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Risk Assessment on the Aflatoxin-induced Hepatocellular Carcinoma in Communities Subsisting on Sorghum Products in the Sudan Savannah Zone of Nigeria

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Abstract

Sorghum is a local drought resistant grain that grows predominantly in the arid, semi-arid and savannah regions of Nigeria and other parts of the world. The Sudan savannah agro-ecological belt of Nigeria was delineated into five sampling districts from where raw and processed sorghum based products were collected. Sorghum based products such as gruel, pap and porridge were sampled using a quantitative food frequency questionnaire (QFFQ) followed by measurement of the body weight and the quantity of food consumed by the respondents. High performance liquid chromatography (HPLC/MS) was used to determine the mycotoxin concentrations in both raw and the sorghum based products. Mycotoxin concentrations determined in both raw and processed samples was used to determine the amount of mycotoxins consumed by respondents from different age groups. Subsequently the burden of aflatoxin induced Hepatocelluar carcinoma(HCC) in communities (within the zone) that subsist on sorghum and sorghum based products was also determined. Average daily consumption of sorghum based products based on age range 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

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elderly respectively . There was a significant difference (P = 0.05) in mycotoxins concentration between the raw and the processed sorghum samples in the area under study. The permissible total daily intake (PTDI) and total dily intake (TDI) levels set by the regulatory agencies for the mycotoxin was however observed not to be attained despite the processing methods employed in the preparation of these products. The predictive incidence of HCC and the burden aflatoxin induced HCC in the HbsAg$^+$ and the HbsAg$^-$ populations was found to be alarmingly high.

Keywords: Mycotoxins, Sorghum, Processing, Hepatocellular carcinoma.

INTRODUCTION

Mycotoxins are poisonous (toxic) secondary metabolites produced by certain filamentous fungi (moulds). These naturally occurring and low molecular weight compounds (usually less than 1000 Daltons) are and practically unavoidable in our food and feeds. They find their way into our food chain either by indirect contamination from the growth of toxigenic fungi on food or directly from plant-based food components contaminated with mycotoxins (Ahmad and Jae-Hyuk, 2017). Mycotoxins can accumulate in maturing corn, cereals, soybeans, sorghum, peanuts, and other food and feed crops in the field and in grain during transportation (Marin *et al*., 2013). Acute or chronic toxicity in human and animals is known to be caused by consumption of mycotoxin-contaminated food or feed. In addition to concerns over adverse effects from direct consumption of mycotoxin-contaminated foods and feeds, there is also public health concern over the potential ingestion of animal-derived food products, such as meat, milk, or eggs, containing residues or metabolites of mycotoxins (Osweiler, 2000). The major mycotoxin producers are the members of three fungal genera, *Aspergillus*, *Fusarium*, and *Penicillium*. While over 300 mycotoxins have been identified, six (aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxins, and patulin) are regularly found in food and feed, posing unpredictable and on-going food safety problems worldwide (Moretti *et al*.,2017).

Unfortunately, about 25% of the world's harvested crops are contaminated by mycotoxins each year, leading to huge agricultural and industrial losses in the billions of dollars (*De* Saeger and Logrieco, 2017). Moreso, mycotoxins threaten human and animal health, hamper international trading, waste food and feeds, and divert resources towards research, enforcement, regulation, and applications to alleviate mycotoxin problems (Antonio *et al*., 2018; *Matumba et al., 2017)*.

Among the mycotoxins, aflatoxins (AFs) are considered the most toxic, with significant economic burden to agriculture. In the United States (US) and European Union (EU) countries, AFs are primarily an economic concern, whereas in the developing countries of Asia and Africa, AFs contribute to hundreds of hepatocellular carcinoma cases each year (Mitchell, *et al*., 2016; Wagacha and Muthomi, 2008). Importantly, the estimated annual losses to the US corn industry due to aflatoxin contamination range from US $52.1 million to US $1.68 billion (Mitchell, *et al*., 2016). Additionally, mycotoxins are the main hazard cited in EU border rejection notifications according to Rapid Alert System for Food and Feed (RASFF), with AFs the specific mycotoxins most commonly associated with the notifications (Marin *et al*., 2013).

