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Prevalence Pattern and Detoxification of Foodborne Aflatoxin and Its Binding Efficiency and Interaction with Different Blood Electrolytes

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Abstract Aflatoxin is a potent carcinogen, occurring from mold growth that contaminates raw agricultural commodities, mostly staple grains. The study was conducted to elucidate the present status of aflatoxin contamination in five commonly consumed foods including rice, wheat, maize, peanut and chickpea in Dhaka city of Bangladesh. The contaminated samples were analysed by HPLC for total aflatoxin (B1, B2, G1, G2). The average percentages of aflatoxin were: maize (90%)> peanut (70%)> chickpea (60%)> wheat (50%)> rice (30%). A high incidence (90%) and concentration of aflatoxin (mean 33.2 µg/kg) was found in maize. The mean concentrations of aflatoxin in 60% chickpea, 50% wheat and 70% peanut were higher than the safe limits proposed by FDA (20 µg/kg). Rice had comparatively lower concentrations (mean 15.4 µg/kg) of aflatoxin of which 30% were beyond safe limits. For detoxification, the samples were treated in either 1% or 5% calcium hydroxide. The 5% calcium hydroxide treatment reduced more toxin than that of 1%. A quantum chemical calculation was done to address the chemical binding of aflatoxin with blood electrolytes. Cationic electrolytes i.e., (Na^+, K^+, Ca^{2+} and Mg^{2+} bound to the O_{18}-O_{22} position of the aflatoxin B1. The divalent cations (Ca2+, Mg2+) bound more strongly to AFB1 than the monovalent cations (Na+, K+). According to the descending order their binding strength was: Mg2+ (-224.5 kcal) > Ca2+ (-156.6 kcal) > Na+ (-67.1 kcal) > K+ (-37.3 kcal). This study gave us an insight about the potential risks of aflatoxin to public health.

Keywords: *aflatoxin, detoxification, electrolyte imbalance, electrolyte binding strength*

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1. Introduction

Aflatoxin are lethal cancer causing elements produced by certain molds (*Aspergillus flavus* and *Aspergillus parasiticus*) which propagate in soil, decaying vegetation, hay and grains [1]. During contaminated food processing, aflatoxin go into the universal food resource of both pet and human foods, as well as in feed-stocks for agricultural animals [2]. Livestock feed with contaminated food can transmit aflatoxin into meat, eggs and milk products [3]. A recent study has demonstrated similar results where contaminated poultry feed are suspected as the cause of aflatoxin impurity of poultry meat and eggs in Pakistan [4]. The occurrence of aflatoxin is influenced by certain environmental factors including terrestrial location, agrarian and agronomic practices, and the exposure of commodities to fungal attack during pre-harvest, packing, and/or processing periods [5].

Although 17 aflatoxin have been identified [6], only 4 of them are well known and have been comprehensively investigated from the toxicological edge. Being strongly fluorescent in ultraviolet light, the four are labelled as B1, B2, G1 and G2 demonstrating their blue and green fluorescence under UV light. Among all aflatoxin, aflatoxin B1 (AFB1) is the most lethal to numerous species [7]. Toxigenic *A. flavus* isolates commonly produce aflatoxin B1 and B2, whereas *A. parasiticus* isolates secret aflatoxin B1, B2, G1 and G2 [8]. Although aflatoxin B1, B2 and G1 are common in the same type of feed, AFB1 constitutes 60-80% of the total aflatoxin content [9]. AFB1 is considered among the most powerful hepatocarcinogenic agents known and more toxic compared to other aflatoxin [10]. Human health hazards by aflatoxin are mainly due to people eating aflatoxin-contaminated food. In 1988, International Agency for Research on Cancer (IARC) classified aflatoxin B1 as a human carcinogen. The median toxic dose of AFBl 0.36 mg / kg body weight is a distinctive

range of extremely lethal poison (aflatoxin animal half of the lethal dose is found in the strongest carcinogens). Its carcinogenicity is 900 times more than dimethyl nitrosamine induced liver cancer and 75 times higher than the 3,4-benzopyrene, an inducer for lung cancer [11].

