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Evaluation of the Levels of Ochratoxin A and Fumonisin B1 Mycotoxins in Herbal Traditional Medicines Selected from Vendor Dealers in Ebonyi State of Nigeria

Richard C. Ikeagwulonu[1*], Charles C. Onyenekwe[1], Ifeanyi O. Oshim[1] Zeal C. Ikeagwulonu[2], Chiedozie K. Ojide[3] and Nkechi A. Olise[4]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[2]Department of Quantity Surveying, Faculty of Environmental Sciences, Enugu State Science and Technology, Enugu State, Nigeria.
[3]Department of Medical Microbiology, Ebonyi State University, Ebonyi, Nigeria.
[4]Department of Medical Laboratory Science, School of Basic Medical Science, University of Benin, Benin City, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RCI, CCO and ZCI designed the study, 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, CKO and NAO managed the literature reviews. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the concentration of Ochratoxin A and Fumonisin B1 mycotoxins in herbal medications available in markets in Ebonyi State.
Study Design: This is a cross-sectional study designed to determine the levels of Ochratoxin A and Fumonisin B1 mycotoxins in herbal traditional medications selected from vendor dealers. One hundred and fourteen (114) herbal medication samples were selectively obtained from local market and stores in Ebonyi state using a multistage random sampling technique.

**Corresponding author: E-mail: rikeagwulonu@gmail.com;*

Place and Duration of Study: This study was carried out at Abakaliki, Ezza-North, Afikpo North, Ohaukwu, Ikwo and Ebonyi metropolises. This study lasted for 12 months.
Methodology: One hundred and fourteen (114) herbal medication samples examined, fifty-seven (57) each of herbal traditional medicine samples, were selectively analysed for the presence of Ochratoxin A and Fumonisin B1 mycotoxins respectively together with the controls. Mycotoxins occurrence and levels were determined using lateral flow immunoassay technique. The data were presented as percentage, mean ± standard deviation. All data were analysed by one sample t-test and descriptive statistics and statistical significant was set at $P \leq 0.05$.
Results: The content of ochratoxin A was statistically significant different ($P < 0.05$) compared to a test value of 5 µg/L (ppb) for all the herbal medications. The concentration in Goodswill, Divine roots, Zaram pile, African Iba, Akwasa and Restorative Tonic herbal medications were significantly higher when compared to 5 µg/L (ppb). Contrary, the presence of this mycotoxin in Goko mixture was not detected. The contamination with Ochratoxin A was recorded in 51(89.47%) out of 57 examined samples of herbal medicine. The highest concentration of Ochratoxin A was found in Goodswill (23.66±3.51 µg/L (ppb) followed by restorative tonic (22.67±2.52 µg/L (ppb).In addition, examination of fumonsin mycotoxin content in the reorder as studied herbal medications. showed that the highest concentration was found in Ukwara (634.33±8.00 µg/L (ppb), followed by Divine roots (353.67±50.40 µg/L (ppb) and Cordel silver (281.33±27.30 µg/L (ppb). There was an absence in Iketo-2 mixture. One sample t-test was computed to compare the various concentrations of Fumonisin-B1 found in the studied herbal medications with a test value of 1000 µg/L (ppb) (the maximum tolerance level of Fumonisin in consumable foodstuffs). The result showed a statistically significant decrease ($P < 0.05$) compared to a test value of 1000 µg/L (ppb) for all the herbal medications studied. This study reported that Fumonisin contaminations in the samples were 47(82.46%) out of 57 herbal medicine examined.
Conclusion: Ochratoxin A and Fumonisin B1 mycotoxins prevalence were very high and these occur in concentrations exceeding permissible limits. The co-occurrence of these mycotoxins in herbal samples analyzed in this study raises further awareness of the health risks consumers of these food commodities are exposed to.

Keywords: Ochratoxin A; Fumonisin, mycotoxins; immunoassay; herbal traditional medicines.

1. INTRODUCTION

Mycotoxins are fungal secondary metabolites that contaminate various feedstuffs and agricultural crops. The contamination of food by mycotoxins can occur before production, during storage, processing, transportation or marketing of the food products. High temperature, moisture content and water activity are among the predisposing factors that facilitate the production of mycotoxins in food [1]. In Africa, mycotoxin contamination is considered to be a major problem with implications that affect human and animal health and economy.