Generally, all crops and cereals that are improperly stored under feverish temperature and prompting humidity for a prolonged time can be subject to mold growth and mycotoxin contamination (Cynthia and Angella,2020). Most mycotoxins are chemically and thermally

stable during food processing, including cooking, boiling, baking, frying, roasting, and pasteurization. Mycotoxins can also come to the human plate via animal products such as meat, eggs, milk as the result of the animal eating contaminated feed (Marin *et al*., 2013; Kaushik, 2015).

This research work therefore set to investigate the effect these processing methods have on the mycotoxin concentration and also determine the daily dietary intake of the five major mycotoxins from sorghum food products in the northern guinea savannah and at the same time infer on the predictive burden of hepatocellular carcinoma that may probably be induced as a result of aflatoxin present in such diets

Materials and Methods

Sampling Briefly, purposive sampling was carried out in 8 communities from the Sudan savannah agro-ecological zone identified to subsist on sorghum almost on daily basis namely: Dumbugga, Kauran Namoda, Roni, Minjibir and Bubaram. In these communities, One hundred and sixty three (163) individuals of various age groups were 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 sorghum based food items consumed which include Gruel (tuwo), Pap/kunu/kamu/ogi, Porridge (Fura), Guinea corn cake(Masa/waina) and Chincoins (Dambu) were the sorghum derived product purposively targeted.

Extraction and Clean-up Procedures

A multi-mycotoxin extraction method (multimycotoxin screen) devised by Patterson and Roberts, (1979) and employed by Makun *et al*.(2011) with modifications was employed for extractions of all the AFs, ZEA, OTA, DON.

Mycotoxin Analysis

AFs (AFB1, AFB2, AFG1 and AFG2) were individually determined using HPLC with fluorescence detection after post column electrochemical derivatization with bromine using KOBRA cell [18]. 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* (2004) 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*(2009). 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*.(2000). 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*. (2008). 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

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 limits as recommended by Araujo,(2009) 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..

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:

$$\left[100\% - \left[\frac{\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{UPS} - \overline{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}}{\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{UPS}}\right] X\ 100\right]$$

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

$\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}$ = 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 Sudan savannah was estimated in accordance to the methods of Kimanya *et al.* (2008) and Bandyopadhyay *et al.* (2007) using the modelled formula thus:

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

where:

$\frac{\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}}{1000}$ = average mycotoxin concentration in processed sample in µg/kg

$\overline{\mathrm{X}}_{\mathrm{m}}{}^{\mathrm{TFK}}$ = average of the amount(weight) of the three food items consumed daily

T,F,K = tuwo, fura and kunu

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

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 (Rauf *et al*., 2018), 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 Ng(given population)

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

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:

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

where 0.01 is the cancer potecy factor for HBsAg negative subjects

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

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

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

The annual HCC cases was calculated 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

Results

Summary On The Effect Of Processing Methods On The Mycotoxin Concentration (µg/Kg) In Sorghum in the southern guinea savannah Agro-Ecological Zones.

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

Agro-ecological zones	Food Sample	Processing Method	MYCOTOXINS (µg/Kg)								
			Aflatoxins				Total Aflatoxins	OTA	ZEA	DON	FB_1
			B_1	B_2	G_1	G_2					
SSD1	[1]Tuwo(Gruel)	Dehulled, Grind, boil in water into semi-solid mass	15.1	9.8	3.7	2.1	30.7	17	620	750	430
SSD2	[2]Fura(porridge	Dehulled, Grind, boil in	48.8	14.9	5.7	23.9	93.3	37.0	618.9	750	429.7

