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Review Article

Contamination Level of Aflatoxins in Traditional Herbal Medicinal Products ("Jamu" and "Makjun") Marketed in Malaysia

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Abstract

Safety assessment for traditional herbal medicinal products such as "jamu" and "makjun" in Malaysia include the laboratory tests for heavy metals, microbiology and scheduled poisons. Test for aflatoxins is not included. Aflatoxin B_1 (AFB_1) is genotoxic and carcinogenic. Its possible contamination in these products should be ruled out. In this study, 12 samples of "jamu" and "makjun", analyzed using IAC clean-up and HPLC were found to have a mean levels of positive samples (incidence) as 0.97 (17%), 0.24 (17%), 0.92 (8%), 0.30 (8%) and 1.82 (17%) µg/kg, and the range was 0.94 - 0.99, 0.06 - 0.42, 0.92, 0.30 and 1.00 - 2.63 µg/kg, for AFB_1, AFB_2, AFG_1, AFG_2 and total AF, respectively. Low contamination level might be attributed to the antifungal effects of some medicinal herbs.

Keywords: *Aflatoxins; Jamu; Makjun*

Introduction

Research has shown aflatoxins (AF) to exhibit carcinogenic, teratogenic and mutagenic properties, and the existing Group 1 (carcinogenic to human) evaluation of naturally occurring AF (AFB_1, AFB_2, AFG_1 and AFG_2) has been reaffirmed and updated by the International Agency for Research on Cancer [1]. The most acutely toxic among the AF is aflatoxin B_1 (AFB_1), which primarily affects the liver and considered as both genotoxic (interfere with DNA) and carcinogenic. The natural occurrence of AF in tropical climate such as in Southeast Asian countries is considered unavoidable due to the favourable conditions for fungal growth and AF production. Low permissible limit for AF is regulated worldwide with the aim to control exposures of humans to as low as possibly achievable. The most frequently occurring limit for AFB_1 and total AF is now 2 µg/kg and 4 µg/kg, regulated mostly in countries belong to EU and 5 µg/kg and 20 µg/kg, regulated in other countries, respectively [2,3]. Malaysia currently regulates the level of 5 µg/kg total AF in all foods [4].

Safety assessment for traditional medicinal products such as "jamu" and "makjun" in Malaysia include the laboratory tests for (i) heavy metals (arsenic, mercury, lead and cadmium) (ii) aerobic microbial content (gram positive and gram negative bacteria, enterobacteria, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* and fungi) and (iii) scheduled poisons, such as agents for sexual dysfunction (sildenafil and tadalafil), slimming agents (sibutramine, fenfluramine and phentramine), antihistamine/antitussive (chlorpheniramine, promethazine, etc), whitening agent (hydroquinone and tretinoin) and steroids (dexamethasone, betamethasone, etc). Test for mycotoxin particularly aflatoxins (AF) is not included in the test regime. As "jamu" and "makjun" are consumed frequently for a prolonged period by consumers, the liver cancer risk associated with AF contamination should be ruled out. This study was carried out to provide the background data on the status of AF contamination in these particular products.

Research Methodology

Analysis of AF in medicinal herbs/plants and products can be difficult due to their complex matrices consisting of various chemical components. Ali., *et al.* [5] had validated the analytical method using IAC clean-up and HPLC with TFA pre-column derivatization and detection by spectrofluorometer for determination of the natural occurrence of AF in commercial "jamu" and "makjun" from Malaysia and Indonesia. Performance of this method to overcome problems of interference at the retention time of AFB1, AFG1 and AFG2 was discussed and photochemical post-column HPLC was also used to confirm the results. Correlation between the contamination level of AF and selected herbs used as ingredients was reported. The report was the first to describe contamination level of AF in commercial traditional herbal medicinal products from Southeast Asian countries, with correlation between the contamination level and the selected herbs used as ingredients to highlight the possible antifungal effects. To date, the report has been cited (> 50) by many researches worldwide and has contributed to the highlight of possible mycotoxins contamination in herbal medicinal products for further studies and evaluation of its safety for frequent and prolonged consumption.

In the present study, using this validated method we intended to further study the level of contamination of AF in commercial traditional herbal medicinal products ("jamu" and "makjun") produced in Malaysia. The result of this study will provide additional data on the status of AF contamination in these particular products and relevant risk assessment of exposure through its frequent and prolonged consumption will be carried out.

Preparation of samples

A total of 12 commercial traditional herbal medicines were purchased randomly in Perak, Malaysia. The samples were locally produced in Malaysia and analyzed on an "as is" basis. All of the samples were stored at -30°C until analyzed.