The liver is the target organ for toxic effects of aflatoxin B1. In liver, AFB1 is processed to diverse metabolites by microsomal enzymes through demethylation, hydroxylation, hydration and epoxidation [12-13]. Aflatoxicol is the only metabolite of AFB1 produced by a soluble cytoplasmic reductase enzyme system [14]. The metabolism of carbohydrates, proteins and lipids in liver is extremely compromised by AFB1. A couple of studies indicate that cytotoxic effects of aflatoxin can restrict RNA and DNA synthesis of information, thereby interfere with cell protein synthesis, resulting in systemic harm to animals [15]. AFB1 toxin hinder RNA polymerase and successive protein synthesis at a quicker rate in ducks than in rats probably due to a faster liver metabolism of AFB1 in ducks than in rats [16]. In day-old chicks, AFB1 diminishes the activity of liver UDP glucose-glycogen transglucosylase resulting in a reduction of hepatic glycogen stores [17]. Furthermore, there is lipid deposition in the liver of chickens and ducklings exposed to aflatoxin. With regard to its lethal effects on liver microsomal enzymes, AFB1 is recognized to lessen microsomal glucose-6-phosphatase activity while stimulation of microsomal enzyme activity by inducers seems to be unaltered by AFB1 [17]. Another consequence of aflatoxin contamination is that it results anticoagulation of blood. This is probably due to the fact that AFB1 impedes synthesis of clotting factors II and VII involved in pro-thrombin synthesis [18]. In addition, aflatoxin have been associated with numerous diseases including aflatoxicosis in livestock, domestic animals and humans throughout the world. Children are predominantly affected by aflatoxin exposure, which leads to stunted growth, delayed development, liver damage, and liver cancer [10]. If aflatoxin is ingested in human body; it can bind with different electrolytes which can lead to fatal kidney failure. The most serious electrolyte disturbances involve abnormalities in the levels of sodium, potassium, and/or calcium.

Because aflatoxin contamination is inescapable, various approaches for their detoxification have been suggested. These comprise physical methods of separation, solvent extraction, and adsorption from solution, thermal inactivation, irradiation, microbial inactivation and fermentation [19]. Chemical methods of detoxification are established as a major approach for effective detoxification. A novel approach in regard to the detoxification of aflatoxins is the addition of inorganic sorbent materials, well-known as chemisorbents, i.e., hydrated sodium calcium aluminosilicate (HSCAS) to the diet of animals. HSCAS are able to tightly bind and immobilize aflatoxins in the gastrointestinal tract of animals and thereby resulting in a subsequent drop in aflatoxin bioavailability [20].

The weather condition including temperature, humidity and moisture content of Dhaka city is very suitable for the growth of aflatoxin creating fungus like *A. flavus* and *A. parasiticus*. These molds can grow rapidly in different stored food grains. The food items examined in this study (chickpea, wheat, peanut, maize and rice) are very closely related to the food habit and nutritional status and among the different types of mycotoxin, aflatoxin are the most prevalent toxin in these food crops [21,22,23]. Therefore, in this study we investigated the prevalence and distribution of different types of aflatoxin in different common food items commonly consumed in Dhaka city, the extent of reduction of aflatoxin by standard detoxification strategies, and the chemical binding of aflatoxin with different blood electrolytes of human body. To monitor aflatoxin contamination in different food grains is, therefore, very important to prevent excessive build-up of the toxin in human food chain, as aflatoxin are severe hepato-carcinogenic agents. In this regard, detoxification of food is very important before intake. The effective methods of reducing aflatoxin in contaminated food items have been estimated in this study. Finally, the preferential binding pattern of most lethal aflatoxin namely B1 with different blood electrolytes has also been investigated in this study.

2. Materials and Methods

2.1. Sampling

Rice, maize, wheat, peanut, and chickpea were collected from 10 different wholesale markets of Dhaka city and analyzed to carry out this work. A total of 50 samples were analyzed for total aflatoxin B1, B2, G1 and G2. For each type of food; 2kg sample were purchased, collected in separate zipper bags and labelled them according to the food type and market name. The Ethical Committee of Dhaka University approved this study (20150106/ERC_biosciencedu).

2.2. HPLC System

An Agilent HPLC (Agilent, 2010) was used in this study. The HPLC system- Agilent: Liquid chromatograph consisted of 4 parts including agilent: solvent delivery system (pumps) series 1100, agilent series 1100 column oven, agilent 1200 series flourosence detector for HPLC and a manual injector [24].

2.2.1. Reagents

Reagents and standards were labelled which included clear identification, concentration, purity, preparation and expiry date and relevant safety information. Water used was either distilled (for extraction) or deionized (for HPLC use). 20 g of lead acetate [$Pb(OAc)_2.3H_2O$] was dissolved by warming in 120 ml distilled water with 12 ml glacial acetic acid in an erlenmeyer flask. The solution was transferred to a measuring cylinder, which was made up to 200 ml with distilled water. The solution was mixed well and kept in a zipette dispenser set to deliver 1 ml. This gave a 10% solution of lead acetate in 6% acetic acid. Sodium sulphate used was AR grade, anhydrous crystalline (not powder). The compound was dried in an oven at 100°C for 2 hours and stored

in a desiccator. Sodium sulphate column was prepared by transferring 1g of sodium sulphate to a 4 ml column and was stored in a desiccator if not used immediately. Both chloroform (AR grade) and methanol (AR grade/HPLC grade) were kept in small amber bottles fitted with zipette dispensers set to deliver either in 4 ml or 10 ml (Merck, Germany) HPLC grade. The ratio of solvents in mobile phase was acetonitrile: methanol: water = 22.5: 22.5: 55.