		water									
SSD3	Tuwo (Gruel)	Dehulled, Grind, boil in water into semi-solid mass	15.1	9.8	3.7	2.1	30.7	17	620	750	430
SSD4	Tuwo(Gruel)	Dehulled, Grind, boil in water into semi-solid mass	15.1	9.8	3.7	2.1	30.7	17	620	750	430
SSD5	[3]Masa/Waina	Grind, Paste, Ferment and Fried	20.8	2.3	7.8	0.0	10.9	11	359	631	0.0
Concentration in raw sorghum sample			**28.6**	**45.7**	**29.1**	**30.1**	**133.5**	**22.2**	**1478**	**1915**	**1648**
Percentage(%) reduction in mycotoxin concentration (µg/Kg)			**47.2 [2]70(higher) [3]27.3**	**[1]78.6 [2]67.4 [3]95.0**	**[1]87.3 [2]80.4 [3]73.2**	**[1]93.0 [2]20.5 [3]100**	**[1]77.0 [2]30.1 [3]91.8**	**[1]23.4 [2]66.7(fold) [3]50.5**	**[1]58.0 [2]58.1 [3]77.7**	**[1,2]60.8 [3]67.1**	**[1]73.9 [2]73.9 [3]100**

SS = Sudan savannah
D 1 – 5 = Five sampling districts in the agro-ecological zone
2 = % mycotoxin reduction in Fura (porridge)
3 = % mycotoxin reduction Masa/Waina (fried sorghum (flour) semi solid paste)

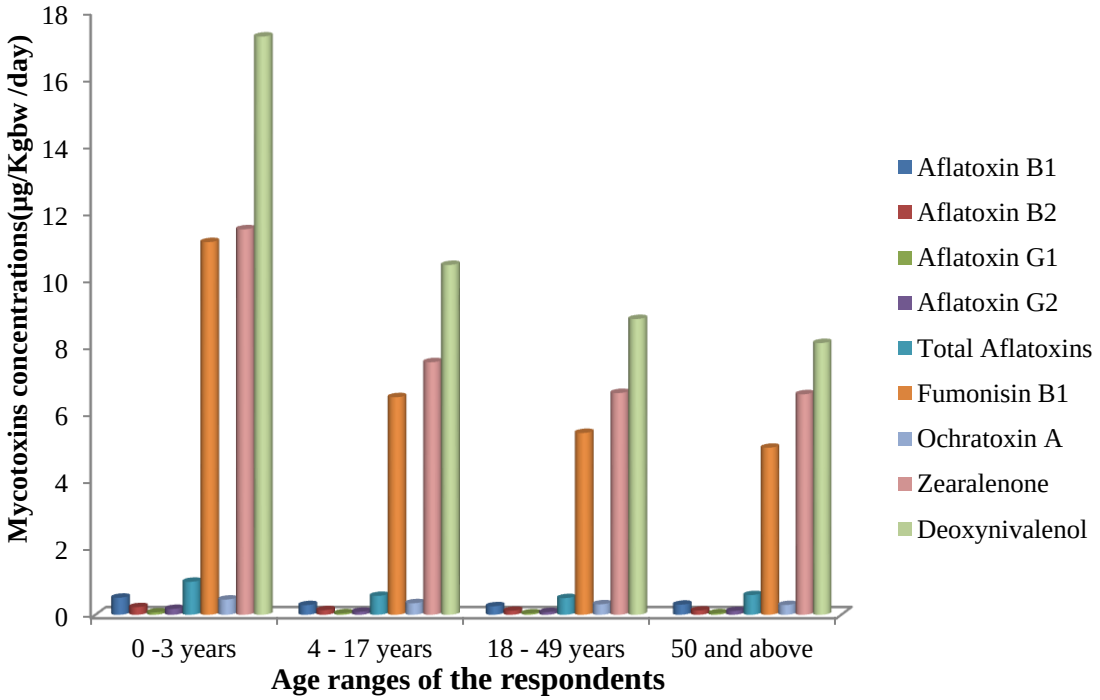

Fig. 3.1 : Average mycotoxins consumption from sorghum based products (**µg/Kgbw /day)** by people from different age groups in Sudan savannah (SS)
Key:

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
Pap (Kunu)	114.6 g	254.0 g	265.3 g	136.0 g

Hepatocellular Carcinoma (HCC) Incidence Attributable To AflatoxinB_1 Consumption From Sorghum Based Products (ng/Kgbw/day) in the Sudan savannah of Nigeria.

