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Investigation of the Levels of Total Aflatoxin in Herbal Traditional Medicines from Selected Vendors Dealers in South-Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RCI and CCO designed the study and performed the statistical analysis. Authors RCI, CCO and IOO managed the protocol and wrote the first draft of manuscript. Authors RCI and CCO managed the analyses of the study. Authors RCI,CCO, IOO,OO and CKO managed the literature reviews. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the levels of total aflatoxin (AF) in herbal medicines from selected vendors in South- Eastern Nigeria.
Study Design: This is a cross-sectional study designed to investigate the levels of aflatoxin (AF) mycotoxin in herbal traditional medicines selected randomly from vendor dealers in Ebonyi State of

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Nigeria. Fifty-seven (57) herbal medicine samples were obtained from local markets and stores in Ebonyi state, Nigeria.

Place and Duration of Study: This study was carried out at Abakaliki, Ezza-North, Afikpo North, Ohaukwu, Ikwo in Ebonyi State, Nigeria. This study covered a period of one year from April, 2018 to April, 2019.

Methodology: Fifty-seven (57) herbal medicine samples were analyzed to detect the level of aflatoxin (AF) mycotoxins with the controls. They were selected with self-administered questionnaire using a multistage random sampling technique. Wet extraction was performed on the liquid herbal samples whereas aflatoxin occurrence and levels were evaluated using lateral flow immunoassay technique. Data were analyzed using One sample t-test and descriptive statistics and statistical significance was set at $P \leq 0.05$.

Results: The incidence of Aflatoxin contamination in the samples were 48(84.21%) out of 57 herbal medicine**s** examined with the highest level seen in African Iba herbal (20.00±2.00 ppb) and the lowest level seen in Dunamis and Divine roots herbals (0.00 ± 0.00). Data from the analysis of herbal medicine samples showed different concentration range of mycotoxins AFs (0-20 ppb).

Conclusion: Although aflatoxins concentration observed in the herbal samples were below Nigerian and European Union (EU) upper limits of 20 ppb, the high incidence rate observed in the study should be of concern to public health. The policy makers in the Nigerian agricultural and health sectors have been provided with research evidence as contained in this study on the dangers posed by consumption of some herbal medicines as a result of their aflatoxin contamination.

Keywords: Aflatoxin; mycotoxins; immunoassay; herbal medicine.

1. INTRODUCTION

Aflatoxin is the third-leading inducer of cancer especially hepatocellular carcinoma (liver cancer) worldwide [1]. It has been estimated that about 600,000 fresh cases occurred each year [2]. Report on aflatoxin-related human health problems in Nigeria is limited. The present work provides for the first time the Aflatoxin mycotoxins of herbal medicines intended for human consumption in Southern East of Nigeria. Aflatoxins are a group of toxic and carcinogenic secondary metabolites of fungal origin produced by strains of *Aspergillus flavus, A. parasiticus* and, in rare cases, *A. nominus and A. pseudotamari* [3]. Toxigenic *A. flavus* generally only produces AFB_1 and AFB_2 while *A. parasiticus* (the most toxigenic species) produce AFB_1, AFB_2, AFG_1 and AFG_2 [4]. The appearance of aflatoxicosis symptoms depends on the level of contamination, length of exposure, type of aflatoxin, degree of combination with several other mycotoxins, individual differences, species- specific resistance, sex, pre-existing pathological and physiological status of the victim [5]. Africa especially Nigeria due to its tropical climate is rich in herbs and several millions of Nigerians both old and young relies on non conventional medicines for their primary health. These medicines are mostly produced locally from herbs grown in the country resulting in ethno pharmacy becoming a fast growing industry in Nigeria and are mostly unregulated, with market in every part of the country having ethno medicinal products). However, According to Ezekwesili-Ofili et al. [6], technological challenges and poor awareness has resulted in a non standardized production and handling of these herbs as improper storage, extended drying times and elevated moisture content has resulted in herbal medicines with questionable quality and safety indices with serious implication for human and animal health. Many Nigerian stored foods and herbs are known to be contaminated with aflatoxins [7,8,9]. The outbreak of aflatoxicosis and the risk of mycotoxin (aflatoxin) in plant, animal, and human systems is contained in the Task force Report of No.139 of the Council of Agricultural Science and Technology [10]. Williams et al. [11] equally investigated the relationship between mycotoxins prone foods and Human immune deficiency syndrome (HIV). In animals, aflatoxin has been reported to cause reduced immunity, reduced egg and milk production, liver dysfunction and also embryo toxicity following long time consumption [12]. The economic and public health implications of aflatoxin contamination of herbal medicines have necessitated the need for this study to unravel the level of contamination so as to provide research evidence to policy makers for subsequent action geared towards standardization and quality.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifty-seven (57) different herbal medications were randomly sighted in all at the end of the survey which lasted for 12 months. The samples in liquid formulation were contained in plastic and bottle containers and such information like herbal product name, manufacturers name and address, production and expiration dates, NAFDAC enlisting and batch numbers were obtained using self- administered questionnaire. All the herbal medicines used in the study were produced in Nigeria and were of liquid (aqueous) formulation. The samples were stored in a cool place, away from light.

