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Mycotoxins in Nigerian foodstuffs

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ABSTRACT: The presence of mycotoxins in a wide variety of Nigerian foodstuffs is a potential health hazard for the human population. The major fungal species implicated in the production of these toxins include *Aspergillus, Penicillium* and *Fusarium.* A random sample of maize and groundnuts harvested from farms in Ilorin and Ogbomoso, Nigeria, revealed the presence of fungal contaminants such as aflatoxins, moniliformin, zearalenone and cyclopiazonic acid in various amounts. Of the four toxins, only cyclopiazonic acid is a known inhibitor of the calcium-pumping adenosine triphosphatase (Ca^{2+}-ATPase) in skeletal muscle sarcoplasmic reticulum. Since the Ca^{2+}-ATPase represents about 85 per cent of the total proteins in the sarcoplasmic reticulum, ingestion of this mycotoxin will definitely have an effect on muscle development and function. We suggest here that the ingestion of cyclopiazonic acid in contaminated foodstuffs could play a role in the aetiology of muscle wasting diseases prevalent in the tropics.

Key Words: Food contaminants; Mycotoxins; Aflatoxin; Moniliformin; Zearalenone; Cyclopiazonic acid; Muscle wasting; Calcium-pumping ATPase (Ca^{2+}-ATPase).

Introduction

Cyclopiazonic acid (CPA) is a mycotoxin produced by some species of *Aspergillus flavus* and *Penicillium cyclopium*. It has been reported to coexist in some foodstuffs with aflatoxin B_1, another toxic metabolite of *Aspergillus flavus* (1).

Several animal diseases have been associated, either directly or indirectly, with mycotoxins (2) and there is increasing interest in the biological effects of the simultaneous presence of two or more of these toxins in foodstuffs. As a result, the interactions between several pairs of mycotoxins, which are known to coexist in foodstuffs, have been studied. These include aflatoxin B_1 and ochratoxin A (3), T-2 toxin and diacetoxyscripenol (4), patulin and rubratoxin B (5), ochratoxin A and zearalenone (6), ochratoxin A and penicillic acid (8,9), ochratoxin A and citrinin (9-11), and citrinin and penicillic acid (9).

The properties of aflatoxin B_1 as a hepatocarcinogen as well as a mutagen have been well studied (12). It has also been shown (13) that cyclopiazonic acid is produced as a metabolite of *Aspergillus flavus* which simultaneously produces aflatoxin B_1. It was further suggested (13) that CPA may have been a contaminant with aflatoxin B_1 in the "Rosetti" meals responsible for Turkey X disease in England between 1960 and 1961. Several studies have also shown the toxicity of CPA in a variety of animal species (14-19).

It is now well established that CPA is a potent inhibitor of the Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (20-25). Since the ATPase represents about 85 per cent of total proteins in the sarcoplasmic reticulum, ingestion of this mycotoxin will definitely have an effect on muscle development and function.

The presence of aflatoxin B_1 as a fungal contaminant of several foodstuffs has been demonstrated (3,12,26,27). However, direct evidence on the occurrence of CPA in foodstuffs has not been provided as a result of inadequate analytical methods for this mycotoxin, hence the health risks associated with CPA have not been well publicized as has been done for aflatoxin B_1.

We report here the presence of mycotoxins, particularly cyclopiazonic acid, in samples of maize and groundnuts harvested from farms in Ilorin and Ogbomoso, Nigeria. We also propose that the ingestion of CPA in contaminated foodstuffs could be the main cause of muscle wasting observed in nutritional marasmus prevalent in the tropics.

Materials and Methods

All the reference standards of the mycotoxins aflatoxin B_1, moliniformin, zearalenone and cyclopiazonic acid were purchased from Sigma Chemical Company, Poole, Dorset, U. K. Silica gel 60HF254 and 60H were products of E. Merck, Darmstadt, Germany. All other chemicals used were of analytical grade and were purchased from various local Scientific Supply Companies.

The maize and groundnut samples were purchased directly from farmers in Ilorin and Ogbomoso in Febreuary, 2000, four months after the harvest period. Visibly mouldy crops were selected from those that had been kept in storage since the harvest. Out of these, 12 samples of each crop (maize and groundnut) were selected for analysis. Mouldy grains were carefully removed from the maize cobs and a 20g portion was ground in a porcelain mortar. The mouldy groundnuts were similarly treated. Extraction of the mycotoxins from the powdered grains was done by a modification of the method of Kamimura (28) as reported by Okoye (29), using methanol/water (95:5, v/v) as the extracting solvent. The extraction procedure was repeated on another sample of each grain, using chloroform/methanol (95:5, v/v) as the solvent. The extract was filtered through a Whatman No. 1 filter paper and the filtrate was subsequently evaporated to about 10 percent of its original volume by placing it in a water bath heated to 70^{o}C.

The crude extract was further purified using minicolumn chromatographic techniques as reported in the literature (28-30). In some cases purification on Amberlite XAD-4 column and Florosil column as described by Kamimura (31) and Okoye (32) was used. The purified extracts were analysed by thin layer chromatography using activated silica gel 60HF254- and 60H-coated plates. Reference standards of the mycotoxins were run on the same plate to aid identification of the components of the mycotoxins in the mouldy grains.