Extraction and IAC clean-up for AF

A 5g sample was added with 1g of sodium chloride, and extracted with 25 ml of methanol-water (80:20, v/v). The mixture was shaken in a mechanical shaker for 60 minutes, allowed to stand for 30 minutes and centrifuged (1400 x g, 10 min). The supernatant was filtered (Whatman No. 1) and diluted (10 times dilution); 5 ml filtrate diluted with 45 ml Tween 20: PBS (1:9, v/v) into 50 ml centrifuge tube. Vortex the solution for 30 second and filtered (microfiber filter); 40 ml of the filtrate was applied to the pre-conditioned (conditioned/washed with 2 x 5 ml distilled water) IAC (AflaTest™). The column was then washed with 20 ml of distilled water. AF was eluted with 1 ml of methanol by gravity into a test tube. The eluate was added with 1 ml distilled water for the determination of AF using HPLC equipped with photochemical reactor. The solution was transferred into two HPLC vials (two replicates) for auto injection. Injection volume was 20 µl.

HPLC analysis

The HPLC system consisted of a PU-980 pump, AS-950 auto-sampler (JASCO, Tokyo, Japan) with a 100 µl loop, and RF-10A XL spectrofluorometer (Shimadzu Co., Ltd., Kyoto, Japan). AF were determined under the following conditions: analytical column was Shim-pack CLC-ODS (Shimadzu Co. Ltd., Kyoto, Japan) with particle size of 5 µm, 250 × 4.6 mm; isocratic mobile phase consisted of water-methanol-acetonitrile (60:30:10, v/v/v) applied at a flow rate of 1 ml/min; and oven temperature was maintained at 40°C. Wavelengths for excitation and emission were 365 and 450 nm, respectively. This HPLC system was equipped with a photochemical reactor (Aura Industries, Inc., New York, USA). The injection solution was methanol:water (50:50). Calibration graph of area against concentration was plotted for series of AF standard solutions from a stock standard solution of 50 ng/ml for AFB1 and AFG1 and 16 ng/ml for AFB2 and AFG2 Concentration of AF in the samples was calculated using a regression line. Limit of detection was 0.01 µg/kg AF (signal to noise ratio, 3:1).

Literature Review

The World Health Organization (WHO) defines traditional medicine (TM) as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in prevention, diagnosis, improvement or treatment of physical and mental illnesses. Herbal medicine is plant derived material or preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants [6]. Preparation of "jamu" and "makjun" in Malaysia may be similar to Indonesia. Traditional "jamu" makers are aware of issues relating to hygiene, sanitation and chemical contaminations from biological or non-biological sources (such as bacterial, fungal toxins and heavy metals). They try to protect raw plant materials and products from contamination, although this is unlikely to comply with international industrial standards [7].

The Malaysian government imposed the Control of Drugs and Cosmetics Regulation 1984 in the year 1992. All herbal products intended to be produced, imported and sold for human consumption must be registered with the Malaysian Ministry of Health in order to ensure and control the quality, safety and efficacy of the herbal products. Manufacturers must be in full compliance with the Code of GMP, which is based on the WHO Code. By law, only registered herbal products are allowed to enter the Malaysian market and these are also subject to regular and random post-marketing surveillance and testing. Safety requirements for herbal medicines include evidence of traditional use without demonstrated harmful effects, compliance with the limits set for heavy metals (mercury, arsenic, lead), testing for microbial and fungal contamination, other physicochemical tests and screening for adulterants. However, the present analytical procedures are rather restricted and minimal, focusing only on several tests for quality and safety [8]. Unsanitary conditions during planting, harvesting, processing, storage and transportation of medicinal plants for medicinal herbal products can result in contamination by various fungi with mouldy growth, spoilage and production of mycotoxins [9].

Findings

The mean level for AFB_1, AFB_2, AFG_1, AFG_2 and total AF contamination found in this study for TM products from Malaysia (0.97, 0.24, 0.92, 0.30 and 1.82 µg/kg) was higher compared to the previous study [5] for samples from Malaysia (0.19, 0.02, 0.12, not detected and

0.25 µg/kg) and from Indonesia (0.29, 0.10, 0.09, 0.03 and 0.41). However the incidence rate for 12 samples analyzed in this study (17%, 17%, 8%, 8% and 17%) was lower compared to those 9 samples from Malaysia (56%, 56%, 22%, 0% and 56%) and 14 samples from Indonesia (79%, 64%, 56%, 7% and 79%) analyzed in the previous study. Different sample's brand was used in this study which possibly has been contaminated by AF at higher levels. The possibility of negative results due to inefficient extraction procedure can be expected because of the technical problem in the laboratory; not well equipped and new staff carrying out the extraction, thus may cause the low incidence rate for positive samples in the present study. Study should be continued as to reanalyze the samples with inconsistent results. A more detailed quality control and another method validation should be carried out at the same time.

Conclusion

Overall results of the present study showed that the contamination levels of AF in TM products ("jamu" and "makjun") marketed in Malaysia were low, which could mean relatively lower exposures of the consumers through intake of these products. Low contamination level might be attributed to the antifungal effects of some medicinal herbs used as ingredients. However only 12 samples were analyzed in this study, thus a larger number of samples should be further analyzed in order to get a more accurate data of AF contamination, and survey for the daily intake of these products should be carried out for risk assessment study. As these products are consumed frequently for a prolonged period by consumers, the liver cancer risk associated with AF contamination should be ruled out.

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