Mycotoxins Consumption and Burden of Aflatoxin-Induced Hepatocellular Carcinoma In People Subsisting On Sorghum Based Products In The Derived Savannah Zone of Nigeria.

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 was 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 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 192.5g/day, 617.0g/day, 810.2g/day and 746.1g/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.

Key words: Sorghum, Mycotoxins, Processing, Consumption, $HbsAg^{+}/HbsAg^{-}$, Hepatocellular carcinoma.

1.0 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 5.35million hectares was cropped in Nigeria with an output of 6.55m 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 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.

2.0 Materials and Methods

2.1 Sampling

Briefly, purposive sampling was carried out in 8 communities from the derived savannah agro-ecological 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), 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 (multi-mycotoxin 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 (100ml) 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 25ml 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 30ml of freshly prepared Sodium bicarbonate ($NaHCO_3$) and another 20ml 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 ($NaSO_4$) 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 1ml Acetonitrile (ACN) and placed into a prepared 10mm dialysis tube, which was knotted from the two ends and placed into a 50ml boiling tube to which 50ml 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 25ml dichloromethane (DCM) into a round bottom flask and dried using rotatory evaporator. This was reconstituted with 1ml DCM and dried using nitrogen gas stream and stored at 4^oC. To the remaining upper layer (from which the neutral fraction forms lower layer), 50ml 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 25ml of DCM. The extract was collected into a round bottom flask and dried

using rotatory evaporator. It was then reconstituted with 1ml DCM and dried using nitrogen gas stream and stored at 4^{o}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 25g was mixed with 50ml 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 5ml methanol (MeOH) and 5ml MeOH/H_2O (3:1v/v). The flow rate was maintained at 2ml/min as described by Sydenham *et al.* [10]. The column was then washed with 5ml of MeOH/H_2O (at 3:1v/v) and subsequently with 3ml MeOH. The fumonisin (FB_1) was finally eluted at the flow rate of 1ml/min with 10ml of 1% acetic acid in MeOH. The eluate was dried under the stream of nitrogen gas at 60^{o}C and stored at 4 – 8^{O}C until further analysis.

2.3 Mycotoxin Analysis

AFs (AFB_1, AFB_2, AFG_1 and AFG_2) were individually determined using HPLC with fluorescence detection after post column electrochemical derivatization with bromine using KOBRA cell [11]. The eluent (Mobile phase) was water/methanol/Acetonitrile (60:20:20) v/v) with addition of 25µL 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 1ml/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 0.1mol/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:

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

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 was estimated in accordance to the methods of Kimanya *et al.* [17] and Bandyopadhyay *et al.*[18] using the modelled formula thus:

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

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

$\overline{X}_m{}^{TFK}$ = 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

2.5 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 $\left| = \mathbf{N} - \left(\frac{13.2}{100}\right.\right.$ **x N (given population)**$\left.\right|$

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:**

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

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:

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

where 0.3 is the AFB_1 cancer potecy factor for HBsAg negative subjects

The annual HCC cases was calculated **thus:**

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

where is N the total population of the individuals

3.0 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.

Table 3.1: Mycotoxin concentration(µg/kg) in sorghum derived products from derived savannah

Agro-ecological zone/Districts	Food Item	Processing Method	MYCOTOXINS **(µg/Kg)** Aflatoxins(µg/kg) B_1	B_2	G_1	G_2	Total Aflatoxins	OTA	ZEA	DON	FB_1
DSD1	Kamu(Ogi)	Soak, wet/dry mill, filter	14.9±0.23	4.0±0.04	5.7±0.13	3.5±0.04	28.1±1.27	37±1.61	620±13.82	750±12.42	430.2±6.51
DSD2	Kamu(Ogi)	Soak, wet mill, filter and boiled into semi liquid	14.9±0.23	4.0±0.04	5.7±0.13	3.5±0.04	28.1±1.27	37±1.61	620±13.82	750±12.42	430.2±6.51
DSD3	[2]Kamu(Ogi)	Soak, wet mill, filter and boiled into semi liquid	14.9±0.23	4.0±0.04	5.7±0.13	3.5±0.04	28.1±1.27	37±1.61	620±13.82	750±12.42	430.2±6.51
DSD4	Kamu(Ogi)	Soak, wet mill, filter and boiled into semi liquid	14.9±0.23	4.0±0.04	5.7±0.13	3.5±0.04	28.1±1.27	37±1.61	620±13.82	750±12.42	430.2±6.51
DSD5	[1]Tuwo/Gruel	Dry mill, boil in water into semi solid paste	9.6±0.14	2.2±0.11	2.1±0.01	2.6±0.03	16.5±1.06	18±0.81	375.8±8.31	129.0±3.53	212±2.86
Concentration in raw sorghum sample (µg/Kg)			**158.1**±3.51	**85.4**±1.41	**64.7**±2.66	**23.1**±0.93	**331.3**±5.81	**69.1**±1.11	**947.5**±17.31	**923**±12.49	**1170**±21.63
Percentage(%) reduction in mycotoxin concentration (µg/Kg)			[2]**90.5** [1]**93.9**±4.51	[2]**95.3** [1]**97.4**±2.31	[2]**91.1** [1]**96.7**±1.39	[2]**84.8** [1]**88.7**±2.02	[2]**91.5** **195.0**±0.79	[2]**46.5** [1]**73.9**±1.64	[2]**34.5** [1]**63.5**±1.55	[2]**18.7** [1]**86.2**±3.81	[2]**63.2** [1]**81.8**±2.31

