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Effect of Processing Conditions on the Aflatoxin Content of Kulikuli-A Groundnut-Based Fried Snack

Adeniran HA*; Ikujenlola AV; Nd'janton GN

Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

***Corresponding Author(s): Adeniran HA**

Professor of Food Science and Technology inDepartment of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Tel: +23480707033; Email: hadeniran@oauife.edu.ng

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Keyword: Groundnut; Aflatoxin content; Kulikuli; Processing conditions; Albino rats.

Abstract

The study investigated effects of processing conditions on the *kulikuli* snack produced from groundnuts contaminated with pre-determined level of aflatoxin, and also assessed the toxicological effect of the *kulikuli* on albino rats. Samples of aflatoxin-free groundnuts were initially spiked with 501.99 and 36.19 mg kg^{-1} Aflatoxins B1 (AFB1) and B2 (AFB2) before being roasted at 120-180 °C for 30-130 min. The treated and untreated samples were processed into *kulikuli*. Aflatoxin content of one set of the product was determined while the second set was fed to albino rats. The effect on the rats and vital organs were also investigated using standard methods. Results show ranges of 27.90 - 56.22 and 17.05 - 47.00% reductions in AFB1 and AFB2 for samples processed at 120 °C for 30-130 minutes. Photomicrographs of vital organs (spleen, kidney and liver) of experimental animals fed with aflatoxin-laden *kulikuli* reveal distortions in architecture depicting the potentials of aflatoxins B1 and B2 as cancer causing agents compared to control. Study has concluded that roasting of groundnut at 120-160 °C for 30-130 min could reduce aflatoxin content of *kulikuli* to safe levels.

Introduction

Groundnut (*Arachis hypogea* L) belongs to the family leguminosae and is one of the most important oil seed crops in the world [1]. It is the third most abundantly cultivated oilseed in the world and over the years, it has played an important role in the economy of Nigeria and many other countries [2]. Groundnut provides an inexpensive source of high quality dietary protein and oil. Groundnut provides food for humans and livestock and in the absence of meat it forms a valuable dietary protein component in many African countries [3]. It is consumed raw, roasted, blanched, as peanut butter, crushed and mixed with traditional dishes as a sauce [4]. Groundnut is an important nutritional supplement to mainly cereal diets of maize, millet and sorghum of many Africans [5]. The vast food preparations incorporating groundnut to improve the protein level has helped to a great extent in reducing malnutrition in the developing countries [6].

Kulikuli is a popular snack in Nigeria but also, widely consumed in other African countries especially by children of school age and young adults. It is made by roasting peanuts and ground into a paste called "Labu". The paste is then mixed with spices including salt and ground pepper. The paste is stripped

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of excess oil with addition of hot water, and made into the desired shape (round balls, cylinders, etc.). The oil removed in this process is then heated and used to fry the shaped peanut paste until it solidifies. It is then removed from the oil and allowed to cool down [7]. It is rich in protein, energy and fat [8]. In Ghana, it is a popular condiment used to flavor grilled meats, roasted plantain and added to soups [9]. Despite its nutritional importance, it is highly susceptible to contamination by numerous moulds which secrete toxins particularly, aflatoxins [7].

Aflatoxins are toxic metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A.nomius* in, or on foods and feeds [10]. They are probably the most widely studied and dangerous mycotoxins. There are about 16 structurally related aflatoxins that have been characterized. Though, only four, aflatoxin B1, B2, G1, and G2 are known to contaminate agricultural commodities and pose a potential health hazard to livestock and humans [11]. Aflatoxin B1 is the most potent natural carcinogen and most commonly occurring form in contaminated foodstuffs. Aflatoxins persist to some extent in food even after the inactivation of the fungi by food processing methods, such as ultra-high temperature products, due to their significant chemical stability [12]. Aflatoxin contamination of food crops is a global problem and occurs in various food products but most commonly in groundnuts and cereals [13]. The production of *kulikuli* from groundnut and the fact that it is widely enjoyed by school children and young adults as a snack in Nigeria put these consumers at risk of ingestion of aflatoxin. The present study isolated and identified the aflatoxin producing strains of Aspergilli present in groundnut seeds samples from markets; investigated the effect of different roasting temperatures and times on aflatoxin infected groundnuts samples; and assessed aflatoxin levels and toxicological impact of the processed *kulikuli* on experimental animal.

Materials and methods

Materials, microbiological media and reagents

Shelled groundnut samples were bought from Sabo Central market Ile-Ife, Nigeria and placed in a well labeled polyethylene bag and transported to the laboratory. Before analyses, authentication of the groundnut was carried out in the Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria. The samples were kept inside desiccators at ambient temperature (28 ± 2.0 ^{O}C) in the Food Microbiology laboratory in the Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria prior to analysis.

