ORIGINAL ARTICLE

Aflatoxin B1 Reported in Herbal and Traditional Medicine and Its Risk Assessment

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ABSTRACT

Introduction: Aflatoxin B1 (AFB1) is a hepatotoxic and carcinogenic mycotoxin produced by *Aspergillus* species of fungi, mainly *A. flavus and A. parasiticus*. Ingestion of AFB1 is followed by gastrointestinal absorption and metabolism in the liver, leading to aflatoxicosis and progression of hepatocellular carcinoma (HCC). Objective: This study aims to perform the risk assessment of AFB1 contamination in herbal and traditional medicines using Margin of Exposure (MOE) approach. **Methods:** Secondary data were collected from animal toxicological data and AFB1 exposure from herbal and traditional medicine products worldwide. Animal dataset with dichotomous HCC endpoint was analysed using Benchmark Dose Software version 3.2 to derive the benchmark dose that gives 10% response ($BMDL_{10}$). The estimated daily intake (EDI) was calculated based on daily assumption of 4.87 g AFB1 and 70 kg of body weight. Risk assessment was performed by calculating MOE with the ratio of $BMDL_{10}$ and EDI for lifetime and 2 week exposure. **Results:** Of 244 samples of herbal and traditional medicine surveyed from the literature, 117 (48%) were contaminated with AFB1 above EU regulatory limit (>5 µg/kg). From this data, 226 of 244 (92%) samples had MOE values below 10,000 for lifetime exposure and the risk was 1950-fold lower for 2 week exposure following the Haber's rule. **Conclusion:** : Majority of the herbal and traditional medicines contaminated with AFB1 had MOE lower than 10,000 indicating an urgency for risk management action. The production of herbal and traditional medicine should be monitored regularly to reduce the risk associated with AFB1.

Keywords: Risk assessment, Aflatoxin B1, Herbal and traditional medicine, Margin of exposure, Benchmark dose

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INTRODUCTION

Aflatoxin B1 (AFB1) is a naturally occurring secondary fungal metabolite produced mainly by *Aspergillus flavus* and *Aspergillus paraciticus* that heavily contaminates plants and medicinal herbs (1). The growth of Aspergillus fungi in food commodities can be affected by many factors including improper storage, drought, natural origin, humidity, and harvesting methods (2, 3). AFB1 is classified as a group one carcinogen by the International Agency for Research on Cancer (IARC) since it has sufficient evidence to cause liver toxicity and cancer in animals and humans (4). AFB1 contamination is considered a public health concern due to its involvement in the pathogenesis of hepatocellular carcinoma (HCC), particularly in developing countries (5). Moreover, chronic exposure to hepatitis B virus (HBV) infection which are prevalent in developing countries can act synergistically with aflatoxins, resulting in a 30 times greater risk of liver cancer than aflatoxins alone (6).

AFB1 is metabolised in the liver by the microsomal cytochrome enzymes (CYP450) following the ingestion of contaminated herbal and traditional medicines. The biotransformation of AFB1 through epoxidation, hydration, hydroxylation and O-demethylation processes in liver produced several types of metabolites including AFB1 exo-8,9-epoxide (AFBO), AFM1, AFQ1, and AFP1 (7). The epoxidation of AFB1 is a key step in

the genotoxic process as it produces reactive AFB1 exo-8,9-epoxides that can react with the N^7 guanine atom to form a highly unstable DNA adduct known as aflatoxin N^7 guanine, which is resistance towards the DNA repair mechanism, resulting gene mutations that lead to the development of HCC (8).The production of this reactive epoxide, in combination with other factors such as fumonisin (9), and HBV infection (10) can increase the potency of AFB1 in humans, thereby increasing the risk of developing hepatocellular carcinoma in human.

Herbal and traditional medicines are increasingly popular worldwide and continue to play an important role in healthcare of the modern world. Globally, herbal and traditional medicines have been used for generations mainly for general health, as home remedies for mild and moderate illnesses, and to treat chronic diseases (11, 12). While some herbal and traditional medicines have promising potential and are widely used, many of them have not been tested in terms of quality and safety, so little information is available on their possible adverse effects (13, 14). The problem with herbal and traditional medicines is lack of toxicological evaluation, insufficient, unacceptable evidence of safety and efficacy, and inconsistent quality (15). The increasing number of herbal medicine consumers will increase the probability of mycotoxin intake amongst them (16). Studies have shown that AFB1 have been detected in some herbal and traditional medicines especially in developing countries such as Brazil (17), India (18), Thailand (19), and South Korea (20), indicating the need for proper safety measures and better implementation of the strategies.