2.2 Aflatoxin Determination

The procedure for mycotoxin determination used for this study was based on Charm EZ-M Rapid One Step mycotoxins as described by Charm Sciences Incorporation [13]. The method is a lateral flow immunoassay technique whose results and sensitivity are comparable with Enzyme linked immunosorbent assay (ELIZA) and high performance liquid chromatography (HPLC) [14]. This method is also similar to that described by Vicam corporations [15] for the analysis of liquids. This method can also be applied in quantitation of other aflatoxins like AFB_1 and B_2 as well as AFM_1 through the use of test strip and substrate buffer specific to the aflatoxin been analyzed. The extraction procedure is same.

2.3 Extraction of Aflatoxins from the Herbal Formulation

At the extraction stage, 50 g of the sample was weighed after mixing using a chemical weighing balance and poured into a beaker. A wet extraction powder (1 Packet for 50 g sample) was added into the liquid sample in the beaker and mixed for 2 minutes using a mixer to obtain homogeneity. The mixture was then filtered using Whatman No. 1 filter paper to obtain a filtrate which was used for the analysis of the total Aflatoxin. Again, 100 µl of the filtrate was mixed with 900 µl of Aflatoxin buffer (1:10 dilution) to obtain a diluted extract.

2.4 Statistical Analysis

Data were analyzed and presented as percentage, mean and standard deviation. One sample t-test and descriptive statistics were used to test the Statistical significance set at $P \leq 0.05$.The statistical package used was SPSS 23 version.

3. RESULTS AND DISCUSSION

The present work provides for the first time the Aflatoxin mycotoxins of herbal medications intended for human consumption in Southern East of Nigeria. The prevalence of Aflatoxin contamination in the samples were 48(84.21%) out of 57 herbal medicine**s** examined as seen in Table 1.The highest concentration of aflatoxins among the herbal medications studied was found in African Iba (20.00±2.00 ppb) followed by Elcocyn Ds (18.00±1.73 ppb) as presented in Table 2. There was an absence of aflatoxins in Dunamis and Divine roots herbal medications. One sample t-test was computed to compare the various concentrations of aflatoxins found in the studied herbal medications with a test value of 20 ppb (the maximum tolerance level of aflatoxins in consumable foodstuffs). The result shows a statistically significant decrease ($P < 0.05$) from a test value of 20 ppb for all the herbal medications with the exceptions of African Iba, Zaram pile, Deep roots, Iketo 2, and Elcocyn Ds which were statistically non-significant ($P > 0.05$). This result is similar with the findings of Ezekwesili-Ofili, et al. [6] and that of Tosun, and Arslan [16]; both of whom detected aflatoxins in varying concentrations in some organic herbs samples in Nigeria. It has to be emphasized that the 82.21% incidence rate of aflatoxin is very significant and even though the samples had AF levels significantly below the acceptable limits (20 ppb) set by the 77 countries that regulate AFs, including the European Union [17], the presence of aflatoxin in almost all the herbal medications studied is appalling and calls for restraints because according to Carlson and Ensley [18], aflatoxin can cause illness when present in relatively low concentration. Also, the high levels of aflatoxin as seen in African Iba herbal, Elcocyn Ds, Zaram pile, Deep roots and Iketo 2 in that order of decreasing concentration show how unsafe these herbal medicines are for human consumption and can be considered potential hazards for public health. However, it is of note that the problem is compounded because in Nigeria the use of herbal medicines is common and more common it is to buy them from local markets and shops. Aflatoxicosis can lead to acute primary aflatoxicosis with symptoms including hemorrhage, acute liver damage, edema, digestion problems and death [19]. Following chronic intake of aflatoxin there could