One of the microbiological media used, Potato Dextrose Agar (PDA), was obtained from Lab M, England while the others, Modified Rose Bengal Agar (MRBA) and 5/2 agar were prepared in the laboratory. Aflatoxin standards, other chemicals were of analytical grade and were obtained from the Sigma Aldrich (MO, USA).

Equipment used included: thin layer chromatography tank, incubator, oven, autoclave, rotor shaker, scanner were available in the Food Microbiology laboratory of Food Science and Technology of Obafemi Awolowo University and at International Institute of Tropical Agriculture, (IITA) Ibadan, Nigeria.

Isolation and counting of Aspergillus species

Fungal species belonging to *Aspergillus* species was isolated from ground groundnut samples by the surface spread plate technique on Modified Rose Bengal Agar (MRBA), a selective medium for *Aspergillus* section *Flavi* [14]. One gram of each sample was suspended in 10 ml sterile distilled water in a 40 ml sterile polystyrene tube and mixed for 2 minutes. To ensure the collection of not more than 10 colonies from plates, appropriate dilutions was plated and spread with a sterile L-rod on MRBA and incubated in the dark for 3 days at 31 ^{o}C. After 3 days of incubation, plates containing 3-10 colonies were observed for colony formation and results recorded in Colony Forming Units (CFU)/g based on the average count of the triplicate. For each sample, isolates were transferred to 5/2 agar (5% V8 juice and 20% agar, pH 6.0) and maintained in the dark at 31 ^{o}C for 5 days, for further characterization.

Identification of Aspergillus species

Isolates were classified into species and strains on the basis of colony characteristics, morphological and cultural features. Slide cultures and agar blocks of each pure isolate were prepared, observed under a light microscope (Leica Galen III), using ×5, ×10 and ×40 objective lens as described by Samson et al., [15]. The quantities of *Aspergillus flavus* in the respective samples were calculated as Colony Forming Units (CFU) per gram.

Inoculation of groundnut seeds with aflatoxigenic spores

Aflatoxigenic *Aspergillus flavus* species were selected for deliberate seeding of mould-free groundnut seeds. The identified isolates were then transferred into 2 ml sterile distilled water from which 50 µl was used to inoculate five grams of aflatoxin free groundnut. The inoculated grains were then incubated in the dark at 31 ^{o}C for seven days. The sporulated grains were then analyzed for aflatoxin producing strains. The *Aspergillus* strain that produced a higher concentration of aflatoxin were cultured on a large scale on 5/2 agar and incubated for 7 days. The spores of the matured culture were then used to spike fresh groundnut seed purchased from a local market. The culture was covered and incubated at 31 ^{o}C for 7 days at the end of which the greenish-yellow sporulation of the mould was widespread in the culture. This was done to ensure uniform distribution of the toxins in the seeds used for this study as it has been reported by Ogunsanwo et al., [16] that spontaneous aflatoxin contamination of the mould-infested groundnut is not always uniformly distributed.

Heat processing of groundnut

Roasting temperatures and processing time of contaminated and uncontaminated groundnut were varied before production of *kulikuli*

Roasting: Six hundred grams of contaminated and uncontaminated seeds were separately roasted using ''Gallenkamp'' hot air oven at specific temperatures of 120 ^{o}C for 30, 50, 70, 90, 110, 130 minutes, at 140 ^{o}C for 30, 50, 70, 90, at 160 ^{o}C for 20 and 30 minutes and at 180 ^{o}C for 10 and 20 minutes. For each processing condition, six hundred grams of groundnut seeds were used. The roasted groundnut seeds were subsequently analyzed for aflatoxin level.

Production of groundnut *kulikuli*: Processing of groundnut to groundnut *kulikuli* was performed using the modified method of Adjoul et al., [17]. Six hundred grams of roasted groundnut were dehulled and blended together with salt (5 g), pepper (2 g) and onions (2.5 g) until a smooth paste was formed using a sterile blender (Excella IS 4250, Japan). Sixty millilitres (60 ml) of hot water was then added to the paste while mixing thoroughly, the addition of hot water helped in the extraction of oil from

the paste. A filter cloth was used for the extraction of oil from the paste. The resultant paste was proportioned into several five gram samples and each was then shaped into long shape of 5 ± 0.75 cm length and fried for about 6 ± 1 min in 600 ml hot vegetable oil (Figure 1).