Southeast Asia and Sub-Saharan countries are the most susceptible regions towards aflatoxin formation (21). Geographically, Southeast Asia's temperature and humidity are very optimum for the growth of fungi since the region has mean temperature ranging from 24-28°C and a tropical rainforest climate. To strengthen the evidence of Asia and Africa's susceptibility towards aflatoxin production, India (1974), Kenya (1981), Kenya (2004), Malaysia (1988), Tanzania (2016) and Tanzania (2017) had experienced aflatoxin outbreaks from 1974 to 2017 involving 106, 12, 125, 13, 20 and 4 deaths, respectively (21). Thailand is exposed to large amount of aflatoxin contamination in food due to the climate and geographical area, with 3,206 food samples highly contaminated with aflatoxin reported in 2001 (22). Malaysia's crops were also widely contaminated with aflatoxins where 72.6% of 95 samples were detected to have AFB1 ranging from 0.54 to 15.33 µg/kg (23).

Lifetime exposure to low levels of AFB1 have been associated with the development of HCC in animals and humans. A study from Wogan et al (24) demonstrated the effects of low dietary intake of AFB1 to male Fischer rats at levels of 1, 5, 15, 50 and 100 µg/kg which resulting in the formation of HCC at all dietary levels. Globally, aflatoxins exposure through the consumption of maize and peanuts ranged from 0.02 to 227 ng/kg body weight/day resulting in a quantitative estimation of 25,200–155,000 of total annual HCC cases attributable to aflatoxin exposure, worldwide (6). Apart from the quantitative liver cancer risk assessment, Margin of Exposure (MOE) is one of the approaches recommended by the European Food Safety and Authority (EFSA) to estimate the risk of genotoxic carcinogens (25). The risk is calculated by the ratio of benchmark dose lower confidence limit (BMDL) to the population estimated dietary intake (EDI). The higher the MOE value, the lower the risk of exposure (26). Hence, using the MOE approach, this study aims to determine the global risk of AFB1 exposure through herbal and traditional medicine consumption.

MATERIALS AND METHODS

This study used secondary data to determine the lifetime exposure to AFB1 among the general population. Animal toxicological data on AFB1 exposure and the contamination rate of AFB1 in herbal and traditional medicine were surveyed and collected from the literature. Benchmark Dose Modelling software (BMDS) version 3.2 by United State Environmental Protection Agency (U.S. EPA) was used to derive point of departure (POD) of AFB1.

Literature search and analysis of rat toxicological data

First, rats' experimental data with dichotomous HCC endpoint were collected based on several standard criteria that has been recommended by the Carcinogenesis Bioassay Program of the National Cancer Institute / National Toxicology Program (27). Dataset obtained have been adjusted and corrected with lifetime and dosing duration by using European Chemicals Agency method by multiplying the dose applied with [(w1/104) (w2/104)] where w1 is the dosing duration and w2 is the observation period, and 104 represent the standard life expectancy for rats in week (28). The datasets from rats' carcinogenicity studies were adjusted to demonstrate two important keys to be included in BMDS software, which were time adjusted dose (µg per kg bw per day) and tumor incidence. This data was analyzed using BMDS software version 3.2 to derive BMD_{10} (benchmark dose) and $BMDL_{10}$ (benchmark dose lower confidence limit). The software was set as dichotomous model type and extra risk 95% confidence level. The acceptance criteria was based on P value more than 0.05 ($p>0.05$) and the BMD: BMDL ratio less than 10 (29).