2.2.2. Preparation of Stock, Intermediate and Working Standard Aflatoxin Solution

Aflatoxin standards were obtained from Sigma Chemicals, USA. Stock standards (100ppm) for all 4 types of aflatoxin were prepared. Also intermediate standards (1000 ppb) were prepared from stock standards for all 4 types of aflatoxin. Working standards (B1=100 ppb, G1=100ppb, B2=20ppb & G2=20ppb) were mixed and from them the intermediate standards (1000 ppb) were prepared. 5-7 levels of mix working standards were prepared from the above mix working standard (B1=100ppb, G1=100ppb, B2=20ppb & G2=20ppb) for calibration. All standards were stored well and sealed in a freezer at < -20°C.

2.2.3. Extraction

Sample weighing in the range of 50g to 200g were taken. The samples were weighed into a blender jar. The calculated volume of water was added for each commodity at 1:2 ratio of sample:water and was blended at high speed for 3 minutes. Water slurry was produced to homogenize the sample to minimize sub-sampling errors in sample division. The resultant slurry was free flowing, homogeneous, and was not separated out into layers within 15 minutes (many slurries remained stable over a 2 hour period or longer). The volume of acetone required was calculated to give a 4:1 ratio of acetone to water. This volume of acetone was added to the slurry and the flask was closed with a stopper. The flask was vigorously shaken in an upside down motion for 20 seconds. The flask was fixed onto the flask shaker and was shaken vigorously for 30 minutes. It was checked whether there was any leakage of stoppers or balling of the sample. This operation was carried out in subdued light. Flasks on the reciprocating shaker were covered with an opaque material, such as black plastic bag. The extracts were filtered through a 24 cm whatman No. 1 filter paper into a 250 ml quickfit flask in a fume cupboard. The operation was completed within 3 minutes to minimize concentration effects. After filtration the flask containing the crude extract was stopped and stored in a dark cupboard prior to use in next step [25].

2.2.4. Injection Sequence

The samples were injected at a volume of 20µL with a flow rate of 1mL/min. The excitation wavelengths were 365 nm with an emission wavelength of 418 nm. The column was heated at 30°C and was run for 30 min. The system was stabilized by running the system with mobile phase for 15-30 min before commencement of analysis. A blank was injected first followed by a 4 ppb standard and then samples.

2.2.5. Calculation

The quantity of samples taken for analysis was 100g. The final extract was reconstituted to 1mL. The calibration curve was made by spiking the blanks followed by extraction and clean-up. The spiking amount was 100 times of the final concentration, hence, the amount shown in the x axis calibration curve was exactly the amount per g of the sample in ng/g.

2.3. Detoxification

For detoxification, each 2 kg of contaminated sample of chickpea, wheat, peanut, maize and rice were taken and 1 kg of each samples were individually soaked in two litre of calcium hydroxide and boiled for 30 minutes. Another 1 kg of each samples were individually soaked in two litre of 5% calcium hydroxide and boiled for 30 minutes [26]. All the calcium hydroxide treated samples were analyzed for aflatoxin.

2.4. Computational Methodology

This study involved quantum chemical calculation to explain the binding of aflatoxin B1 with cations i.e., Na^+, K^+, Mg^{2+}, Ca^{2+}. At first aflatoxin B1 molecule was built wth GaussView 5.0 [27] in a PC. Then full optimization of AFB1 was performed using Gaussian 09 program [28] at the HF (Hartree-Fock) level of theory. The basis set employed was 6-31G [29] for all atoms. Cation-AFB1 complexes were built by placing cations near the probable binding sites. Then full optimization of all bond lengths and bond angles was performed at HF (Hartree-Fock) level using the same basis set as mentioned above.

2.5. Statistical Analysis

In this study some statistical method was used for the interpretation of observed data. Graphpad prism (version 5) was used for bar diagram and whiskers plot generation. SPSS 21.0 was used for ANOVA test and Post Hock Test for the analysis of the significance of aflatoxin concentration in different food items. χ^2 test was done to interpret the association of detoxification strategy with reduction in contaminated samples. Microsoft excel 2013 was used to develop the tables.