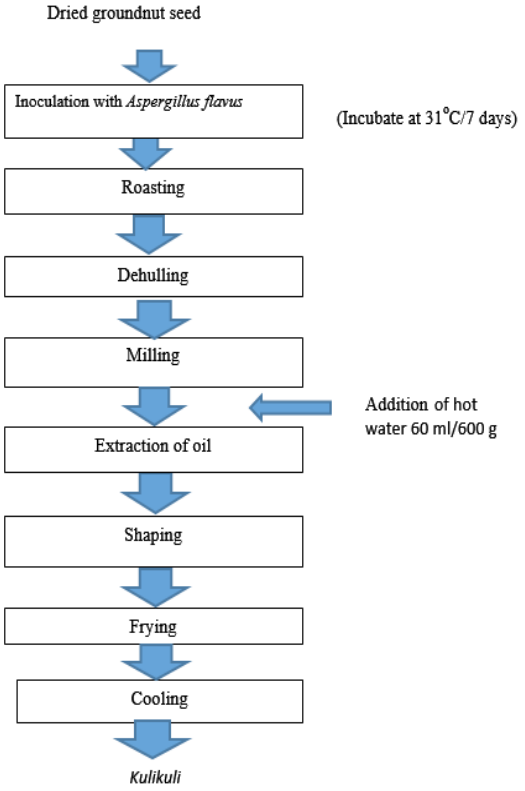

Figure 1: Flow chart of kulikuli production.
Source: Adjoul et al., [17], (modified).

Determination of aflatoxin content of *kulikuli* samples

Extraction of aflatoxins from samples: Aflatoxin was extracted from grain samples (groundnut and *kulikuli*) before and after processing. Aflatoxin was extracted from approximately 20 g of a representative sample by grinding the grains or product in 100 ml of 80% methanol for 3 minutes, mixed on a Roto-Shaker Genie (Scientific Industries, Bohemia, NY) for 30 minutes. This was then filtered and the filtrate was diluted with 40 ml of 10% NaCl and 25 ml of n-hexane. The collected filtrate was partitioned by adding 35 ml of dichloromethane (methylene chloride). The filtrate was passed through anhydrous sodium sulphate (Na_2SO_4) and evaporated to dryness in a fume hood. The residue was dissolved in 1 ml. of dichloromethane and subjected to thin layer chromatography [14].

Detection and quantification of aflatoxin: In a single determination, 4 µl aliquot of the sample extract was spotted directly (1 cm apart) on pre-coated silica gel glass plates, 20 × 10 cm (HPTLC Silica gel 60 F254, EMD Chemicals Inc., Germany) alongside 4 µl aliquots of the working aflatoxin standard (Supelco, Bellefonte, PA, USA). The spots were dried up in a fume hood for 3 minutes, and the plates developed in a saturated chamber with diethyl ether-methanol-water (96:3:1) for about 10 minutes as described by Garber & Cotty [18]. Aflatoxin spots was visualized under long-wave ultraviolet light (λ = 366 nm) and each sample identified qualitatively for the presence (and type) or absence of aflatoxin by comparison with the spots of the aflatoxin standard. Aflatoxin was quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with win CATS 1.4.2 software).

Animal experiments

Thirty-five (35) Wistar rats (*Rattus norvegecus*) weighing about 100 to 130 g were obtained from the Animal house, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria and were kept in the animal house in the Department of Food Science and Technology of Obafemi Awolowo University, Ile-Ife, Nigeria where they were fed with standard diet UAC® Grower's mash and water for seven days before experimentation [19]. The protocol of the Department of Food Science and Technology and the Animal Ethical Committee of Obafemi Awolowo University, Ile-Ife, Nigeria was observed in the course of the study. During the seven days of acclimatization, ambient temperature 30 ± 2 °C in a 12 h light/dark cycle. At the end of the seven days, the animals were randomly weighed and divided into 7 groups of five with an average weight of 120.4 g. In the course of the study, the animals were cared for in accordance with Helsinki declaration. The first group served as control and was given only maize as the protein free diet (AA). The second group (A) was fed on raw contaminated groundnut that was used for the production of *kulikuli*, containing 501.99 mg kg^{-1} AFB1 and 36.19 mg kg^{-1} AFB2. The third group (B) was given *kulikuli* free from aflatoxin. The fourth group (C) was fed *kulikuli* roasted at 180 °C for 20 minutes containing respectively 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2, respectively while the fifth group (D) was given *kulikuli* sample processed for 160 °C for 20 minutes containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2, respectively. The sixth group (E) was given *kulikuli* roasted at 140 °C for 50 minutes containing 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2, respectively.

