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Original Article

A Possible Link Between Consumption of Sorghum based Products and Prevalence of Hepatocellular Carcinoma In The Sahel Savannah Zone of Nigeria: A Risk Assessment

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ABSTRACT

The study area (Sahel savannah zone of Nigeria) was delineated into five sampling districts, which were further delineated into three localities from where raw and processed sorghum based products were collected. Sorghum based products such as gruel, pap and chincoins "dambu" were sampled using a quantitative food frequency questionnaire. Anthropological measurements of volunteer subjects along with the quantity of food consumed by the respondents was recorded. The mycotoxin concentration in both raw and processed sorghum products was determined using high performance liquid chromatography (HPLC/MS). The determined mycotoxins concetrations in both the raw and processed samples were further used to determine the amount of mycotoxins consumed by respondents in different age groups. Aflatoxin induced Hepatocelluar carcinoma (HCC) in was subsequently determined in communities (within the zone) that subsist on such products. Cumulative average daily consumption of sorghum based products was found to be 192.5±8.32g/day, 617.0±16.45g/day, 810.2±23.24g/day and 746.1±21.02g/day for the infants, children, adults and elderly respectively. A significant difference (P = 0.05) exists between the mycotoxins concentration in raw and the processed sorghum-derived products in the study area. Despite the processing methods employed, the values for PTDI and TDI for mycotoxins, were found to be far above the limits sets by the regulatory bodies. The predictive incidence of HCC and the burden aflatoxin induced HCC in the $HbsAg^+$ was found to be 479.9/100000, 30.0/100000, 28.8/100000, 172.2/100000 in the infants, children, youth and the elderly respectively.

Introduction

Mycotoxins are secondary metabolites, that are usually toxic compounds, produced by various moulds that contaminate many staple foods and cause a broad range of detrimental health effects in animals and humans through chronic exposure or acute toxicity (Richard, 2007). Therefore, quite a significant problem arises due to the worldwide contamination of food and feed with mycotoxins, especially in sub-Saharan Africa (Shephard, 2008). Mould growth can occur often under warm, Warm, damp and humid conditions promotes mould growth either before harvest or after harvest, during storage, on/in the food itself. Chemical stability and resistance to food processing are common characteristics of most mycotoxins. Aflatoxins, ergot alkaloids, ochratoxins, 3-nitropropionic acid, fumonisins, trichothecenes, and zearelenone, are the most important economically, although dozens of other mycotoxins can also be associated with human health risks (Rocha *et al.*, 2014).

The prevalence of mycotoxins in food and feeds worldwide, has constituted an enormous problem to both human and animal populations. Less industrialized countries and rural areas within some developed countries are the most affected by this predicaent. Problems such as liver cancer, reduction of immunity, alterations in the protein metabolism, gangrene, convulsions, and respiratory problems, among others are provoked by both acute and chronic adverse effects of mycotoxins on human health, (Resanovic *et al*., 2013) in the form of Burdens in the form of increased health care costs and premature deaths are some of the economic impact of mycotoxins in foods incurred by the developing countries. Some environmental conditions, such as storage, that can be controlled without too much expense are some factors which influence the presence of mycotoxins in foods. The storage facilities in the developing countries are mostly substandard and helps to promote, instead of limiting the mould growth and proliferation (Shephard, 2008). Proper cleaning and handling of contaminated foods, on the other hand, is rarely implemented in developing nations such as Nigeria due its economic implications, when compared to the developed nations.. Moreover, despite international attempts to improve and implement Due to dwindling economic situation, exacerbated by corruption, implementation of legislation to control the presence of mycotoxins in foods, has been ineffective in developing countries where the main burden is passed to the consumers particularly, in the rural settings (Jeswal and Kumar, 2015).

The main ways through which human body can be exposed to mycotoxins includes contact, ingestion, and inhalation Mycotoxins, depending on their nature and toxicity, may exert different effects which may include but not limited to, carcinogenic, endocrine disorders, teratogenic, mutagenic, hemorrhagic, estrogenic, hepatotoxic, nephrotoxic, and immunosuppressive effects (Sofia *et al*., 2019)

Because of their serious impact on human and animal health, AFs are the best known among all mycotoxins, Based on IARC in classification, (1987), (Sofia *et al*., 2019; Jeswal and Kumar, 2015). AflatoxinB_1 is a carcinogenic substance (category 1A). The risk of liver cancer may increase by up to 30 times on exposure to chronic hepatitis B virus infection and aflatoxin, compared to the risk in individuals exposed to aflatoxin only (Udovicki *et al*., 2016). More so, more than 4.5 billion people are prone to the risk of exposure to contaminated foods with varying levels of AFs worldwide (Blankton *et al*., 2019). Though at present, approximately 100 countries have established levels of AFs in food and feed Assaf *et al*., 2019); the EU have even set legal limit for AFB1 in processed cereal foods to be 0.02 µg/kg (Blankton *et al*., 2019), similar concerted effort is yet to be made in most Sub Saharan African countries including Nigeria.