Results and Discussion

Tables 1 and 2 show the frequencies of occurrence and semi-quantitative analysis of mycotoxins in mouldy maize and groundnuts harvested from Ilorin and Ogbomoso, Nigeria. Aflatoxin B_1, moniliformin, zearalenone and cyclopiazonic acid were present in the mouldy maize examined with frequencies of 3/12, 5/12, 7/12 and 4/12 respectively. Some yet unidentified mycotoxins were also seen in nearly all the specimens examined. For mouldy groundnuts, the frequencies of occurrence were 10/12, 0/12, 0/12 and 8/12 respectively. In this case, some unidentified mycotoxins were also present.

Attempts were made to detect mycotoxins in some processed Nigerian staple foods such as gari, rice and corn starch. However, we could not find any evidence of the presence of mycotoxins in these foodstuffs, probably due to the limitations of the methodology in detecting minute quantities of mycotoxins. Further work is in progress to employ more sensitive chromatographic techniques to quantify mycotoxins which may be present in minute quantities.

Table 1: Mycotoxins in mouldy maize harvested from farms in Ilorin and Ogbomoso, Nigeria.

Sample No.	AFB_1	Moniliformin	Zearalenone	Cyclopiazonic acid	Unidentified
1.	–	++	++	–	++
2.	–	+++	++	–	+
3.	–	–	++	–	+
4.	+	–	–	++	++
5.	+	++	–	–	+
6.	+	–	–	++	–
7.	–	–	+	–	++
8.	–	+++	++	–	–
9.	–	++	+	+	–
10.	–	–	–	–	+++
11.	–	–	+	–	++
12.	–	–	–	+	+++

– = Not detected; + = 100 – 400 μg/kg; ++ = 400 – 800 μg/kg; +++ = > 800 μg/kg.

Table 2: Mycotoxins in mouldy groundnuts harvested from farms in Ilorin and Ogbomoso, Nigeria.

Sample No.	AFB_1	Moniliformin	Zearalenone	Cyclopiazonic acid	Unidentified
1.	+++	–	–	++	+
2.	+++	–	–	++	–
3.	++	–	–	+++	–
4.	–	–	–	+++	–
5.	++	–	–	++	–
6.	–	–	–	–	+++
7.	++	–	–	–	++
8.	+++	–	–	++	–
9.	++	–	–	++	+
10.	+	–	–	–	++
11.	++	–	–	–	+++
12.	+++	–	–	++	–

– = Not detected; + = 100 – 400 μg/kg; ++ = 400 – 800 μg/kg; +++ = > 800 μg/kg.

The occurrence of aflatoxin, zearalenone and moliniformin in some Nigerian farm products have previously been reported (26, 32). However, there have been no previous reports of the detection of cyclopiazonic acid in Nigerian foodstuffs, probably because this mycotoxin is present in minute quantities which could not be detected using available chromatographic techniques.

Intracellular calcium plays a key role in the regulation of many cellular responses such as excitation-contraction coupling in muscle. The sarcoplasmic reticulum membrane in muscle is also specialized for intracellular Ca^{2+} uptake and release. The Ca^{2+}-pump protein (Ca^{2+}-ATPase) which mediates the energy-dependent Ca^{2+} uptake comprises 85 – 90 percent of membrane proteins from skeletal muscle sarcoplasmic reticulum. Cyclopiazonic acid is a very potent inhibitor of the Ca^{2+}-pump (21,22). Its potency is second only to that of thapsigargin, a naturally occurring sesquiterpene lactone which has been report to be a tumour promoter (23). The presence of cyclopiazonic in foodstuffs commonly consumed by man, therefore, poses a health to human populations.

It has long been suggested that the major cause of nutritional marasmus is a prolonged deficiency of both protein and energy in the diet (33). The term protein-energy malnutrition (PEM) refers to a spectrum of clinical disorders the most well known of which are marasmus and kwashiorkor. Marasmus arises as a result of prolonged restriction of both dietary energy and protein. Kwashiorkor is due to a quantitative and qualitative deficiency of protein, although energy intake may be adequate.

Marasmus has been shown to occur in areas where there is famine when infants are weaned from breast milk and placed on inadequate diets. This is a very crucial stage of growth for infants when both energy and protein intake are needed in abundance. The disease is characterized by arrested growth, extreme muscle wasting, general weakness and anaemia. It is usually complicated by deficiencies in vitamins and minerals. Energy deficiency in early childhood, even if ultimately alleviated with an abundant supply of food later in life, leaves a permanent, irreversible deficit in body growth.

It is possible that there could be an interplay between energy deficiency and the presence of mycotoxins in foodstuffs in producing the symptoms manifested in nutritional marasmus. Since the main target of cyclopiazonic acid in the Ca^{2+}-pump protein in the sarcoplasmic reticulum of skeletal muscle, this mycotoxin could play a role in skeletal muscle wasting observed in nutritional marasmus. Further studies will be carried out to see the effect, on skeletal muscle development, of feeding rats on energy-suffucient diets containing sublethal quantities of cyclopiazonic acid.

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