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 figure 3.1

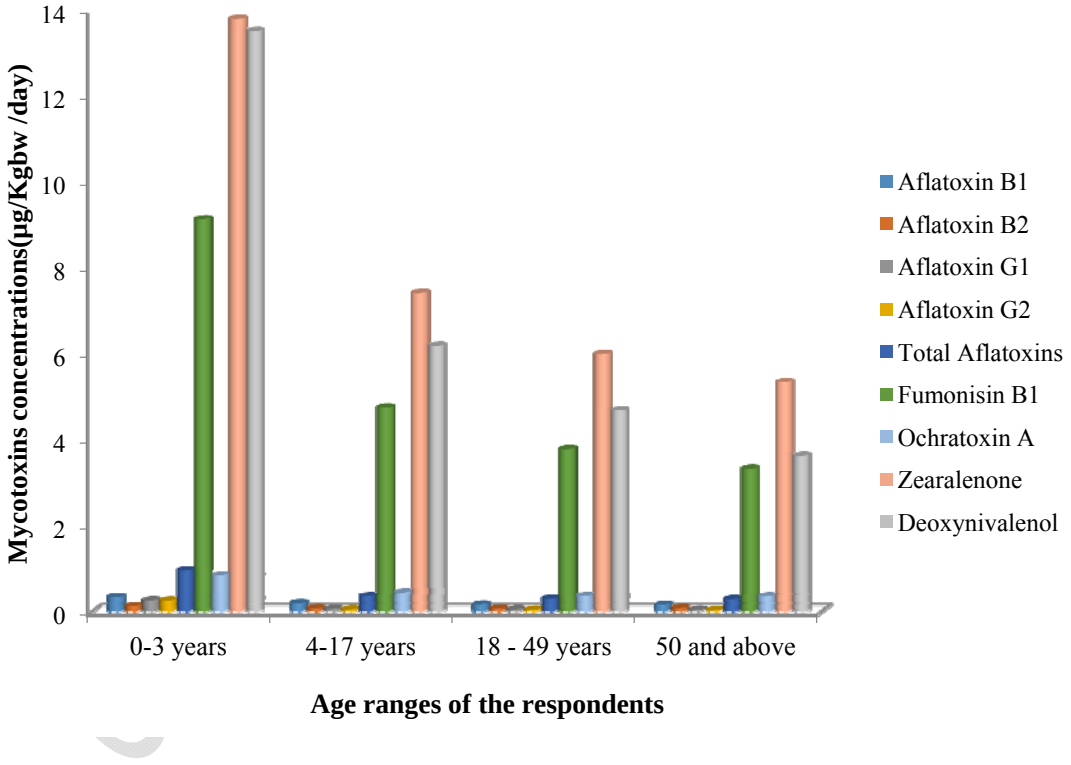

Fig.3.1: Average mycotoxins consumption from sorghum based products (µg/Kgbw /day) by people from different age groups in Derived savannah (DS)

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±1.10	294.8 g±11.00	432.8 g±23.01	371.0 g±6.51
Porridge (Fura)	22.6 g±0.39	68.2 g±2.43	112.1 g±3.17	239.1 g±4.27
Pap (Kunu)	114.6 g±3.60	254.0 g±13.28	265.3 g±1.28	136.0 g±2.17

3.3 Hepatocellular Carcinoma (HCC) Incidence Attributable To AflatoxinB_1 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 3.2 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.