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

Age range (years)	Aflatoxin exposure(ng/kgbw/day)	Estimated annual HCC(per 100,000)	
		HBsAg negative	HBsAg positive
0 – 3	516.0	5.16	154.8
4-17	293.0	2.93	87.9
18 -- 49	258.0	2.58	77.4
50 and above	306.0	3.06	91.8

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 Sudan savannah agro-ecological region of Nigeria

Age range (years)	Population (millions	Annual HCC cases	
		HBsAg negative	HBsAg +vepositive
0 – 3	3,400,675	152.3	694.9
4-17	12, 356,786	314.3	1433.2
18 -- 49	12,709,110	284.6	3512.9
50 and above	2,451,567	65.1	297.1

Note for Tables 3.2 & 3.3* :
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 of Nigeria as reported by Fasola *et al.* (2008).

Discussion

The processed commodities from the SS agro-ecological zones are mainly the gruel and the Masa/waina. While the gruel or tuwo has simple method of preparation, the masa or waina involves some extra procedures involving fermentation and frying/ toasting. As reviewed by Petr *et al.,*(2016), fermentation, has no any significant effect on the mycotoxin concentration in the commodities so far studied. In fact, only fermentation using yeast *Saccharomyces cerevisiae* in beer making (Petruzzi *et al*. 2014) and *Rhizopus oligosporus* used in the production of "Tempeh" (Nakazato *et al*. 1990) were shown to exhibit slight decrease in

the mycotoxin concentration. The frying/toasting further applied were also shown by Conway *et al*. (1978) and Raters and Matissek (2008) to reduce the aflatoxin coentrations by 50- 70% and 100% (at 160^{o}C) respectively. While Oliveira *et al*. (2013) reported 97% reduction in OTA in coffee beans, and Boudra *et al*. (1995) reported the same OTA degradation on subjection to high temperatures. The additional effects of these processing methods (particularly the frying/toasting) could be seen to bring about a significant decrease in the mycotoxin concentration (Table 3.1) as reduction levels of 47.2, 27.3 were observed in AFB_1 while 70.0% (fold increase) of the same mycotoxin was observed in porridge/fura. This increase could be attributed to the possible breakage and subsequent release of these mycotoxins from the "Masked mycotoxin" by one or combination of these processing methods. Other reason is the fact that, this food sample is commonly consumed by the Fulani and Hausa people that migrated from the far north to settle in these areas purposely for farming reasons. To these people, the family head's (Maigida) calabash into which he is served is never allowed to be empty and dried, as this portrays a sign of hunger or insufficiency in the family or household. For this reason, mycelial growth of different fungal species(particularly the fusarium spp) at the upper surface of the calabash is a common sight. The perpetual growth of these fungi and the subsequent production of their respective mycotoxins might well explain the reason behind such manifold increase in the ZEA observed.

Reduction by 23.4, 50.5% in tuwo and waina/masa were observed in the case of OTA except the porridge/fura that showed 66.7% increase compared to the original sample.(Table 3.1)

Despite the application of the aforementioned processing methods, the observed mycotoxin levels in the samples are above the Provisional tolerable daily intake (PTDI) of the mycotoxins as reported by Antonio *et al*.(2018). This implied that, consumption of various cocktails of mycotoxins in this agro- ecological zone is presently an unavoidably common practice that has the potent danger of predisposing the consumers to series of disorders associated with the consumption of such mycotoxins and their respective cocktails.