3. Result and Discussion

3.1. Concentrations of Aflatoxin in Different Foods

The concentration of aflatoxin aggregate of B_1, B_2, G_1, G_2 and total was determined in chickpea, wheat, maize and rice. Aflatoxin B1 was found highest in every food items whereas B2 found the lowest. Among five types of crop samples, the highest concentration of aflatoxin found in maize (33.2µg/kg) and the lowest concentration of aflatoxin found in rice (15.4 µg/kg). Out of them, four types of food grains were higher than permissible limit, only rice was within permissible limit (Figure 1).

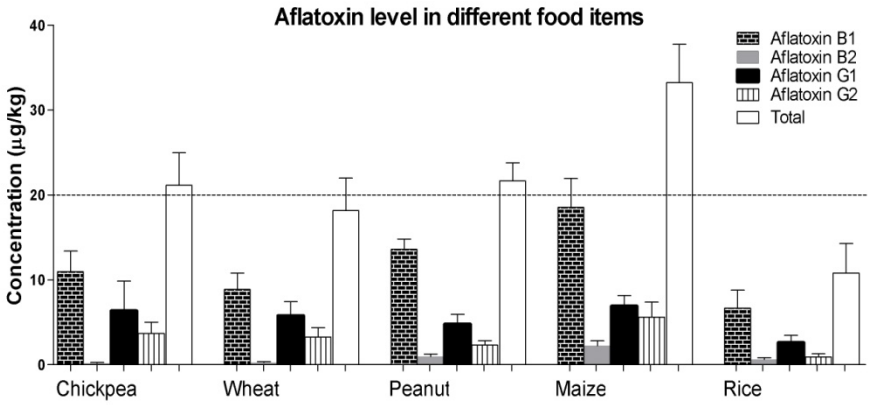

Figure 1. Concentrations of different types of aflatoxin (B1, B2, G1, G2 and total) in different food crops. Samples were collected from 10 different markets to analyse the presence of aflatoxins with HPLC. Data are expressed in mean±SEM

In chickpea, the mean concentrations of aflatoxin B1, B2, G1, and G2 were 12.2, 0.35, 9.1 and 5.2µg/kg respectively. The mean concentration of aflatoxin B1, B2, G1, and G2 in wheat were 11.1, 0.38, 7.2 and 4.1µg/kg respectively. In Peanut, the mean concentrations of aflatoxin B1, B2, G1, and G2 were 13.6, 0.97, 4.8 and 2.6 µg/kg respectively. In maize, the mean concentrations of aflatoxin B1, B2, G1, and G2 were 18.5, 2.3, 6.9 and 5.6 µg/kg respectively. In rice, the mean concentrations of aflatoxin B1, B2, G1, and G2 were 9.5, 0.88, 3.7 and 1.3 µg/kg respectively (Figure 1). The mean concentration of aflatoxin B1 was the highest in every types of food items whereas the mean concentration of aflatoxin B2 was the lowest. According to descending order; the mean concentration of total aflatoxins in different types of crop samples were: maize (33.2 µg/kg)> wheat (22.7 µg/kg)> peanut (21.7 µg/kg)> chickpea (21.2 µg/kg)> rice (15.4 µg/kg).

3.2. Aflatoxin Level within or above Safety Level

In chickpea, 60% of the samples crossed the safety level. Highest number (90%) of samples crossed the safety level in maize (Figure 2). Only 30% of the samples in rice crossed the safety level of aflatoxin. Out of 50 samples used in the study, about 60% samples (30) crossed the safety level and 40% samples (20) were within the safety level set by FDA (20µg/kg) (Figure 2). According to descending order; percentages of samples crossed the safety level for total aflatoxins in different food items were: maize (90%)> peanut (70%)> chickpea (60%)> wheat (50%)> rice (30%).

3.3. Detoxification of Aflatoxin

Before detoxification the mean concentration of total aflatoxin were 21.2, 22.7, 21.7, 33.2 and 15.4 µg/kg in chickpea, wheat, peanut, maize and rice samples, respectively (Figure 3). After treatment with 1% calcium hydroxide; the mean concentration of aflatoxin detoxified to 8.8, 10.2, 8.94, 15.1 and 7.3 µg/kg in chickpea, wheat, peanut, maize and rice samples respectively which was equivalent to a reduction of 57.5, 53.9, 57.6, 57.3, 51.6% of aflatoxin in chickpea, wheat, peanut, maize and rice samples respectively (Figure 3). After treatment with 5% calcium hydroxide the mean concentration of aflatoxin detoxified to 6.0, 6.3, 6.1, 9.5 and 4.4 µg/kg in chickpea, wheat, peanut, maize and rice samples respectively which was equivalent to a reduction of 69.1, 72, 69.5, 70.7 and 69.4 % aflatoxin in chickpea, wheat, peanut, maize and rice samples respectively. So, the reduction of aflatoxin by 5% calcium hydroxide was more efficient than 1% calcium hydroxide.

