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Mycotoxins Consumption and Burden of Aflatoxin-Induced Hepatocellular Carcinoma in People Subsisting on Sorghum Based Products in the Derived Savannah Zone of Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Sorghum is a local grain that grows predominantly in the semi-arid, savannah and grassland region of Northern Nigeria and other parts of the world. Sorghum samples were collected from five districts in each of the six agro-ecological zones, while the sorghum based products such as gruel, pap and porridge were also sampled using A quantitative food frequency questionnaire (QFFQ) followed by measurement of the body weight and the quantity of food consumed by the respondents. The mycotoxin concentrations in both raw sorghum sample and the sorghum based products were determined using High performance liquid chromatography (HPLC). Mycotoxin concentrations determined from the two different samples was used to determine the amount of mycotoxins consumed by respondents from different age groups. Subsequently the burden of aflatoxin induced Hepato celluar carcinoma (HCC) in communities (within the zone) that subsist on sorghum and sorghum based products was also determined. There was a significant difference (P = 0.05) between the concentration of the mycotoxins in the raw and the processed sorghum

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samples in the area under study. The processing methods employed in the in the preparation of these products could not reduce the toxin level below the PTDI and TDI levels set by the regulatory agencies. Average daily consumption of sorghum based products based on age range was found to be 192.5 g/day, 617.0 g/day, 810.2 g/day and 746.1 g/day for the infants, children, adults and elderly respectively The incidence of HCC and the burden aflatoxin induced HCC in the HbsAg⁺ and the HbsAg- populations was alarmingly high. Sorghum is a major grain corn in the world agricultural economy and represents an important staple food for the populations of many developing countries. Nevertheless, the nutritional value of sorghum as human food, as well as a feed material for animals, is impaired by its susceptibility to infection by fungi and fungal metabolites and this calls for urgent mitigation strategies to avoid health emergencies particularly in the poverty stricken countries of the sub Saharan Africa where this crop is a common staple.

Keywords: Sorghum; mycotoxins; processing; /HbsAg_ ; hepatocellular carcinoma.

1. INTRODUCTION

In the world agricultural economy, sorghum is the fifth largest most important cereal after wheat, maize, rice and barley, and ranked the second after maize in sub-Saharan Africa. Global area of 42.3 million hectares was cropped with sorghum in 2013 giving an output of 61.5 million metric tonnes. According to the USDA data, sorghum production was 63.08 million metric tonnes in 2016, while a total area of 5.35 million hectares was cropped in Nigeria with an output of 6.55 m metric tons in the 2017/18 cropping season. The Nigeria's sorghum consumption for the year 2017/18 stood at 6.45M and 150,000 metric tons for food and feed respectively. In Nigeria,
sorghum is a local grain that grows is a local grain that grows predominantly in the semi-arid, savannah and grassland region of Northern Nigeria and other parts of the world [1].

Unlike in the developed economies, sorghum together with millet, represents a main source of energy and protein for about one billion people in the semi-arid region of tropics (Sub Saharan Africa) and it is part of the staple diet of more than 300 million people in developing countries (of which Nigeria forms an integral part), representing their major source of energy and nutrients [1]. Sorghum is a basic staple food for many rural communities, in Africa, and Nigeria in particular especially in drought prone areas, which makes it a subsistence food crop for many food insecure people [2].

The proven presence of toxigenic fungi, toxic and carcinogenic mycotoxins in sorghum a highly consumed grain makes it a major source of mycotoxins exposure to animals and human beings and therefore of great public health concern. With regards to economic impact of mycotoxins on human health, the loss of 40% labour productivity in Africa due to diseases and deaths exacerbated by Aflatoxins [3,4] is quite worrisome.

Studies so far carried out ranked aflatoxins as the most potent carcinogens in animal and human populations [5] and were confirmed to be potent immune-suppressors [6]. In 1993, the International Agency for Research on Cancer (IARC) assessed and classified naturally occurring mixtures of aflatoxins as a class1 human carcinogen [7].

This research work therefore set to establish the effect of processing and the daily dietary intake of the five major mycotoxins (Aflatoxin, Ochratoxin, Fumonisin, Deoxynivalenol and Zearalenol) from sorghum derived food products in Nigeria and at the same time determine the HCC burden that may arise therein from its consumption.

