. AHMAD and other studies have found that the extracts of Andrographis paniculata and Phyllanthus chinensis can significantly reduce the genotoxicity and mutagenicity of AFB1. Therefore, AFB1 extracted from Jianqu was given to mice by gavage. Compared with AFB1 group produced in PDA solid medium with the same concentration, AFB1 group had a certain increase in liver index, but the increase level was very limited ($p>0.05$). Both groups of AFB1 group had yellow liver and obvious pathological changes. These results indicated that although Jianqu could reduce AFB1 production during fermentation, Jianqu had no obvious inhibition on AFB1 toxicity. The investigation found that in order to save costs, manufacturers generally take out and dry at 36h. At this time, it is difficult to observe the growth of Aspergillus flavus on the surface of the starter, but the AFB1 concentration is 9.1 μ g/kg, which is a higher level [10]. Therefore, it is necessary to detect aflatoxin in the subsequent production process of Jianqu, which provides theoretical support for the improvement of quality standards of Jianqu.

6. CONCLUSIONS

A strain of Aspergillus flavus isolated and purified from natural fermentation starter was analyzed in this experiment. It was identified as a toxin producing strain by PCR and AFB1 by liquid chromatography. This strain was extremely harmful. Compared with the growth characteristics and toxin production characteristics of this strain of Aspergillus flavus in the starter, it grows very rapidly in the starter, and the toxin production is as high as 55μg/kg; when mixed with other molds, its growth is inhibited, and the toxin production level is greatly reduced to 12μg/kg, but its toxin content is still high. The results of animal experiments showed that Jianqu had no obvious inhibitory effect on AFB1 toxicity. In short, the natural fermentation process of Jianqu is easy to pollute Aspergillus flavus to produce aflatoxin, which brings safety risks to product quality, and explains the necessity of directional fermentation of Jianqu.

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REFERENCES

[1] Jiang Yun, Huang Qinwan.Research on processing of modern Chinese medicine [J]. Scientific Press, 2010.

[2] Wang Junyao, Cheng Xianlong, Li Ting, et al. Quality control status and problems of fermented traditional Chinese medicine [J] Food and Drug Administration of China, 2022, 217 (2): 60.

[3] Huang Li, Zhang Hao, Ding Weiqin, Shan Qiyuan, Chen Chen, Zheng Kaiyi, Hu Yuzhu. Contamination of Mycotoxins in Traditional Chinese Medicine [J]. Strait Pharmacy, 2009, 21 (06): 95-99.

[4] Risk assessment of aflatoxin B1 in soy sauce [J]. Chinese Journal of Microecology, 2010 (8): 4.

[5] Wei R, Qiu F, Kong W, et al. Co-occurrence of aflatoxin B1, B2, G1, G2 and ochrotoxinA in Glycyrrhiza uralensis analyzed by HPLC-MS /MSIJJ [J]. Food Control, 2013, 32(1):216-223.

[6] Zhang L, Dou X, Kong W, et al. Assessment of critical points and development of apractical strategy to extend the applicable scope of immunoaffinity column cleanup foraflatoxin detection inmedicinal herbsl [J]. J Chromatogr A, 2017, 1483:56-60.

[7] Bai Feirong, Yao Su, Ling Kong, etc. Polyphasic identification of Aspergillus flavus and Aspergillus oryzae [J]. Bulletin of Microbiology, 2018, 45 (01): 215-226. DOI: 10.13344 / j.microbiol.china.170025.

[8] Minman, Pan Huanyu, Li Feng. Research progress on detection technology of aflatoxin in traditional Chinese medicine [J]. Guangdong Chemical Industry, 2021, 48 (05): 223-224.

[9] Liang Yueqiu.Detection and Analysis of Aflatoxin B1 Contaminated by Traditional Chinese Medicine [J]. Chinese Journal of Modern Applied Pharmacy, 2000, 17 (3): 224.

[10] Zhou Heng, Wang Shaomin, Ji Shen. Current situation and prevention and control measures of mycotoxin pollution in traditional Chinese medicine [J]. China Food and Drug Administration, 2022, (03): 110-118.

[11] Zhao S P, Zhang DTan L H, et al. Analysis of aflatoxins in traditional Chinese medicines: classification of analytical method on the basis of matrix variationsIJJ [J]. SCI RepUK.2016.6:308-311.

[12] Ling L, Ye Z M, Wang S Y, et al. Isolation and Study on the Aflatoxin Genes of Aflatoxin-producing Fungi in Paprika Samples in Chengdu [J]. Journal of Sichuan University(Medical Science Edition), 2015.

[13] Abdullah N A, Mohammad S W, Abdullah Z A, et al. Fast and efficient immunoaffinity column cleanup and liquid chromatography-tandem mass spectrometry method for the quantitative analysis of aflatoxins in baby food and feeds [J]. Journal of separation science, 2020, 43(11):2079-2087.

[14] ABDEL-WAHHAB M A, HASSAN N SEL-KADY AA, et al Red ginseng extract protects against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats [J]. Food and Chemical Toxicology, 2010, 48(2):733-742.

[15] Ren Fenglan, Ma Hongwei. Aflatoxin B1 in Chinese medicinal materials and Chinese patent medicine was detected by kit [J]. Journal of Pharmaceutical Analysis, 1997, 17 (4): 280.

[16] AGAR G, ASLAN A, SARIOGLU E Ket al. Protective activity of the methanol extract of Usnea longissima against oxidative damage and genotoxicity caused by aflatoxin B1 in vitrol [J]. Turkish Journal of Medical Sciences, 2014.416):1 043-1 049.

[17] MO H ZZHANG H, WU Q H, et al. lnhibitory effects of tea extract on aflatoxin production by Aspergillus flavus [J]. Letters in Applied Microbiology, 2013, 56(6):462-466.

[18] SCARPARI M, PARRONI A, ZACCARIA M, et al. Trametes versicolor bioactive compounds stimulate Aspergillus flavus antioxidant system and inhibit aflatoxin synthesis [J]. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 2016, 150(4):653-659.

[19] Ji Wenhua, Li Xuemei, Yin Xiaoyan, et al. Research progress on detection methods of aflatoxin [J]. Journal of Henan University of Technology (Natural Science Edition), 2022,43 (05): 14-21. DOI: 10.16433 / j.1673-2383.2022.05.003.

[20] AHMAD M S, BANO S, ANWAR S. Cancer ameliorating potential of Phyllanthus amarus: ln vivo and in vitro studies against aflatoxin B1 toxicityl [J]. Egyptian Journal of Medical Human Genetics, 2015, 16(4):343-353.

[21] AHMAD M SAHMAD SARSHAD M, et al. Andrographia paniculata a Miracle Herbs for cancer treatment: ln vivo and in vitro studies against aflatoxin B1 toxicity [J]. Egyptian Journal of Medical Human Genetics, 2014, 15(2):163-171.