Members of the seventh group was given (H) were sacrificed before the experiment and their organs harvested and preserved for further analysis. The animals were weighed at four-day intervals during the experiment and were also observed for appetite and physical behavior. After 21days of experiment, the animals from the other groups were sacrificed to examine the effect of aflatoxin on the tissues of their vital organs (liver, kidney and spleen).

Histopathological study: At the end of the experiment animals were sacrificed under chloroform anaesthesia after 24 hours of the last feeding during which organs (kidney, liver, spleen) were excised and fixed immediately in 10% neutral buffer formaldehyde solution for 48 hours and were further processed for routine Haematoxylin and Eosin (H&E) for general histology. The tissues were trimmed to about 3-5 mm thick sections and processed via paraffin wax embedding method of Drury& Wallington [20]. The tissues were dehydrated at ambient temperature through ascending grades of ethanol from 70% ethanol, 90% ethanol, Absolute ethanol I and Absolute ethanol II and allowing a residence time of 1 h in each case.

Dehydrated tissues were cleaned at ambient temperature in two changes of xylene for one hour in each change. The tissues were then infiltrated in two changes of molten paraffin wax at 60 °C for one hour in each change and finally embedded in paraffin wax using multi-block plastic embedding moulds. The paraffin blocked tissues were trimmed and mounted on wooden block for sectioning on a rotary microtome. Sections of 5 µm thickness were produced from the tissue blocks using a rotary microtome (Bright B5143, Huntington, England). The sections were transferred into water bath (maintained at 40 °C) to allow spreading of the folded ribbons of sections. These sections were mounted on new clean glass slides. These were then dried at 40 °C on a slide drier to enhance adherence of the sections

to the slides.

Staining procedures and microscopy

Procedures of H and E as described by Drury & Wallington [20] was employed which involved dewaxing in two changes of xylene, and rehydration in descending grades of alcohol from absolute I to 50% and final rinsing in distilled water. The sections were further stained in haematoxylin for 15-20 minutes and later in 1% aqueous eosin. Stained sections were examined under a LEICA research microscope (LEICA DM750, Switzerland) equipped with a digital camera. Digital photomicrographs of the stained organs were taken at various magnifications (×100; ×400; ×1000) [21].

Statistical analysis

Experiments were performed in triplicates, and data analyzed were mean subjected to Analysis of Variance (ANOVA). Means were separated by the Tukey's multiple range test. (SPSS version 17) while results were taken to be significant at ($p < 0.05$).

Results & discussion

Aflatoxin content of *kulikuli* produced from groundnut samples not deliberately inoculated with aflatoxigenic spores

Table 1 reveals the effect of processing conditions on uncontaminated groundnut obtained from market samples. The starting groundnut samples obtained from the market contained 138.17 and 24.34 mg kg^{-1} of AFB1 and AFB2, respectively. This level of aflatoxin exceeded EU (EFSA) and US (FDA) standards of 20.0 and 4.0 ppb of aflatoxin [22,23] showing that posed serious health if consumed without exposure to temperatures of 120 oC and above for a minimum of 30 minutes. However, groundnut cake *kulikuli* produced in the laboratory from these groundnut samples were found to be free from aflatoxin. This shows that all the processing conditions employed in this study effectively reduced the aflatoxin content below detection level.

Concentration of aflatoxin in groundnut and *kulikuli* produced from deliberately inoculated with aflatoxigenic spores

Effects of processing on aflatoxins content of *kulikuli* are presented in Tables 1, 2, 3 and 4. AFB1 and AFB2 content of the product (*kulikuli*) significantly decreased when groundnut samples deliberately inoculated with aflatoxin were processed with increasing temperatures and exposure times. The highest reduction for samples produced from groundnut samples roasted at 120 °C was observed in sample roasted for 130 minutes with a total aflatoxin level decreased from 501.99 mg kg^{-1} (AFLB1) to 56.57 mg kg^{-1} of sample; and from 36.19 mg kg^{-1} (AFLB2) to 4.98 mg kg^{-1} of AFLB1. For *kulikuli* produced from groundnut roasted at 140 °C, the highest reduction was observed in the sample produced from groundnut roasted for 90 minutes with a reduction from 501.99 to 29.97mg kg^{-1} of AFLB1 and from 36.19 to 4.49 mg kg^{-1} of AFLB2, respectively.