2. MATERIALS AND METHODS

2.1 Sampling

Briefly, purposive sampling was carried out in 8 communities from the derived savannah agroecological zone identified to subsist on sorghum almost on daily basis. In these communities, One hundred and sixty three (163) individuals of various age groups were also purposively targeted. Their body weights, age and the weight of the sorghum products consumed per day were recorded. Individuals were categorised as infants, children, adults and elderly based on the following age range thus: $0 -3$, $4 - 17$, $18 - 49$ and 50 and above years. The main food items
consumed which include Gruel (tuwo). consumed which include Gruel (tuwo), Pap/kunu/kamu/ogi, Porridge (Fura), Guinea corn cake (Masa/waina), Chincoins (Dambu) and the

local alcoholic beverage, burkutu/fito were the sorghum derived product purposively targeted.

2.2 Extraction and Clean-up Procedures

A multi-mycotoxin extraction method (multimycotoxin screen) devised by Patterson and Roberts [8] and employed by Makun et al. [9] with modifications was employed for extractions of all the AFs, ZEA, OTA, DON. Twenty five (25) grammes of the pulverised sorghum based food substance was placed in a volumetric flasks. Then One hundred millilitres (100 ml) of Acetonitrile**/**KCl (at a ratio of 9:1) was added and thoroughly shaken and filtered using No1 filter paper. The filtered portion was then placed in a separating funnel held on a tripod stand. Fat impurities were removed from the filtered sample by washing three times with 25 ml of 2,3,4, Trimethylpentane (Iso-octane). The lower layer (Pellet) was carefully filtered into another flask while the supernatant (Iso-octane+fat) was discarded. The defatted extract was returned back into the separating funnel and 30 ml of freshly prepared Sodium bicarbonate (NaHCO $_3$) and another 20 ml of distilled water was added into the defatted extract (this separates into two layers). The lower /bottom layer (Neutral fraction) was passed through a bed of anhydrous Sodium Sulphate (NaSO₄) overlaid on a filter paper into a receiving flask. This filtered portion was placed into a rotary flask and dried using rotatory evaporator. The dried extract was reconstituted using 1 ml Acetonitrile (ACN) and placed into a prepared 10 mm dialysis tube, which was knotted from the two ends and placed into a 50 ml boiling tube to which 50 ml of 30% acetone has been added. The test tube was then sealed firmly with a Parafilm to avoid leakage. The sealed boiling tube was placed into a conical flask and placed on a shaker overnight. After overnight shaking, the dialysis tube was removed from the test tube and discarded while the 30% acetone solution was again placed back into the separating funnel and held on a tripod stand and washed/back extracted three times with 25 ml dichloromethane (DCM) into a round bottom flask and dried using rotatory evaporator. This was reconstituted with 1 ml DCM and dried using nitrogen gas stream and stored at 4°C. To the remaining upper layer (from which the neutral fraction forms lower layer), 50 ml of 1 molar (M) sulphuric acid (H_2SO_4) was added to form acid fraction. This was moderately shaken with slight opening of the valve of the separation funnel. The bubbling was allowed to subside and washed three times with 25 ml of DCM. The extract was collected into a round bottom flask and dried using rotatory evaporator. It was then reconstituted with 1 ml DCM and dried using nitrogen gas stream and stored at 4°C.

Because fumonisins (FB) are only soluble in lower alcohols especially methanol and water, a different extraction method of Sydenham et al. [10] without modification was employed. Sub sample 25 g was mixed with 50 ml of methanol/water (3:1) in a volumetric flask and shaken for one hour and then filtered through Whatman No1 filter paper. The pH of the extract was adjusted to 6-6.5 using acetic acid (to enable the binding of the fumonisin on the SAX column). The SAX cartridge was first conditioned by washing with 5 ml methanol (MeOH) and 5ml MeOH/H₂O (3:1v/v). The flow rate was maintained at 2 ml/min as described by Sydenham et al. [10]. The column was then washed with 5 ml of MeOH/H₂O (at $3:1v/v$) and subsequently with 3ml MeOH. The fumonisin $(FB₁)$ was finally eluted at the flow rate of 1ml/min with 10 ml of 1% acetic acid in MeOH. The eluate was dried under the stream of nitrogen gas at 60 $^{\circ}$ C and stored at 4 – 8 $^{\circ}$ C until further analysis.