Analysis of herbal products containing AFB1 and estimated dietary exposure

Literature searches were conducted using Google Scholar, Science Direct, Sage, and EBSCOhost. The reference lists of retrieved studies were searched using the snowball system or manual to find other

relevant publications. The terms and keywords used in the literature search included "aflatoxins", "herbal medicine" and "traditional medicine". The literature search was conducted from March 2021 to June 2021. The titles and abstracts of the retrieved studies were screened to assess their relevance. Of 812 abstracts screened, 649 were excluded due to irrelevant topics. Of 163 potentially eligible studies whose full articles were reviewed, 154 were excluded since the paper did not include information on AFB1 contamination. Therefore, only 9 eligible studies from the search and 2 studies from the snowball technique were considered for further assessment. The selected data comprised of AFB1 analysis in herbal and traditional medicine for oral use and the products studied were obtained from local markets or purchased online, raw products or any herbs supplement taken by consumers. One sample T-test and Signed test were performed using IBM SPSS Statistics 25 to compare the level of AFB1 contamination of each county with the regulatory limit of 5 µg/kg (30). Next, the dietary exposure or EDI of AFB1 consumption was calculated based on 70 kg default bodyweight of adults as recommended by EFSA (31) and 4.87 g average daily consumption of herbal and traditional medicines (32) using the following formula (33):

$$EDI = \frac{\text{Contamination level (µg/kg) x Daily amount consumed (µg/kg.bw/day)}}{\text{Body weight (kg)}}$$

Calculation of MOE values

MOE approach is a harmonized approach to compare the margin between a dose and an exposure that causes cancer to humans or animals (34). This approach used animal dose and dietary exposure in humans to derive a value which indicate the necessity for risk management action. To derive the MOE value, the point of departure or reference point from animal data was divided by the estimated dietary exposure as shown below:

$$MOE = \frac{BMDL_{10}\ (ng/kg.bw/day)}{\text{Estimated Daily Intake (ng/kg.bw/day)}}$$

Haber's rule

Haber's rule was applied in this study to calculate the risk by assuming the short-term exposure of 2 weeks to AFB1 from herbal and traditional medicine consumption (29). The equation below shows the MOE value would be 1950 higher and the risk would be 1950 lower compared to lifetime exposure.

$$\text{Haber's rule} = \frac{75 \text{ years x } 52 \text{ weeks per year}}{2 \text{ weeks}}$$

RESULTS

$BMDL_{10}$ value from rat toxicological data

Dataset from Wogan et al., (24) was selected to calculate points of departure (POD) of AFB1. Table I shows the results of BMD analysis of AFB1-induced HCC incidence in male Fischer rats using BMDS software version 3.2. Multistage Degree 5 was chosen as the fittest model with P value > 0.05 and BMD_{10} and $BMDL_{10}$ ratio is less than 10. This model is recommended because it gives the lowest value of $BMDL_{10}$ which is 63.57 ng/kg b.w./day to evaluate the worst-case scenario.

Table I: Results from BMD analysis of AFB1 tumor incidence in Male Fisher rats (27) using BMDS software

Model	p-value	Accepted[a]	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Dichotomous Hill	0.02	No	0.50	0.40
Gamma	<0.0001	No	0.15	0.08
Logistic	0.01	No	0.32	0.24
Log-Logistic	0.01	No	0.43	0.31
Log-Probit	0.01	No	0.44	0.31
Multistage Degree 5	0.28	Yes	0.45	0.06
Multistage Degree 4	0.04	No	0.42	0.08
Multistage Degree 3	0.00	No	0.20	0.07
Multistage Degree 2	0.01	No	0.39	0.18
Multistage Degree 1	0.00	No	0.08	0.06
Probit	0.01	No	0.29	0.22
Quantal Linear	0.00	No		0

[a]The criteria of acceptance was chosen p > 0.05 and BMD: $BMDL_{10}$ ratio < 10 (32)

Standard Regulatory Limit

A total of 244 samples collected from nine countries which are China, Morocco, Egypt, Malaysia, Indonesia, South Korea, Taiwan, Turkey and Thailand as shown in Table II (19, 20, 32, 35-41). Level of AFB1 contaminated in samples were compared with 5 µg/kg of European regulatory limit (Fig. 1). Samples from Indonesia, Malaysia, South Korea, and Thailand had levels lower than the regulatory limit. In total, 117 out of 244 samples were found to exceed the regulatory limit.

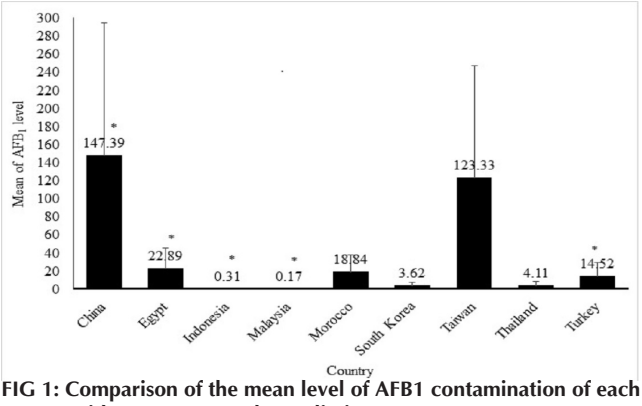

FIG 1: Comparison of the mean level of AFB1 contamination of each county with European regulatory limit.