3.4. Bond Lengths and Binding Energy

All cations' (Na^+, K^+, Mg^{2+}, Ca^{2+}) binding with the aflatoxin B1 through O_{18} and O_{22} were stronger than their binding through O_{10} and O_{13} (Table 1 and Table 2). Again all cations bound with the AFB1 through both oxygen atoms O_{18} and O_{22} rather than either of the oxygen atom alone which was evident from the cation-oxygen distance (Table 1). The cation-O_{18} and cation-O_{22} distance was almost same. The distance between Na^+ and O_{18} was only 2.6% different than that between Na^+ and O_{22}. In case of Mg^{2+} binding with AFB1, the difference was only 1% whereas in case of Ca^{2+} binding, it was 1.5% different (Table 1). Larger cations' bindings were less strong than that of corresponding smaller cations. Binding energy of K^+ with AFB1 was 16.94 kcal less than the binding energy of Na^+ with AFB1 through O_{18} and O_{22} (Table 1). Binding energy of Ca^{2+} was 67.87 kcal less than the binding energy of Mg^{2+} with AFB1 through O_{18} and O_{22} (Table 1).

3.5. Carbon-oxygen Bond Length

The C_{15}-O_{18} and C_{19}-O_{22} bond distances were lengthened by 2-3% in case of Na^+-AFB1 complex compared to AFB1 molecule itself (Table 1). In K^+-AFB1 complexes, similar behaviour was observed but with lower percentage lengthened in bond distance compared to Na^+-AFB1 complex (Table 1). For divalent cation-AFB1 complexes, increase in bond length was much higher, approximately 4-6%. The increase in bond length was slightly lower in Ca^{2+}-AFB1 complex than Mg^{2+}-AFB1 complex.

3.6. Charges and Binding Energy

Although O_{10} and O_{13} was more negative than O_{18} and O_{22} in AFB1, binding energy of cations with AFB1 through O_{18} and O_{22} was much higher (Table 1, Table 2).

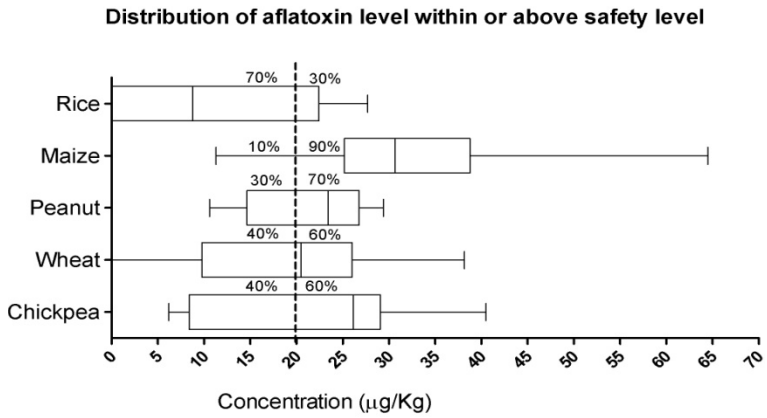

Figure 2. Concentration and percentage of total aflatoxin in chickpea, wheat, peanut, maize and rice within or above the safety level. Data are expressed in a box and whisker plot where the ends of the box are the upper and lower quartiles and the median is marked by a vertical line inside the box

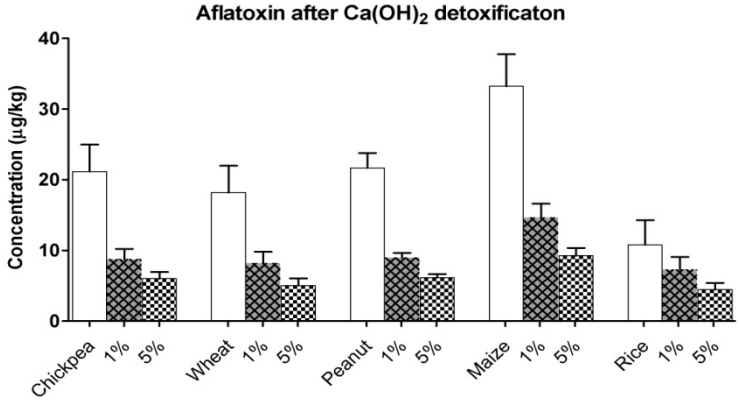

Figure 3. Concentration of total aflatoxin in different food items before and after treatment with 1% and 5% calcium hydroxide. Each samples were individually soaked in different concentration of calcium hydroxide and boiled for 30 minutes. After that samples were analyzed for aflatoxins using HPLC. Data are expressed in mean±SEM