Various diseases, such as aflatoxicosis in animals, pets, and humans around the world are linked to Aflatoxins (Karunarathna, *et al*., 2019; Adeyeye, 2016), and due to their carcinogenic, mutagenic (DNA damaging), teratogenic, and immunosuppressive effects they are considered to be particularly harmful. Vomiting, abdominal pain, jaundice, pulmonary edema, coma, convulsions, and death are the commonest symptoms of acute aflatoxin poisoning in humans (Strosnider, *et al*., 2006), while chronic aflatoxicosis occurs via cancer, immune system inhibition, and liver damage. In many areas of the world, where liver cancer occurs in large numbers in the population (e.g., in southeast Asia and sub-Saharan Africa), chronic hepatitis C infection and aflatoxin exposure are considered important risk factors, since they are likely to interact synergistically (Gross-Steinmeyer and Eaton, 2012). Around the world and particularly in Africa, the problem of AFs is very important, where aflatoxin contamination is reported in raw cereals with 50% incidence, with infestation reaching 1642 µg/kg and in some exceptional cases higher in rice and maize (Adeyeye, 2016; Lee and Ryu, 2017).

Investigating the extent to which processing methods applied in the preparation of sorghum-based products helps in reducing the concentration of mycotoxins in such products and how the leftover of the mycotoxins (particularly aflatoxins) contribute to the escalation of hepatocellular carcinoma in the zone under study is the main focus of this study.

Materials and Methods

Sampling

This was carried out base on the method described by Garba *et al*. (2021a). Briefly, purposive sampling was carried out in 5 communities from the Sahel savannah agro-ecological zone identified to subsist on sorghum as a major source of their di*et al*most on daily basis namely: Goronyo, Sabon Birni, Roni, Kirikasamma and Guri/Nguru. In these communities, Two hundred and fifty (250) individuals of various age groups from the five communities were purposively targeted (n= 50) using a quantitative food frequency questionnaire (QFFQ) followed by measurement of the body weight and the quantity of food consumed by the respondents.

Extraction and Clean-up Procedures

A multi-mycotoxin extraction method (multimycotoxin screen) devised by Patterson and Roberts, (1979) and employed by Garba *et al*. (2021b) without modifications was employed for extractions of all the AFs, ZEA, OTA, DON. A different extraction method was used for fumonisins (FBs). Extraction of FBs and clean-up were done according to the method of Sydenham *et al.* (1992) without modification.

Validation of Mycotoxins Analytical

In order to be sure of the reliability of the results, the typical parameters for validation methods such as: specificity, accuracy, linearity and detection and quantification limits as recommended by Araujo, (2009) and employed by Anthony *et al*. (2016) were used in addition to ensuring that, validated methods were employed in the course of the determination process. Both internal and external quality control experiments were conducted.

Quantification of Dietary Intake of Mycotoxin In Sorghum based products

The determined mean values of aflatoxins, fumonisins, ochratoxinA, zearalenone and deoxynivalenol in sorghum based products samples, the average amount of these samples consumed and the concentration of toxins removed by processing methods from the raw sorghum grains were the three pieces of information used and was calculated thus:

$$\left[100\% - \left[\frac{\overline{X}_m UPS - \overline{X}_m PS}{\overline{X}_m UPS}\right] x\ 100\right]$$

Where: $\overline{X}_m UPS$ = Mean of mycotoxin concentration in unprocessed sample

$\overline{X}_m PS$ = Mean of mycotoxin concentration in processed sample

Based on the values (data) for the mycotoxins concentrations obtained in section 2.3 the average daily mycotoxin exposure

per person from sorghum based products in 5 districts that forms the sampling sites and invariably the northern guinea savannah was estimated in accordance to the methods of Kimanya *et al.* (2008) and Bandyopadhyay *et al.* (2007). using the formula thus:

$$\frac{\sum \left[\frac{\bar{X}_m PS}{1000} X \bar{X}_m\right]^{T,F,C}}{\bar{X}bw}$$

where: $\frac{\bar{X}_m PS}{1000}$ = mean mycotoxin concentration in processed sample in µg/kg

$\bar{X}_m{}^{TFK}$ = mean of the amount(weight) of the food items consumed daily

T,F,C = tuwo, fura and chincoins(dambu)