Table II: Contamination of AFB1 in herbal and traditional medicines collected from the literature.

Herbal Medicine (compound/ species)	Country of Origin	Product Presentation	Level of AFB1 (µg/kg)	Ref
Gastrodia elata	China	Powder	0.758	(40)
Radix bupleuri	China	Raw	10-26.85	(42)
Thyme	Egypt	Powder	16.8	(38)
NA	Indonesia	Powder/ paste	0.58	(33)
NA	Malaysia	Powder/ paste	0.45	(33)
NA	Morocco	Powder	6.7	(41)
Nelumbinis Semen	Korea	Raw	6.27	(23)
Areca Semen	Korea	Raw	10.4	(37)
Polygalae Radix	Taiwan	Raw	0.7-8.1	(39)
Garcinia	Thailand	Capsule	2.7	(22)

NA: Not available

Risk assessment of exposure to AFB1 from consumption of herbal and traditional medicine using MOE approach

The level of AFB1 contamination found in the positive samples ranged from 0.01 to 1268.8 µg/kg. The calculated EDI obtained ranged from 0.01 to 88.27 µg/kg bw/day. Next, the MOEs calculated based on the EDIs and the lowest $BMDL_{10}$ of 63.57 ng/kg bw/day were summarized in Table III based on respective country. The range of MOE obtained from the analysis was 0.7 to 91,373. From the calculations, 226 out of 244 (92.2%) samples were found to have MOE values less than 10,000. MOE value lower than 10,000 indicates a priority for risk management.

The MOE approach recommended by EFSA is used to estimate the risk of AFB1 in lifetime exposure (75 years) but considering that the consumption of herbal and traditional medicine may not be that long, Haber's rule is applied to estimate short-term exposure of AFB1. Fig. 2 shows the comparison of samples with MOE values between lifetime and 2 weeks' exposure where Fig. 2 (A) represents the samples with MOE values from lifetime exposure while Fig. 2 (B) represents MOE values of the samples from 2 weeks' exposure. Generally, the number of samples with MOE value less than 10,000 decreased when Haber's rule is being applied to reflect the short-term exposure to the AFB1. In total, 233 (95%) out of 244 samples had MOE values more than 10,000 when the Haber's rule was applied.

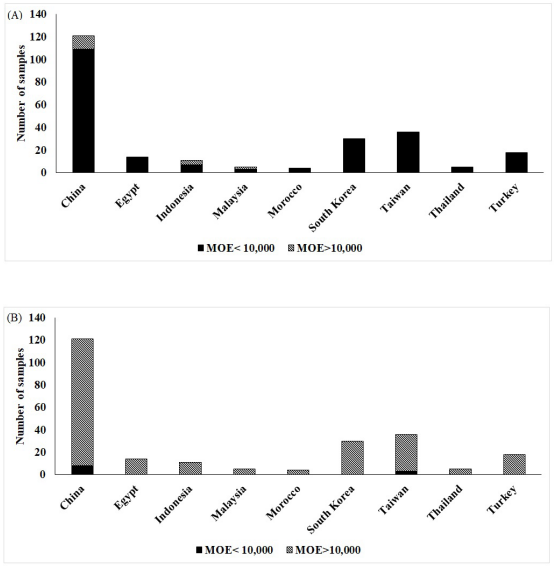

FIG 2: MOE values of AFB1 contaminated in herbal and traditional medicines between lifetime exposure (A) and short-term exposure (B) to AFB1 with the respective country.