Table 1. Optimized Binding Energy of Cation with AFB1, Cation-oxygen Distance in Cation-AFB1 complex, Carbon-oxygen distance and Charges of Different Atoms in the Complex (for the Binding Site of AFB1:O18-O22)

Binding sites of AFB1: O18-O22									
Bond	Energy of the cation (a.u)	Energy of cation-AFB1 complex	Binding energy (Kcal)	Cation-oxygen distance (Å)	Distance in AFB1	Distance in cation-AFB1 complex	Percentage of change (%)	Atoms	Charge
Na+									
Na+-O18-	-161.66	-1259.99	-67.14	2.194				O18	-0.72
Na+-O22				2.252				O22	-0.676
C15-O18					1.194	1.232	+3.17	Na	+0.88
C19-O22					1.204	1.23	+2.11		
K+									
K+-O18	-598.97	-1697.27	-50.2	2.598				O18	-0.69
K+-O22				2.672				O22	-0.645
C15-O18					1.937	1.225	+2.63	K	+0.93
C19-O22					1.204	1.225	+1.73		
Mg2+									
Mg2+-O18	-198.81	-1297.39	-224.51	1.893				O18	-0.945
Mg2+-O22				1.912				O22	-0.929
C15-O18					1.194	1.276	+6.89	Mg	+1.689
C19-O22					1.204	1.272	+5.65		
Ca2+									
Ca2+-O18	-676.11	-1774.57	-156.64	2.25				O18	-0.922
Ca2+-O22				2.284				O22	-0.89
C15-O18					1.937	1.259	+5.5	Ca	+1.865
C19-O22					1.204	1.255	+4.24		

Results presented in table-1 reveal that all cations bind with the aflatoxin B1 through O_{18} and O_{22} much strongly. Again all metal ions bind with the AFB1 through both oxygen atoms O_{18} and O_{22} rather than either of the oxygen atom alone (detailed explanation given in result section).

Table 2. Optimized Binding Energy of Cation with AFB1, Cation-oxygen Distance in Cation-AFB1 complex, Carbon-oxygen Distance and Charges of Different Atoms in the Complex (for the Binding Site of AFB1:O10-O13)

Binding sites of AFB1: O10-O13									
Bond	Energy of the cation (a.u)	Energy of cation-AFB1 complex	Binding energy (Kcal)	Cation-oxygen distance (A0)	Distance in AFB1	Distance in cation-AFB1 complex	Percentage of change (%)	Atoms	Charge
Na+									
Na+-O10	-161.66	-1259.94	-37.27	2.313				O10	-0.856
Na+-O13				2.258				O13	-0.8
C9-O10					1.454	1.46	+0.15	Na	+0.911
C9-O13					1.421	1.433	+0.8		
K+									
K+-O18	-598.97	-1697.23	-25.1	2.774				O10	-0.83
K+-O22				2.676				O13	-0.784
C9-O10					1.454	1.453	+0.12	K	+0.946
C9-O13					1.421	1.429	+0.55		
Mg2+									
Mg2+-O10	-198.81	-1297.26	-142.25	1.972				O10	-1.004
Mg2+-O13				1.96				O13	-0.935
C9-O10					1.454	1.484	+2.13	Mg	+1.74
C9-O13					1.421	1.459	+2.67		
Ca2+									
Ca2+-O10	-676.11	-1774.47	-90.3	2.363				O10	-0.976
Ca2+-O13				2.333				O13	-0.92
C9-O10					1.454	1.469	+1.05	Ca	+1.889
C9-O13					1.421	1.448	+1.86		

Results presented in Table 2 reveal that cations also bind through O_{10} and O_{13} but binding through O_{10} and O_{13} is weaker than O_{18} and O_{22} (detailed explanation given in result section).