2.3 Mycotoxin Analysis

AFs $(AFB_1, AFB_2, AFG_1, and AFG_2)$ were individually determined using HPIC with individually determined using HPLC with
fluorescence detection after post column fluorescence detection after electrochemical derivatization with bromine using KOBRA cell [11]. The eluent (Mobile phase) was water/methanol/Acetonitrile (60:20:20) v/v) with addition of 25 µL of Triflouroacetic acid (TFA) per litre at a flow-rate of 1.00 ml/min (isocratic). The AFs were detected using a scanning Photo diode array (PDA) detector (λex.= 365 nm, λem.=500 nm). ZEA in its own case was analysed by fluorescent detector at excitation and emission wavelengths of 274 nm and 455 nm respectively, in accordance with the method of Abdulkadar et al. [12]. The injection volume was set at 20 μl, while the mobile phase used was acetonitrile/water/Methanol, (46: 46: 8 v/v) was pumped at the rate of 1 ml/min. OTA analysis was performed accordingly, by fluorescence detection as described by Ghali et al. [13]. The mobile phase (acetonitrile/water/acetic acid, 50:48:2 v/v/v) was pumped at a rate of 0.8 ml/min. Respective fluorescence excitation and emission wavelengths of 333 nm and 443 nm was set and used. Residues for FB analysis were reconstituted in methanol and aliquots derivatized with o-phthaldialdehyde (OPA) prior to separation on a reversed-phase HPLC system using fluorescence detection at excitation and emission

wavelengths of 335 and 440 nm respectively Shephard et al. [14] The isocratic mobile phase made up of 0.1 mol/L sodium dihydrogen phosphate/methanol (80:20) that had its pH adjusted to 3.5 using Acetic acid, was pumped at a rate of 1 ml/min. DON was also analysed on a photodiode array detector (PDA) at 220 nm according to the method described by Igor et al. [15]. The mobile phase was Water/Methanol (85:15 v/v) and was pumped at a flow rate of 0.4 ml/min. The injection volume 20 μl. Mycotoxins were quantified using peak area and external calibration curves.

2.4 Validation of Mycotoxins Analytical Methods

In order to be sure of the reliability of the results, the typical parameters for validation methods such as: specificity, accuracy, linearity and detection limits as recommended by Araujo, [16] 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. Visual determination was used to asses the limit of detection. This was as thus: Known concentrations of mycotoxin standards were prepared and successively diluted and subjected to TLC and HPLC until the minimum concentration at which the analyte can be detected was established. This was taken as the limit of detection. Sorghum samples that were known not to contain mycotoxins or those with known concentrations were spiked with 100 µg/kg of AFs, OTA, ZEA, FM and DON for determination of recoveries. For DON and FUM, a spiking level of 500 µg/kg was used. Correlation coefficients of the calibration curves of the known concentrations of the standards, was used to check the linearity of the HPLC method. The RF values and retention time of the mycotoxin standards are indicative of the specificity of the methods. The low detection limit, recoveries of 71.2 – 96.4% for the various mycotoxins and the chromatographic separation indicated that the sensitivity and reliability of the methods employed was sufficient for the purpose of food analysis.

2.5 Determination of Dietary Intake of Mycotoxins in Sorghum

Three pieces of information were used to estimate these parameters. Firstly, the levels i.e comprehensive mean values of aflatoxins,

fumonisins, ochratoxinA, zearalenone and deoxynivalenol in sorghum based products in Nigerian communities that subsist on this grains that was determined. The average amount of food(s) made from the grain consumed by the populace in different district of the sampling areas was the second information utilised. The concentration of toxins removed by processing methods was the third parameter that was taken into account and was calculated thus:

$$
\left[\,100\% - \left[\frac{\overline{x}_{\text{m}} \text{ups}}{\overline{x}_{\text{m}} \text{ups}}\right] x \, 100\right]
$$

Where:

 $\overline{X}_{\rm m} UPS$ = 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 was estimated in accordance to the methods of Kimanya et al. [17] and Bandyopadhyay et al. [18] using the modelled formula thus:

$$
\frac{\Sigma\,\left[\frac{\overline{\rm X}_{\rm m} {\rm PS}}{1000}\,X\,\,\overline{\rm X}_{\rm m}\right]^{T,F,K}}{\overline{\rm X}bw}
$$