Ochratoxins are produced by several species of the fungal genera *Aspergillus* and *Pencillium* of which *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum* have been mostly implicated [2]. Ochratoxin formation by *Aspergillus* species appears to be limited to conditions of high humidity and temperature, whereas at least some *Pencillium* species may produce ochratoxin at temperatures as low as 5°C [3]. However, OTA is the most toxic and most prevalent member of the ochratoxins [4]. Ochratoxin A is a nephrotoxin to all animal species studied to date and is most likely toxic to humans, who have the longest half-life for its elimination of any of the species examined [5].

Fumonisins are fungal metabolites produced by various Fusarium species, primarily by *Fusarium verticillioides*, formerly F. moniliforme [6] and *Fusarium proliferatum*, which are global contaminants of maize and maize products. However, the occurrence of ochratoxin A and Fumonisin mycotoxins detection in herbal medicine is rare in our locality. This study equally aims to detect and quantify Ochratoxin A and Fumonisin contaminations in liquid herbal medications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Two samples were collected each for fifty-seven (57) different herbal medications and a total of one hundred and fourteen (114) herbal medications were sighted in all at the end of the

survey which lasted for 12 months using multistage random sampling technique. 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 ascertained with self-adminstration questionnaire.

2.2 Mycotoxins Determination

The procedure for mycotoxin determination used for this study was based on Charm EZ-M Rapid One Step mycotoxin assay as described by Meulenberg [7]. The method is a lateral flow immunoassay technique whose results are comparable with Enzyme linked immunosorbent assay, high performance liquid chromatography and liquid chromatography with tandem mass spectro-photometry [8]. Results obtained from this method are in conformity with the European regulations and is similar to that described by Vicam corporations [9] for the analysis of milk. The principle is based on mycotoxin in the sample interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ®-M system and displayed as ochratoxin or ppm (parts per million) fumonisin.

2.3 Test Procedure

The test strip for the mycotoxins was placed in Charm EZ®-M system. Appropriate test, commodity and dilution were carefully selected. The tape was peeled and 300 µL of the Diluted Extract was pipetted into sample compartment and the tape resealed. The result was read with Charm EZ-M system after 5 minutes incubation for fumonisin and 10 minutes for ochratoxin A. The quantitative range using the Charm EZ-M system is 0 – 30 ppb for ochratoxin A and 0 – 6ppm (0 to 6000 ppb) for fumonisin. Prior to the above procedure, the negative Control and positive control extract were tested to verify performance of equipment and test strips. Control values obtained were valid within the specified ranges {Negative Control: Less than 100 ppb (0.1 ppm) and Positive Control Extract: 1400 to 2600 ppb (1.4 to 2.6 ppm) fumonisin. All analyses were run in triplicates.

2.4 Statistical Analysis

The data were presented as percentage, mean and ±standard deviation. All data were analysed by one sample t-test and descriptive statistics. Differences were considered at $P \leq 0.05$.The analyses were carried out using Statistical Package of Social Science (SPSS) 23 version.

3. RESULTS AND DISCUSSION

The present work provides for the first time the mycotoxins of herbal medications intended for human consumption in Ebonyi state with respect to two of the most important mycotoxins worldwide, namely ochratoxin A (OTA) and Fumonisin B1 (FB1). The contamination with Ochratoxin A was recorded in 51(89.47%) out of 57 examined samples of herbal medicine. The highest concentration of Ochratoxin A was found in Goodswill (23.66±3.51 µg/L (ppb) followed by Restorative Tonic (22.67±2.52 µg/L (ppb). Contrary, the presence of this mycotoxin in Goko mixture was not detected. One sample t-test was computed to compare the various concentrations of Ochratoxin A found in the studied herbal medications with a test value of 5ppb (the maximum tolerance level of Ochratoxin A in consumable foodstuffs). The result shows a statistically significant different ($P < 0.05$) compared to a test value of 5 µg/L (ppb) for all the herbal medications. The concentrations in Goodswill, Divine roots, Zaram pile, African Iba, Akwasa and Restorative Tonic herbal medications were significantly higher when compared to 5 µg/L (ppb) are presented in Tables 1. The occurrence of these mycotoxins could be attributed to the various s raw materials used in the preparation of these medications which are not under the scope of this work but need to be further studied and also due to contamination of the samples or these raw materials by mycotoxigenic fungi *Aspergillus* and *Fusarium* [10].