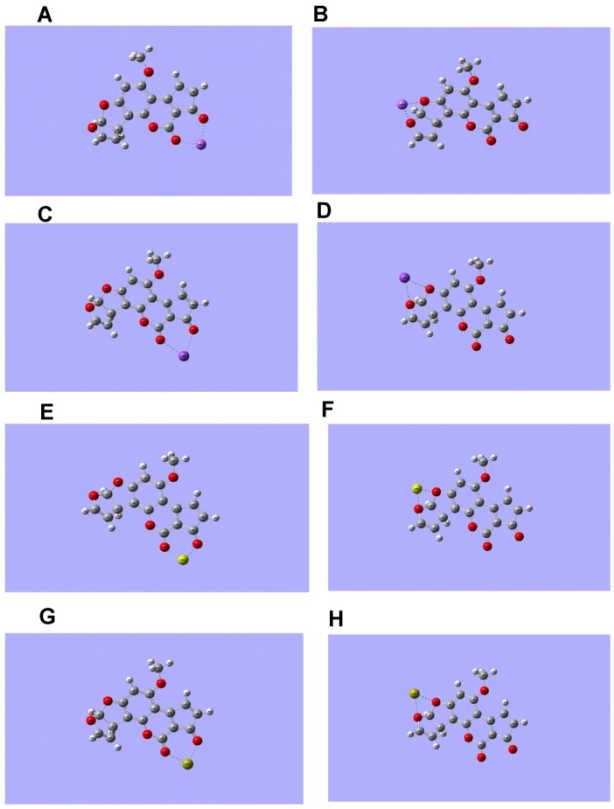

Figure 4. Binding of Na^+ (figure-A), K^+ (figure-C), Mg^{2+} (figure-E) and Ca^{2+} (figure-G) with AFB1 in O_{18}-O_{22} position. Binding of Na^+ (figure-B), K^+ (figure-D), Mg^{2+} (figure-F) and Ca^{2+} (figure-H) with AFB1 in O_{10}-O_{13} position. Cations bind through O_{10} and O_{13} forming a four- membered ring whereas binding through O_{18} and O_{22} occurs by the formation of six-membered ring. In six membered ring, cations get closer to both oxygen atoms resulting in higher binding energy

In the cation-AFB1 complex, atomic charges on O_{10} and O_{13} were also more negative than the atomic charges on O_{18} and O_{22}. Cations binding energy with AFB1 was higher while cations bound through O_{18} and O_{22} simultaneously. This was probably because of the fact that, cations bound through O_{10} and O_{13} forming a four-membered ring whereas binding through O_{18} and O_{22} occurred by the formation of six- membered ring. In six membered ring cation got closer to both oxygen atoms resulting in higher binding energy (Figure 4).

The atomic charges on O_{18} were more negative in case of Na^+- AFB1 complex than that of K^+ - AFB1 complex. Correspondingly the atomic charges on sodium were less positive than potassium ion. Similar trends were also observed in case of Ca^{2+}-AFB1 and Mg^{2+}-AFB1 complex (Figure 4).

So from the above discussion it can be concluded that different blood cation can bind with aflatoxin B1 molecule.

And according to descending order the binding strength was: Mg^{2+} (binding energy -224.5 kcal) > Ca^{2+} (binding energy -156.6 kcal) > Na^+ (binding energy -67.1 kcal) > K^+ (binding energy -37.3 kcal).

4. Discussion

In this study, it has been found that all five commodities (rice, wheat, maize, peanut and chickpea) collected randomly from different markets of Dhaka city were contaminated with aflatoxin. Maize was found to be highly contaminated with aflatoxin. The mean concentration of total aflatoxin found in maize samples was 33.2µg/kg which was above the safe limit set by FDA (Food and Drug Adminstration) and JECFA (Joint FAO/WHO expert committee on Food

Additives). The total aflatoxin concentration in 90% samples of maize crossed the safe limit. Among these five types of food, all of the food items except rice crossed the safe limit of aflatoxin set by FAO and JECFA (20 µg/kg). In chickpea, wheat, peanut, maize and rice about 60%, 50%, 70%, 90% and 30% of the samples crossed the safe limit. Out of 50 samples, 45 samples were contaminated with aflatoxin. Among them, 30 samples crossed the safe limit of aflatoxin. The average percentage of different aflatoxin (B1, B2, G1, G2) in different food items according to the descending order were found: aflatoxin B1(54.7%)> aflatoxin G1(26.3%)> aflatoxin G2(11.5%)> aflatoxin B2(5.7%). A study conducted by BCSIR in Bangladesh also found similar result in 2013 [26].