Where,

 $\frac{\overline{X}_{m}PS}{1000}$ = average mycotoxin concentration in $\frac{1000}{1000}$ average in yeoloxing \bar{X}_{m}^{TFK} = average of the amount(weight) of the three food items consumed daily $T.F.K =$ tuwo, fura and kunu $\overline{X}bw$ = Mean body weight of the studied

group/age range

2.6 Determination of Burden of Aflatoxin-Induced Hepatocellular Carcinoma

The two standard values for cancer potency factors for aflatoxin 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 employed by the IPSC/WHO, which was based on one cohort study that estimated cancer potency in individuals positive for the HBV surface antigen (HBsAg; a biomarker of chronic HBV infection) and in HBsAg-negative individuals asreporetd by Yeh et al. [19], was adopted. These values were used in the course of our estimation throughout and was multiplied by the aflatoxin exposure in ng/kgbw/day to determine the annual HCC cases per 100,000 individuals, while the values for the annual HCC cases/100,000 in both the HBsAg negative and positive indiduals were multiplied by their respective population (determined) to arrive at the burden of the disease in the agro-ecological region.

The formulae below were modelled and employed as thus:

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

While population of the HBsAg negative $|= N -$ 13.2100 \times N (given population

where is N the total population of the individuals

13.2% is the determined percentage of Nigerian population that are HBsAg positive

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

Aflatoxin exposure
$$
\left(\frac{\frac{ng}{kgbw}}{day}\right) x 0.01
$$

where 0.01 is the cancer potency factor for HBsAg negative subjects

While estimated annual HCC cases per 100,000 for HBsAg positive subjects is given by:

Aflatoxin exposure
$$
\left(\frac{\frac{ng}{kgbw}}{day}\right)x
$$
 0.3

where 0.3 is the AFB₁ cancer potency factor for HBsAg negative subjects

The annual HCC cases was calculated thus:

$$
\frac{\text{Aflatoxin exposure x AFB1 potency factor} (0.01 \text{ or } 0.3)}{100,000} \times N(\text{HBsAg}-\text{ve or}+\text{ve})
$$

where is N the total population of the individuals

3. RESULTS

3.1 Summary on the Effect of Processing Methods on the Mycotoxin Concentration (µg/Kg) in Sorghum from the Derived Savannah Agro-Ecological Zone

Processing methods seems to have profound effect on the mycotoxin concentration in sorghum and the daily mycotoxin intake from sorghum derived products in the agro-ecological zone. There appeared to be significant reduction in the mycotoxin concentration in all the food samples.

3.2 Average Mycotoxins Consumption from Sorghum Based Products (µg/Kgbw /day) by People from Different Age Groups in the Derived Savannah Agro-Ecological Zones

The purposive sampling carried out in 8 communities identified to subsist on sorghum almost on daily basis and the subsequent categorisation of individuals as infants, children, adults and elderly based on the following age range thus: $0 -3$, $4 - 17$, $18 - 49$ and 50 and above years forms the preliminary data. Based on these data the average daily mycotoxin exposure per person from sorghum in 5 districts that forms the sampling sites was estimated as shown in Fig. 1, Table 2.

3.3 Hepatocellular Carcinoma (HCC) Incidence Attributable to AflatoxinB1 Consumption from Sorghum Based Products (ng/Kgbw/day) in Nigeria

Burden of Aflatoxin-Induced Hepatocellular Carcinoma was assessed in people that subsist on sorghum and sorghum based products within the derived savannah. region of the country. Table 3 clearly revealed the estimated aflatoxin exposure level and the annual Hepato cellular carcinoma (HCC) per 100,000 persons in both HBsAg negative and HBsAg positive in the derived savannah region of the country.

4. DISCUSSION

The case of high sorghum consumption can be expected to be quite high in Nigeria which, apart

Table 1. Mycotoxin concentration (µg/kg) in sorghum derived products from derived savannah

Age ranges of the respondents

Fig. 1. Average mycotoxins consumption from sorghum b frombased products (µg/Kgbw /day) by bypeople from different age groups in Derived Savannah (DS)

Table 3. Estimated HCC incidence attributable to aflatoxin B toB1 from sorghum based products consumption (ng/kgbw/day) in the derived savannah agro derivedagro-ecological zone in ecological Nigeria

Note* : 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 of Nigeria or *AEZ*

Table 4. $\,$ Estimated annual burden of HCC cases attributable to aflatoxin B $_1$ exposure due to **derived savannah agro agro-ecological zone of Nigeria**

Note* : 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 of Nigeria or *AEZ*

from being the second largest world producer of this grain crop, is, at the same time, the highest consumer of this grain in form of human foods. The high consumption rate is attributable to the widespread cultivation of the crop and probably, the extreme poverty level in the country of which relatively low cost of the grain attracts the poor masses towards it. Findings made from this study clearly revealed that, there is occurrence of AF/OTA/FB/ZEA/DON in the samples from Derived savannah (DS).