Table 1. Frequency and concentration data of the mycotoxins in herbal medications from Ebonyi State

Mycotoxin	Number of samples analyzed	Frequency of positive samples	Concentration (ppb) in herbal medications (Mean ± SD)	Range (ppb)
Total Aflatoxin	57	48(84.21%)	7.35 ± 1.86	0 – 20

Key: ppb: Part per Billion

Table 2. Concentration of mycotoxins in commonly used herbal medications in Ebonyi State, Nigeria

	Concentration in ppb (Part per Billion) N=57 (Mean ± SD)
Herbal Medications	**Total Aflatoxin**
Goko mixture	6.67±3.06[a]
Goodwills	3.67±1.15[a]
Dunamis	0.00±0.00
Divine roots	0.00±0.00
Bitter extra	8.33±2.52[a]
Zaram pile	16.00±5.00
Deep roots	15.67±3.51
Blood purifier	5.67±2.52[a]
Ezinne herbal	9.00±1.73[a]
Cordel silver	5.33±2.52[a]
Iketo	13.50±6.36
African iba	20.00±2.00
Restorative tonic	6.33±2.89[a]
Akwasa	2.33±3.21[a]
Chindus	6.00±3.00[a]
Ukwara	6.33±3.06[a]
Asheitu adams	4.00±1.41[a]
Elcocyn-Ds	18.00±1.73
Golden seed	6.67±3.06[a]

Values are means ±standard deviation of triplicate values. Means with similar letter(s) differ significantly according to the least significant different at $p < 0.05$

be a synergistic action with hepatitis B and other carcinogens to elicit primary liver cancer as contained in the work of [20] who examined the relationship between hepatitis B virus (HBV) infection and biomarkers of aflatoxin exposure in West African children. Aflatoxin in humans has been linked to infertility in both male and females [21] as well as been widely known as a potentialcarcinogen [2]. The various raw materials used in the preparation of these herbal medicines as well as the pre processing, processing, post processing and storage methods employed could attribute to the high level of aflatoxins found in the study. The relationships between these raw materials and aflatoxins though not part of this study could be found in similar studies [22,23]. The technological and awareness challenges seen mostly in Africa and Nigeria could explain the assumption made by [24] that 'mycotoxin contamination in food is not completely preventable'.

4. CONCLUSION

The high incidence of Aflatoxin found in this study though, in levels lower than the maximum tolerance level in consumable foodstuffs pose a greater risk to the health of the consumers of these herbal products, and calls for increased surveillance and also, the need for a further study on the raw materials and the processing protocols used in the herbal preparation to identify the source of the contamination so as to prevent or reduce it. Since this study has shown that herbal medications contain mix contaminants, there is need for a further study to explain the effect and danger posed by the mycotoxins and other contaminants on humans following consumption.

CONSENT

As per international standard guideline, participant's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The ethical approval for this study was obtained from the Faculty of Health Sciences And Technology Ethics Committee, College Of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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