Table 3.2: Estimated HCC incidence attributable to aflatoxin B_1 from sorghum based products consumption (ng/kgbw/day) in the derived savannah agro-ecological zone in Nigeria

Age range (years)	Aflatoxin exposure(ng/kgbw/day)	Estimated annual HCC(per 100,000)	
		HBsAg negative	HBsAg positive
0 – 3	392.0	3.92	117.6
4-17	191.0	1.91	57.3
18 -- 49	157.0	1.57	47.1
50 and above	152.0	1.52	45.6

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 3.3: Estimated annual burden of HCC cases attributable to aflatoxin B_1 exposure due to sorghum based foods consumption in HBsAg-positive and HBsAg-negative populations in the derived savannah agro-ecological zone of Nigeria

Age range (years)	Population (millions	Annual HCC cases	
		HBsAg negative	HBsAg positive
0 – 3	2,530,405	86.1	392.8
4-17	9,440,432	156.5	714
18 -- 49	13,090,058	178.4	809.3
50 and above	2,828,124	37.3	170.2

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.

4.0 Discussion

The case of high sorghum consumption can be expected to be quite high in Nigeria which, apart 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±2.17g/day, 617.0±8.26g/day, 810.2±7.43g/day and 746.1±12.05g/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], 120ng/kgBW/PTWI [23], 2000ng/kgBW PTDI/TDI [24] [25], 1000ng/kgBW TDI/PTDI [26][27], 500ng/kgBW/day or 250ng/kgBW/day PTDI/TDI [28] [29] for ochratoxin, fumonisinB_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 Aflatoxin-induced 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 3.1 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.

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.

References

[1] Ilaria Proietti, Chiara Frazzoli, and Alberto Mantovani (2015). Exploiting Nutritional Value of Staple Foods in the World's Semi-Arid Areas: Risks, Benefits, Challenges and Opportunities of Sorghum. Healthcare (Basel) 3(2): 172 193 doi: 10.3390/healthcare3020172

[2] De Cardoso L.M., Pinheiro S.S., da Silva L.L., de Menezes C.B., de Carvalho C.W., Tardin F.D., Queiroz V.A., Martino H.S., Pinheiro-Sant'Ana H.M. (2015) Tocochromanols and carotenoids in sorghum (*Sorghum bicolor L.*): Diversity and stability to the heat treatment. Food Chem. 2015;172:900–908. doi: 10.1016/j.foodchem.2014.09.117.

[3] Felicia, W. & Pornsri, K. (2011). Evaluating the technical feasibility of aflatoxin risk reduction strategies in **Africa**. *Food Additives and Contaminants,* (Part A Chemical Analysis and Control) Expo Risk Assessment.27(5),658–676. doi: 10.1080/19440041003639582

[4] Miller, J. D. (1995). Fungi and mycotoxins in grains: implications for stored product research. *Journal of Stored Products Research,* 31(1), 1–16

[5] Thomas, W., Kensler, B., Roebuck, S. Gerald, N., Wogan, J. & Groopman, D. (2011). Aflatoxin: A 50-Year odyssey of mechanistic and translational toxicology. *Toxicological* Science. 120(Suppl 1),28–S48. doi: 10.1093/toxsci/kfq283

[6] Björn, L. D. M., Brücher, I. & Jamall, S. (2014). Epistemology of the origin of cancer: A new paradigm. *BMC Cancer*, 14, 331. doi: 10.1186/1471-2407-14-331

[7] IARC (2016). Mycotoxin Control in Low- and Middle-Income Countries*:* A Working Group of world-leading experts convened by the International Agency for Research on Cancer (IARC) with support from the Bill & Melinda Gates Foundation (BMGF. Lyon, France, 17 February 2016. PRESS RELEASE N° 242

[8] Patterson, D. S. P. & Roberts, B. A. (1979).Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxin, ochratoxinA, sterigmatocystin, zearalenone and T2 toxin. *Journal of.Association of Official Anaytical Chemists*, *62*, 1265–1267.

[9] Makun, H. A., Michael F. D., Patrick, B. N., Mulunda, M. & Kabiru, A.Y. (2011). Natural multi- mycotoxin occurrence in rice from Niger State, Nigeria. DOI: 10.1007/s12550-010-0080-5. *Mycotoxin Research*, 27 (2), 97-104.

[10] Sydenham, E. W., Marasas, W. F. O., Shephard, G. S., Thiel, P. G. & Hirooka, E. Y., (1992). Fumonisin concentrations in Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicoses. *Journal of Agric. and Food Chemistry*, 40, 994-997.