The co-occurrence of mycotoxins has been reported to worsen the already complicated situation of toxicity exerted by these mycotoxins, as a result of their synergistic and in some cases, additive effects. Therefore, the occurrence of some unusual (strange) ailments with some unfamiliar symptoms which are often attributed to witchcraft or spiritual attacks in the study area, may be attributed (based on our opinion) to the consumption of these matrix of mycotoxins, which either synergistically or additively interact to cause such an usual events. Various processing methods employed in the preparation of the sorghum based product seems to be ineffective in reducing the OTA (46.5%), ZEA (34.8%) and DON (18.7%) in the Derived savannah (DS).

Our finding revealed that, probably, due to the gloomy economy and extreme poverty experienced from 2015 to 2017, consumption of sorghum based products has surpassed the values in previous findings by Makun et al. [9]; Odoemelam and Osu, [20]. We found an average daily consumption of sorghum based products based on age range as 192.5±2.17 g/day, 617.0±8.26 g/day, 810.2±7.43 g/day and 746.1±12.05 g/day for the infants, children, adults and elderly respectively. The implication of these high levels of consumption of sorghum based products may also have to be correlated to the findings again thus made in this study in which it was found that despite the seeming efficiency of the processing methods observed, the mycotoxins concentration still remain much higher than the Health based guidance value (HBGV) such as: as low as possible for aflatoxins [21,22], 120 ng/kgBW/PTWI [23], 2000 ng/kgBW PTDI/TDI [24,25], 1000 ng/kgBW TDI/PTDI [26,27], 500 ng/kgBW/day or 250 ng/kgBW/day PTDI/TDI [28,29] for ochratoxin, fumonisin B_1 , deoxynivalenol and zearalenone set by the regulatory agencies respectively. Due to high sorghum consumption across all the age groups observed in this study and the observed

inefficiency of the processing methods to significantly reduce the mycotoxins concentration to levels lower than the PTDI, coupled with the high value of daily consumption determined. It became apparent that, the burden of Aflatoxininduced hepatocellular carcinoma may probably be a source for serious concern, particularly with Nigeria ranking third on the Estimates of HBV prevalence in select countries based on HBsAg seroprevalence with 13.2% [30] after the Gambia with 15-20% [31] and Sudan with 6-26% [32] respectively. Despite the fact that it is unclear the extent to which aflatoxin exposure alone causes HCC in exposed populations, findings by Qian et al. [33] and Ross et al. [34] has shown it (aflatoxin) to modify the risk associated with persistent hepatitis B infection by increasing it by about tenfold.

Findings made by Joseph et al. [35] revealed that, there is a marked geographical difference in the prevalence of HBsAg among HBsAg-positive patients in Nigeria, with the savanna zone of the broadly northern Nigeria showing a higher prevalence when compared with the southern forest zone. Results obtained in this studies follows similar trend as indicated in Table 1 in which the incidence was observed to increase in the derived savannah (DS) compared with results observed from the studies conducted in the forest zones.

Taken collectively and following documented evidence in Nigeria, it is clear that high infectivity of the virus is widespread among Nigerians with HBV infection and whenever HBsAg was present in Nigeria and most developing countries, it was associated with active liver disease.

5. CONCLUSION

From the findings thus made from this work, it suffice to assert that, three fundamental issues come to fore, which include, high concentration of mycotoxins in the grain sorghum from all the agro-ecological zones and invariably Nigeria, insufficiency of the processing methods to lower the mycotoxins to tolerable limits, high consumption of sorghum based products across all the age groups. These taken together will certainly translate into a precarious emergency situation with regards to mycotoxicosis and aflatoxin-induced burden of Hepatocellular carcinoma (HCC) in all the agricultural zones studied and invariably Nigeria. Therefore, a necessary mitigation strategy by all the stake holders becomes imperative.

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CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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