Availability of toxicological data and data on the occurrence of mycotoxins on a particular commodity provide the necessary information for hazard and exposure assessment (Wild and Gong, 2010). As could be observed from figure 3.1 following the outlined exposure and hazard analysis procedure, the aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins concentrations were all at a level that based on "our opinion" could be regarded as unsafe, since these are toxins where carcinogenicity is the basis for concern, due to the fact that a no-effect concentration limit cannot be established for these genotoxic compounds by virtually all the safety regulatory agencies such as EFSA, JECFA and the CAC/WHO, because any small dose was found to have a proportionally small probability of inducing a genototoxic and carcinogenic effect (Petr *et al.,* 2016). Additionally, since the immune-competence varies from one individual to another (De Saeger and Logrieco, 2017), it will be ideal not to assign Health-based guidance value (HBGV) as any reasonably low quantity of the toxin can elicit carcinogenic/genetotoxic response (Ahmad and Jae-Hyuk, 2017). Careful scrutiny of the figures 3.1 revealed that, despite a significant reduction in mycotoxins levels achieved through various processing methods, the quantities of the toxins was found to be significantly higher ($P \leq 0.05$) than the HBGV set by the regulatory bodies (Moretti *et al.*, 2017).

It has been observed by Wild and Gong (2010) that, aflatoxin exposure in food is a significant risk factor for HCC. Unfortunately again, hepatocellular carcinoma (HCC), or

liver cancer, is the third leading cause of cancer deaths world wide, (WHO, 2008), with roughly 550,000 - 600,000 new cases observed annually. It has been reported by Strosnider *et al.* (2006) that >5.5 billion people worldwide suffer from uncontrolled exposure to aflatoxin. Therefore it remains imperative to put in more effort towards determining how many cases of liver cancer can be attributed to this aflatoxin exposure worldwide.

Considering the above information on the factors that promote HCC prevalence. It suffice to say that, Nigeria, a country known to be among the major producers of sorghum in the world and from the findings thus made from the present study and the previous findings made by Makun *et al*.,(2007; 2009 and 2011) will certainly be faced with the burden of aflatoxin induced HCC. As clearly indicated from Table 3.1, people within the age ranges 0 – 3 years were the most prone to the HCC incidence attributable to aflatoxin B_1 with 218.4 individuals/100,000 while those within the age ranges of 18 – 49 years had the least incidence, this is in agreement with the findings reported by Rauf *et al*. (2018). However, Table3.1 portrays that, the estimated annual burden of HCC cases attributable to aflatoxin B_1 exposure due to sorghum based foods consumption (ng/kgbw/day) in HBsAg-positive and HBsAg-negative populations in Nigeria to be higher in the age ranges of 0 -3 and 50 years and above in both the HBsAg(+) and HBsAg(-), this observation concides with similar report by Fasola *et al.* (2008).

Alternatively, when the estimated HCC incidence attributable to aflatoxin B_1 from sorghum based products consumption (ng/kgbw/day) in the Sudan savannah is taken into consideration, it will interesting to note from Table 3.1 that, the figures of the HCC cases/100,000 across all the age group in the Sudan savannah is indeed fearful.
This study represents a first step in attempting to estimate the burden. Our findings in this study follows a similar trend to the findings made by Liu and Wu.(2010) where they found aflatoxin to play a role in about 4.6% of total annual HCC cases and 28.2% of al the HCC cases worldwide. Due to poverty, drought and starvation in the Sub Sahara Africa, South east Asia and China often times people have no choice but to eat mouldy food or starve (Williams 2008).

Conclusion

Of the multiple public health interventions that exist to control the burden of aflatoxin in the body and to prevent HCC, the most prominent which include agricultural, dietary, and clinical interventions (Wu and Khlangwiset ,2010), it is petinent to mention that, these interventions be strictly adhered to particularly in the sub saharan Africa, Southeast Asia, and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in the food.

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