$\bar{X}bw$ = Mean body weight of the studied group/age range

Determination of Burden of Aflatoxin-Induced Hepatocellular Carcinoma

Based on IPSC/WHO cohort studies, in which the cancer potency factors of 0.01 and 0.30 cases/100,000/ year/nanogram/kilogram body weight per day aflatoxin exposure for individuals "without" and "with" chronic HBV infection were employed.in HBsAg-negative individuals (Yeah *et al.*, 1989) was adopted. These values were employed in the course of our estimation throughout and was multiplied by the aflatoxin exposure in ng/kgbw/day to arrive at the annual HCC cases per 100,000 individuals, while the burden of the disease in the agro-ecological region was determined by multiplying the values for the annual HCC cases/100,000 in both the $HBsAg^-$ and $HBsAg^+$ individuals by their respective total population (determined).

The modelled formulae below were applied thus:

Population of HBsAg positive = $\frac{13.2}{100}$ **x N(given population)**

While population of the HBsAg negative $= \mathbf{N} - \left(\frac{13.2}{100}\right.$ **x N (given population)**$\left.\right|$

where is N the total population of the individuals

13.2% reported by Rauf *et al.* (2018) as the determined percentage of Nigerian population that are $HBsAg^+$ was applied.

Estimated annual HCC cases per 100,000 for HBsAg negative individual is given **by:**

AFflatoxin exposure(ng/kgbw/day**) × 0.01**

where 0.01 is the cancer potecy factor for HBsAg negative subjects

While estimated annual HCC cases in every 100,000 HBsAg positive subjects is given by:

Aflatoxin exposure$(ng/kgbw/)$ **x 0.3**

where 0.3 is the AFB_1 cancer potency factor for HBsAg positive subjects as determined by the IPSWICH/WHO.

The annual HCC cases was therefore, determined **thus:**

$$\frac{\text{Aflatoxin exposure x AFB1potency factor(0.01 or 0.3)}}{100{,}000} \text{ x N(HBsAg} - \text{ve or} + \text{ve)}$$

where is N the total population of the individuals determined

Results

Effect of different processing methods applied on two sorghum based products.

As revealed in Table 1, the various treatment methods applied in the preparation of different diets determine to a great extent how much of the mycotoxin will be removed from a given grain or food material. The mycotoxin concentration is much reduced in chincoin "Danbu" compared to gruel "tuwo" Effective reduction of fumonisins to almost zero level in the chincoins could also be noted from the table 1.

Table 1. Mycotoxin concentration(µg/kg) in sorghum-based products in Sahel Savannah.

Agro-ecological zones	Food Sample	Processing Method	MYCOTOXINS (µg/Kg)								
			Aflatoxins								
			B_1	B_2	G_1	G_2	Total Aflatoxins	OTA	ZEA	DON	FB_1
SHSD1	Tuwo Gruel	Dehulled, Grind, boil in water into thick paste	82.0	49.2	9.6	8.0	148.8	10.3	742	307	71
SHSD2	[1]Tuwo (Gruel)	Dehulled, Grind, boil in	82.0	49.2	9.6	8.0	148.8	10.3	742	307	71
SHSD3	Tuwo (Gruel)	Dehulled, Grind, boil in water into thick paste	82.0	49.2	9.6	8.0	148.8	10.3	742	307	71
SHSD4	[2]Dambu (Chicoins)	Coarsely milled, sieved, Steam cooked	3.2	1.3	0.4	1.2	6.1	1.3	1802.2	237	0.0
SHSD5	Dambu (Chicoins)	Coarselyg rind, sieve, Steam cooked	3.2	1.3	0.4	1.2	6.1	1.3	1802.2	237	0.0
Concentration in raw sorghum sample (µg/Kg)			123.5	61.5	62.2	96.6	344	62.6	3092	2252	1378
Percentage(%) reduction in mycotoxin concentration (µg/Kg)			[1]33.6 [2]97.4	[1]20.0 [2]97.8	[1]84.6 [2]99.4	[1]91.7 [2]98.7	[1]56.7 [2]98,2	[1]83.5 [2]97.9	[1]76.0 [2]58.28	[1]86.36 [2]89.5	[1]94.8 100

SHS = Sahel savannah Super scripts 1&2 = % mycotoxin reduction in Tuwo (gruel)
D 1 – 5 = Five sampling areas

3.2 Average mycotoxins consumption from products derived from sorghum (µg/Kgbw /day) by people in different age groups in Sahel savannah agro-ecological zones

Figure 3.1 depicts the average daily mycotoxin consumption and/or exposure in the following age range thus: 0 -3, 4 – 17, 18 – 49 and 50 and above years in the study area, figure 3.1:

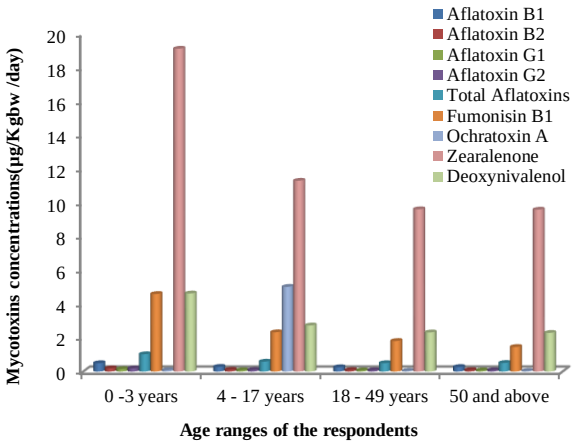

Fig. 3.1. Average mycotoxins consumption (µg/Kgbw /day) by people from different age groups in Sahel savannah (SHS) that subsists on sorghum products.

Key

Average daily consumption of the three common sorghum derived products

Sorghum product	Age range			
	0 – 3 years	4 – 17 years	18 – 49 years	50 year and ≥
Gruel (Tuwo)	55.3 g	294.8 g	432.8 g	371.0 g
Porridge (Fura)	22.6 g	68.2 g	112.1 g	239.1 g
Chincoins (Dambu)	64.6 g	254.0 g	265.3 g	136.0 g

AflatoxinB$_1$ Consumption and the Resulting Hepatocellular Carcinoma (HCC) Incidence due to consumption of Sorghum Based Products (ng/Kgbw/day) in the Sahel savannah of Nigeria

The susceptibility of people that subsist on sorghum based products to the burden of Aflatoxin-Induced Hepatocellular Carcinoma was assessed within the Sahel savannah agro-ecological region Table 2 & 3.

Table 2. Estimated HCC incidence attributable to aflatoxin B$_1$ from sorghum based products consumption (ng/kgbw/day) in the Sahel savannah agro-ecological regions in Nigeria.

Age range (years)	Aflatoxin exposure (ng/kgbw /day)	Estimated annual HCC(per 100,000)	
		HBsAg negative	HBsAg positive
0 – 3	1763.0	17.63	528.9
4-17	1095.0	10.95	238.5
18 -- 49	698.0	6.98	209.4
50 and above	878.0	8.78	236.4

Table 3. HCC cases attributable to aflatoxin B$_1$ exposure due to consumption of sorghum based products in HBsAg-positive and HBsAg-negative populations in the Sahel savannah agro-ecological regions of Nigeria.

Age range (years)	Population (millions	Annual HCC cases	
		HBsAg negative	HBsAg positive
0 – 3	2,298,439	351.7	1604.7 (479.9/100000)
4-17	10,312, 100	980.1	3246.5 (30.0/100000)
18 -- 49	12,565,842	761.3	3473.3(28.8/100000)
50 and above	1,861,110	141.8	580.8 (172.2/100000)

Note* : For tables 2 & 3

Cancer potency for HBsAg –ve is 0.01

Cancer potency for HBsAg +ve is 0.3

Population of HBsAg -ve = Total population – population of HBsAg +ve

Population of HBsAg +ve = 13.2% of total population in the Sahel savannah region as reported by Fasola *et al.* (2008).

Discussion

In Nigeria and most part of the world, sorghum is one of the most highly consumed grain. Of 80% of the 6.7 million MT of sorghum produced, is locally used as food in form of paste (tuwo), pap (akamu) and beverages (kunu, burukutu and pito) in Nigeria (Daniel *et al*., 2016).

As revealed in table1, the mean value of aflatoxin B1(AFB1) in both the gruel and chincoins were well above the Health-based guidance values (HBGV) set by the regulatory agencies of 0.1µg/Kgbw for aflatoxin (Petr *et al*., 2016), More to the unfortunate situation, is the high concentration of AFB1 (82.0 ±3.15µg/Kg) observed in gruel, a highly and routinely consumed product, compared to the 3.2± 0.03 µg/Kg concentration observed in chincoins. Correlating this finding to the prevailing situation of incidence of stunted growth and kwashiorkor in children from the Sahel savannah region, it can be said that, though, the aetiology and pathogenesis of kwashiorkor still remain obscure, but much higher aflatoxins have been found in the blood, urine and livers of children with the disease than similar age-matched children (Resanovi *et al*., 2013) and the presence of the toxin was established in the autopsy brain tissue of some Nigerian children (Bankole, 2003).