The result of the fumonisin in the herbal medications studied showed that the highest concentration of fumonisin was found in Ukwara (634.33±8.00 µg/L (ppb), followed by Divine roots (353.67±50.40 µg/L (ppb) and Cordel silver (281.33±27.30 µg/L (ppb). Contrary, the presence of this fumonisin in Iketo-2 mixture was not detected. One sample t-test was computed to compare the various concentrations of fumonisin-B1 found in the studied herbal medications with a test value of 1000 µg/L (ppb) (the maximum tolerance level of fumonisin in consumable foodstuffs). The result showed a statistically significant decrease ($P < 0.05$) compare to a test value of 1000 µg/L (ppb) for all the herbal medications studied which are also presented in Table 1.

Table 1.Concentration of Ochratoxin A and Fumonisin B1 mycotoxins in commonly used herbal traditional medications in Ebonyi State, Nigeria

Herbal medications	Ochratoxin A (ppb) (µg/L)	Fumonisin B1 (ppb) (µg/L)
Goko mixture	0.00±0.00	0.33±0.58[a]
Goodwills	23.66±3.51[b]	6.67±11.55[b]
Dunamis	2.67±1.15	250.00±2.00[c]
Divine roots	12.00±1.73[d]	353.67±50.40[d]
Bitter extra	10.00±2.00	3.33±5.77[e]
Zaram pile	12.33±2.52[f]	109.67±10.02[f]
Deep roots	1.33±1.53[g]	156.67±20.82[g]
Blood purifier	7.33±2.52	76.67±25.17[h]
Ezinne herbal	7.67±3.21	55.33±21.50[i]
Cordel silver	2.67±1.15	281.33±27.30[j]
Iketo	2.50±.707	0.00±0.00[k]
African iba	14.33±2.08[l]	76.67±25.17[l]
Restorative tonic	22.67±2.52[m]	170.00±51.96[m]
Akwasa	12.67±2.08[n]	140.00±10.00[n]
Chindus	6.00±3.00[o]	49.00±4.58[o]
Ukwara	6.33±3.06[p]	634.33±8.23[p]
Asheitu adams	4.00±1.41[q]	165.00±21.21[q]
Elcocyn-Ds	18.00±1.73	107.33±6.43[r]
Golden seed	1.33±.577[s]	133.00±58.03[s]

Values are means ±standard deviation of triplicate values. Means with dissimilar letter(s) differ significantly according to the least significant different at p < 0.05; Key: ppb: Part per Billion

Table 2. Frequency and concentration data of the mycotoxins in herbal medications from Ebonyi State

Mycotoxin	Number of samples analyzed	Frequency of positive samples	Concentration (ppb) (µg/L) in herbal medications (Mean ± SD)	Range (ppb) (µg/L)
Ochratoxin A	57	51(89.47%)	6.25 ± 0.26	0 – 23
Fumonisin B1	57	47(82.46%)	116.88 ± 56.79	0 – 634

Key:SD: Standard Deviation, ppb:Part per Billion

Table 2 shows the frequency and concentration data of the mycotoxin contaminants in the herbal medications. The contamination with ochratoxin A was recorded in 51(89.47%) out of 57 examined samples of herbal medicine while Fumonisin B_1 was also recorded in 47(82.46%) out of 57 examined samples of herbal medicine. The contamination of Nigerian feeds and foods by Zealenone [11] and FBs [12] has been reported by several authors. However, the present study seems to be the first one reporting the occurrence of FBs in liquid herbal medications from Nigeria. FBs levels in the herbal medications were all below common maximum levels, including the European Union acceptable limit. However, the occurrence of these toxins in herbal medications should be taken seriously. This evaluation of mycotoxins in Nigerian herbal medications reveals the quality of the herbal drugs with regards to its acceptability for human and animal consumption. The demonstrated presence of Ochratoxin A at concentrations above the limits acceptable to world mycotoxin regulatory agencies and the co-occurrences of toxins with possible toxic synergistic effects make this studied herbal medications of low quality for human and animal consumption and in fact raises preliminarily national public health concerns. Although the level of FB contamination found in this study in herbal medications for human consumption (up to 20 ppb) are lower than the levels (1,600–12,000 ppb) that caused deaths in the two fatal outbreaks of AF poisoning in Kenya [13].

4. CONCLUSION

Ochratoxin A and Fumonisins are major mycotoxins involve in the developments of certain cancers in kidney and liver toxin and also, this present work provides for the first time the mycotoxins of herbal medications studied in Nigeria. The result consequently, revealed the quality of the herbal drugs with regards to its

acceptability for human and animal consumption since the synergistic effect of these mycotoxins increases their toxicity and effect. However, there is need to sensitize the general public on the danger posed by the presence of mycotoxins in Nigerian food stuffs, most especially as regards to herbal medications.

CONSENT

It is not applicable.

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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