Rice is the staple food in Bangladesh. Chickpea, wheat, peanut and maize are also consumed here in a huge amount. Although rice in Asian countries like Bangladesh usually contains no or very low amount of aflatoxin [30], there is a lack of proper storage facility and there is a chance of the rice getting infected with aflatoxin producing fungus. The seasonal temperature and humidity accelerates the growth. Huge amount of wheat is imported by Bangladesh every year but there are no available data about imported and home-grown wheat. Maize is found highly contaminated and may be transferred to cattle and in poultry feed as it is a main source of animal feed. In one study in Nepal, 22% of maize samples from foothills of the Himalayan mountain contained aflatoxin [31]. Another study in India showed that 89% of maize and 20% of sorghum were contaminated with aflatoxin in the range of 5-120 µg/kg [32]. Countries with similar climatic conditions also experience similar problem. The African countries also found to experience these problems frequently in peanuts and maize [33]. In Nepal, one third samples of maize and its products were infested, and 20% of them showed level of aflatoxin which was more than the recommended value [34]. Survey conducted in Nigeria showed slightly higher percentage of aflatoxin contamination in maize and its products [35]. In contrast to the present study, which showed grains with aflatoxin (more than recommended value), developed countries also experience contamination in the same food items though below the recommended value [36]. The reason behind this may be due to the availability of better storage facilities, proper screening and regular monitoring for these contaminants.

This study demonstrated that peanut, its product, and maize were the main items contaminated with aflatoxin. In this study, aflatoxin contamination in chickpea was new. Chickpea are consumed a lot during Ramadan in Bangladesh but till date, there is no study done for checking the aflatoxin contamination in chickpea. Because aflatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These include physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, microbial inactivation, and fermentation [37]. Chemical methods of detoxification are also practiced as a major strategy for effective detoxification.

In this study, for detoxification of aflatoxin contamination the simplest and the most practical chemical method has been applied. The detoxification of highly contaminated samples was achieved by 1-5 % calcium hydroxide with 30 minutes of boiling. The detoxification reduced contamination in chickpea samples to an average 57.5% and 69.1% followed by 1% and 5% calcium hydroxide treatment respectively. In contaminated wheat, peanut, maize and rice samples 53.9 %, 57.6%, 57.3 % and 51.6 % aflatoxin were reduced respectively after treatment with 1% calcium and 72.0 %, 69.5 %, 70.7 % and 69.4 % aflatoxin reduced with 5 % calcium hydroxide treatment. The 5% calcium hydroxide treatment reduced more aflatoxin than the 1% calcium hydroxide treatment. The detoxification strategy in a study conducted by BCSIR, Bangladesh showed average reduction of 58.3 % and 68 % aflatoxin in maize with 1 % and 5 % calcium hydroxide treatment respectively [26].

Electrolytes are substances that produce an electrically conducting solution when dissolved in water. They carry a charge and are essential for life. In our bodies, electrolytes include sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), bicarbonate (HCO_3^-), magnesium (Mg^{2+}), chloride (Cl^-), hydrogen phosphate (HPO_4^{2-}) and hydrogen carbonate (HCO_3^-); deficiency of which can cause electrolyte imbalance [6,10]. Electrolyte imbalances can develop by diminished ingestion or excessive elimination of an electrolyte. The most common cause of electrolyte disturbances is kidney failure in human. In this study, chemical binding of different blood electrolytes with aflatoxin B1 was performed by a quantum chemical method. Aflatoxin B1 is the deadliest toxin than all other types of aflatoxins and was found more prevalent than other types of aflatoxin. For this reason, aflatoxin B1 was selected for this part of the study. From the binding energy found by quantum chemical calculation, it has been suggested that blood electrolytes including sodium Na^+, K^+, Ca^{2+} and Mg^{2+} bind in the O_{18}-O_{22} position of the aflatoxin B1. Divalent electrolytes including Ca^{2+} and Mg^{2+} bound more strongly than monovalent electrolytes like Na^+ and K^+. According to the descending order their binding strength was: Mg^{2+} (binding energy -224.5 kcal) > Ca^{2+} (binding energy -156.6 kcal) > Na^+ (binding energy -67.1 kcal) > K^+ (binding energy -37.3 kcal).

5. Conclusion

This study gives us an insight about the potential risks of aflatoxin to public health. Food items stored for a long time before its distribution are prone to get contaminated with aflatoxin. On top of that, toxins can be transmitted to the food products from its raw food. To reduce contamination, it is, therefore, necessary to introduce regulatory bodies to check this problem. Bangladesh Government has no data on aflatoxin contamination in the food of its people. Proper knowledge and concept on harvest and storage management is required to reduce the risk of aflatoxin in feed and foods. Cost-effective detoxification strategy is necessary to reduce the economic losses and to minimize hazards to human health. Different mold inhibitors could be used for effective conservation and control of aflatoxin production. This can be done along with proper education/awareness to the farmers, grain handlers and people engaged in marketing for safety and minimizing loss. The use of heat treatment for decontamination in food is also another approach to control aflatoxin. The contamination of commonly used

food and feed is an important unrecognized risk factor to public health and can have long-term health implications.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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