[11] Reif K, Metzger W (1995) Determination of AFs in medicinal herbs and plant extracts. J Chromatogr A 692:131–136

[12] Abdulkadar AHW, Al-Ali AA, Al-Kildi M, Al-Jedah JH (2004) Mycotoxins in food products available in Qatar. Food Control 15:543–548

[13] Ghali R, Hmaissia-Khlifa K, Ghorbel H, Maaroufi K, Hedilli A (2009) HPLC determination of OTA in high consumption Tunisian foods. Food Control 20:716–720

[14] Shephard GS, Marasas WFO, Leggott NL, Yazdanpanah H, Rahimian H, Safavi N (2000) Natural occurrence of FBs in corn from Iran. J Agric Food Chem 48:1860–1864

[15] Igor J, Vuric V, Abramovic B (2008) First survey of DON occurrence in crops in Serbia. Food Control 19:545–550

[16] Araujo P (2009) Key aspects of analytical method validation and linearity evaluation. J Chromatogr B 877:2224–2234

[17] Kimanya ME, De Meulenaer B, Tiisekwa B, Ndomondo-Sigonda M, Devlieghere F, Van Camp J, Kolsteren P: Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008; 25: 1353–1364.

[18] Bandyopadhyay, R., Kumar, M. & Leslie, J. F. (2007). Relative severity of aflatoxin contamination of cereal crops in West Africa. *Food Additives and Contaminants,* 24**,** 1109–1114.

[19] Yeh, F. S., Yu, M.C., Mo, C. C., Luo, S., Tong, M. J. & Henderson, B. E. (1989). Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Research,* 49(9),2506–2509

[20] Odoemelam, S. A. and Osu, C. I. (2009). Aflatoxin B1 Contamination of Some Edible Grains Marketed in Nigeria. E-Journal of Chemistry. 6(2), 308-314 ISSN: 0973-4945; CODEN ECJHAO http://www.e-journals.net 2009,

[21] IARC, International Agency for Research on Cancer (2012). Monographs on the evaluation of carcinogenic risks to humans volume 100F: chemical agents and related occupations. PRESS RELEASE N° 374

[22] EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2007) European food safety authority (EFSA) scientific opinion related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. EFSA J 446:1–127

[23] EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2006) EFSA scientific opinion on ochratoxin a in food. EFSA J 365:1–56

[24] JECFA, Joint FAO/WHO Expert Committee on Food Additives (2012) Safety evaluation of certain food additives and contaminants, Prepared by the 76th JECFA meeting, WHO Technical Report Series, No. 974

[25] SCF, Scientific Committee for Food (2003) Updated opinion of the Scientific Committee on Food on fumonisin B1, B2 and B3.

[26] SCF, Scientific Committee for Food (2002) Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol (adopted on 26 February 2002). http://ec.europa.eu/food/fs/sc/scf/out123_en. pdf. Accessed 16 February, 2018

[27] EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2004) EFSA scientific opinion on deoxynivalenol (DON) as undesirable substance in animal feed. EFSA J 73:1–42

[28] JECFA, Joint FAO/WHO Expert Committee on Food Additives (2000) Safety evaluation of certain food additives and contaminants, Prepared by the 51st JECFA meeting. WHO Technical Report Series, No. 891

[29] EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2011b) EFSA scientific opinion on the risks for public health related to the presence of zearalenone in food. EFSA J 9:2197

[30] Fasola F, Kotila T, Akinyemi J. 2008. Trends in transfusion-transmitted viral infections from 2001 to 2006 in Ibadan, Nigeria. Intervirology 51(6):427–431.

[31] Kirk GD, Lesi OA, Mendy M, Akano AO, Sam O, Goedert JJ, *et al*. 2004. The Gambia Liver Cancer Study: infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. Hepatology 39(1):211–219.

[32] Elsheikh R, Daak A, Elsheikh M, Karsany M, Adam I. 2007. Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. Virol J 4:104; doi:10.1186/1743-422X-4-104 [Online 24 October 2007].

[33] Qian GS, Ross RK, Yu MC *et al.* (1994) A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, Peoples Republic of China. *Cancer Epidemiology, Biomarkers and Prevention* 3, 3–10

[34] Ross RK, Yuan JM, Yu MC *et al.* (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*. 339, 943–946.

[35] Joseph C. Forbi, Odunayo H Iperepolu, Timothy Zungwe, and Simon M. Agwale (2012). Prevalence of Hepatitis B e Antigen in Chronic HBV Carriers in North-central Nigeria J Health Popul Nutr. 2012 Dec; 30(4): 377–382.