Table III: Summary result of total 244 herbal medicine samples contaminated with AFB1 from literature

Country	N	AFB1 range (µg/kg)	Dietary Exposure[a] (ng/kg bw/day)	MOE[b]	Ref
China	121	0.01 – 1268.8	0.001 – 88.27	0.7 – 91373	(40, 42)
Egypt	14	1.4 – 75	0.097 – 5.22	12.2 – 652.7	(38)
Indonesia	11	0.02 - 1	0.001 – 0.07	913.7 – 45686.9	(33)
Malaysia	5	0.03 – 0.45	0.002 – 0.031	2030.5 – 30457.9	(33)
Morocco	4	1.87 – 41.8	0.130 – 2.91	488.6 – 21.9	(41)
South Korea	30	0.1 – 105.5	0.007 – 7.3434	8.7 – 9137.4	(23, 37)
Taiwan	36	0.2 - 592	0.014 – 41.19	1.5 – 4568.7	(39)
Thailand	5	1.1 – 11.3	0.077 – 0.79	80.9 – 830.7	(22)
Turkey	18	2.4 – 51.6	0.167 – 3.59	17.7 – 380.7	(36)

[a]Dietary exposure is estimated based on consumption of 4.87g daily (33) and 70 kg bw
[b]MOE is calculated using the lowest $BMDL_{10}$ value for AFB1 induced liver tumor formation 63.46 ng/kg bw/day derived from BMDS software 3.2 version

DISCUSSION

MOE is a harmonized approach recommended by EFSA for risk assessment of genotoxic and carcinogenic substances. This approach is used in this study to perform risk assessment of AFB1 resulting from intake of herbal and traditional medicine. From the literature search, 117 (48%) samples were positive with contamination level above the European Regulatory limit of > 5 µg/kg for AFB1. The herbal medicine samples were contaminated with AFB1 ranging from 0.01 to 1268.8 µg/kg. Indeed, the highest contamination of AFB1 that was reported was more than 1,000 µg/kg and this finding shows that other commodities besides peanuts, maize, and staple food, are susceptible to fungi growth and subsequently the production of AFB1.

Study of food samples from many regions revealed that the hazelnuts samples originating from Asian countries had the highest aflatoxin contamination (mean value = 0.33 µg/kg) compared to European (0.14 µg/kg) and other countries including USA (0.19 µg/kg) (42, 43).To compare with AFB1 level in other food commodities, AFB1 was found in maize samples from Indonesia ranging from 0.3 to 6171.0 µg/kg (44). In Uganda, peanut samples had 7.3 to 12.4 µg/kg of AFB1 (45), while in China, home-made peanut oil samples had the highest amount of AFB1 ranged from 0.26 to 283 µg/kg (46). In Malaysia, AFB1 contamination found in food, particularly in nut products ranged from 0.40 to 222 µg/kg (47). This shows that the level of AFB1 contaminated in foods were comparable to AFB1 in herbal and traditional medicine found in this study, which were 0.01 to 1268.8 µg/kg indicating the need for further investigation.

In this study, 48% of the samples collected worldwide were highly contaminated, which shows that mycotoxin contamination is another emerging problem that needs to be addressed. The current findings also revealed that 92% of the samples had MOE values lower than 10,000, indicating the need to prioritise management of both food and supplements. In view of the unrealistic estimates for herbal and traditional medicine consumption over their lifetime, a period of two weeks for PFS was assumed to be a reasonable minimum estimate (29). Therefore, Haber's rule was applied to calculate the less-than-lifetime exposure. The MOE values for a 2 weeks exposure were 1950 times lower, which resulting in 233 samples rather than only 18 samples with MOE values of greater than 10,000 for lifetime exposure. Therefore, a proper risk management action is important considering that risk can be reduce by reducing the exposure.

To support these findings, more research should be initiated to increase awareness and to find the best strategy to combat this issue. In terms of regulatory management, good agriculture practices and good manufacturing practices should be implemented and sustained to reduce the growth of harmful fungi as recommended by The Codex Alimentarius Commission (48). Primary, secondary and tertiary prevention should also be considered to prevent mycotoxin production. Elias (48) also suggested to use controlled humidity during storage and fungicide as primary prevention measures in developing fungi resistance. Secondary and tertiary prevention include seed contamination removal and detoxification of mycotoxin to the minimum level.

CONCLUSION

This study revealed that majority of the herbal and traditional medicines contaminated with AFB1 had MOE lower than 10,000, emphasizing the urgency for worldwide priority of risk management. More research and improvement in regulatory management are recommended in order to control the toxicant production and prevent AFB1 outbreak.

LIMITATIONS OF THE STUDY

The MOE was calculated based on secondary data available in the literature. Hence, the way the study was conducted, and the number of samples used were not standardised.

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