IARC, (1993) has classified aflatoxin as a very powerful hepatocarcinogen, and eventually classify naturally occurring mixtures of aflatoxins as a class 1 human carcinogen. Since it was predicted by Nazari *et al*. (2014) that, at most 9 ng·g^{-1} aflatoxin in foodstuff would cause a corresponding increase of 1 HCC case per 10,000 population; with high HBsAg-positive prevalence rate of 13.2% in Nigeria (Turner *et al*., 2012) concerted efforts towards aflatoxin mitigation in the sorghum value chain should be of greater priority and faced with all seriousness. Tolerable limits need to be set and enforced to provide safe food for the population especially for infants young and children (IYC) which will translate to the lower risk of aflatoxin-mediated HCC. However, extreme poverty level and food shortage in the Sahel savannah zone of Nigeria, are other bigger problems to contend with for such limits enforcements to be successful.

Despite the reduction in the concentrations of the individual mycotoxins by the processing methods employed when compared to the raw samples, it however suffice to state that, the concentrations is far above the provisional maximum tolerable daily intake (PMTDI) (JEFCA, 2012) and the values of 2.0, 17.0, 1.0 and 0.25 µg·kg−1 bw·day−1 for

total aflatoxin, Ochratoxin, Deoxynivalenol and Zearalenone respectively reported by Adetunji *et al*. (2014).

Ochratoxin A (OTA) was first isolated by Van der Merwe *et al*., (1965b), from cultures of Aspergillus ochraceus it was found to be produced by Penicillium. OTA has also been found to be a natural contaminants in many foodstuffs including cereals, dried fruits, cocoa, wine poultry eggs and milk. The high concentrations of 10.3± 0.51 and 1.3 ±0.25 $\mu g \cdot kg^{-1}$ determined in this study is in tandem with the report by Sedmikova *et al.* (2001) that, it is frequently associated with crops grown in semi-arid (where our sampling area falls) and temperate regions. Values obtained from this zone far exceeds the provisional tolerable weekly intake (PTWI) of 120 ng/kg bw/day (EFSA, 2006) and 100 ng/kg BW/day (JEFCA, 2000) for ochratoxin. Since it has been considered to be immunosuppressive, teratogenic, genotoxic and mutagenic, and classified as group 2B (possibly carcinogenic to human) by IARC, (1993), level of concentration in processed samples revealed in this study is a cause for concern, particularly with the report by Sedmikova *et al.* (2001) that, ochratoxin A can increase the mutagenic ability of aflatoxin B1 in the case of the two simultaneously occurring in the same crop.

Simultaneous contamination with all the five mycotoxins (AF/OTA/ZEA/DON/FB) in the gruel (tuwo) samples and four of the five mycotoxins (AF/OTA/ZEA/DON) in chincoins (dambu) has been observed in this study. Despite the obscurity of the exact health implications of such toxin "cocktails" on human health, however, synergistic, additive or antagonistic interractions in host organisms are most probable (Miller 1995). Other combinations such as: AFB1 and the trichothecenes (Placinta *et al*. 1999), FB1 and OTA (Creppy *et al*. 2004), and FB1 and ZEA (Luongo *et al*. 2008) was also observed in this study.

Aflatoxin can penetrate cell membrane and attach to its DNA where it causes irreversible mutations, as such, aflatoxin B_1 has been reported as the highest carcinogenic mycotoxin among the different mycotoxins. Aflatoxins can be absorbed from the site of exposure such as from the gastrointestinal and respiratory tract to the blood stream where it can move throughout the body courtesy of its chemical nature that makes it a highly liposoluble compound (Muwaffaq *et al*., 2017). As can be seen from table 2 and 3, it suffice to state that, the revelation is shocking when the ages of 0 – 3years is considered and goes to confirm the report of Hendrickse, (1984). According to Peraica *et al*. (2014) young ones including animals and children are highly sensitive and show reactions to effect of mycotoxins than adults due to their lower body mass, higher metabolic rate, and underdeveloped organs and detoxification mechanisms (Francis *et al*., 2021). Thus far, stricter regulatory measures and improved mitigation strategies remain the viable options in effectively reducing the risk of mycotoxin consumption across the age group in the study population.

Conclusion

As a result of the insufficiency of the common processing methods applied in the preparation of the sorghum based diets in the Sahel savannah, consumption of mycotoxins at levels much above the set maximum limits (MLs) has clearly manifested in the study. Of greater concern is the glaring risk of exposure to HCC due to incidence of aflatoxin at quite an alarming level in products. Mobilisation of various mitigation strategies through the governmental and non-governmental agencies remains the viable options.

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