For groundnut cake *kulikuli* produced from groundnut roasted at 160 °C, the highest reduction was observed in samples produced from groundnut roasted for 30 minutes with a reduction from 501.99 to 10.31 mg kg^{-1} AFB1 and from 36.19 mg/kg to a non-detectable level for AFB2. However, groundnut samples processed at 180 °C for beyond 20 minutes could not be processed into *kulikuli* because oil could not be extracted from the groundnut processed for it. Samples processed at the same temperature for 20 minutes showed a significant reduction in the AFB1 and AFB2 content to 9.60 and 0.00 mg kg^{-1} respectively. A good percentage of 25-60 % reduction was also observed after roasting and dehulling of groundnut seed.

It was also established that dehulling which is an important unit operation in the processing of groundnut to *kulikuli* also contributed to reduction of AFB1 and AFB2 in the final product. A range of 27.90-56.22 and 17.05-47.00% reductions in AFB1 and AFB2 were observed for whole groundnut samples processed at this temperature for 30-130 minutes. However, for dehulled groundnut samples, a range of 45.13-77.81 and 44.21-70.76% reductions in AFB1 and AFB2 were observed. This clearly shows that after the removal of the hulls, there were more reductions in the aflatoxin content indicating that some of the aflatoxins could have been harboured in the hulls. Processing of the treated groundnut into *kulikuli* evidently caused further reductions in the aflatoxin content. A range of 62-.52-88.73 and 62.03-86.23% reductions were detected for the groundnut processed into *kulikuli* after roasting at 120 °C for 30-130 minutes. Similar trends were observed in Tables 2 and 3 for groundnut roasted at 140 and 160 °C.

This study has shown that roasting at different temperatures and times, and the unit operations of dehulling, and processing of groundnut into *kulikuli* could reduce the level of aflatoxins in the product and this agrees with the submissions of Hamada & Megalla [24] and Ogunsanwo et al., [16] that reported that heating reduced aflatoxins in agricultural products. Three main reasons could be adduced for the aflatoxin loss as a result of application of heat. Heat lability of aflatoxins; thermodynamically enhanced reactions between the aflatoxins and other constituents of the peanut seeds; and thermal destruction of other constituents of the peanut seeds and less extractability of the aflatoxins from these products [16]. In the study of Ogunsanwo et al., [16], positive correlations between loss of aflatoxins in the peanut seeds and the roasting conditions were reported.

Body weight response of rats

Average body weight responses of rats during the experiment are presented in Figure 2. Group AA rats fed on maize (protein-free diet) alone experienced slight increase in average weight from 119.70 (initial weights) to 120.32 g (final weight) possibly due to the lack of protein and fat in their diet which could have made the diet complete. Group A rats (fed with groundnut containing 501.99, 36.19 mg kg^{-1} of AFB1 and AFB2, respectively) did not experience any increase in weight rather their weight slightly decreased from 120.40 (initial) to 118.76 g (final weight). This could be as a result of high dose of the AFB1 and AFB2 present in their diet as protein and fat in their diet should have caused weight gain. The ingested high levels of AFB1 and AFB2 could have interfered with metabolism of the nutrients present in the treated feed. Group E rats also experienced weight loss from 119.16 to 99.56 g probably due to the high dose of AFB1 and AFB2 despite the presence of relatively high amount of protein and fat content in their feed. Significant reduction in fat content occasioned by conversion of groundnut into *kulikuli* could have contributed to the effect of aflatoxin being felt more in terms of weight gain compared to what happened to rats of group A despite receiving more aflatoxin than members of group E. This agrees with the submission of Gong et al., [25] who reported stunted growth in humans after ingestion of aflatoxigenic fungal strains along with meal among young children in Benin and Togo. Rotimi et al., [26] also reported lipid and lipoprotein metabolism in rats administered with 0.5-1.0 mg kg^{-1} of AFB1 for 7 days. Rats in groups C fed with *kulikuli*

containing low level of aflatoxins 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2, respectively showed average weight gain during the course of the experiment. The average weight of the animals increased during the period from 120.52 to 138.74 g indicating that the low level of aflatoxin in their diet did not adversely affect their growth. The residual aflatoxin was obviously tolerated by the rats with no observable negative effect on their nutrient metabolism. The diet contained some amounts of protein and fat which could have been assimilated to cause weight gain in the experimental rats. This trend was also observed in rats of group D probably because of the low content of AFB1 and AFB2 in their diet. Group B rats fed with aflatoxin free diet showed greatest increase in weight (120.95 to 144.24 g) during the period of experiment indicating that the available balanced nutrients were well utilized and there was no aflatoxin to interfere with their metabolism.

Effects of aflatoxin in animal organs

Spleen: Looking at the photomicrographs of spleen tissue in Figures 3a and b, it was observed that there was minimal disruptive effect on the architecture of spleen tissue (groups H, AA and B). However, a decrease in lymphoid follicles was observed in the spleen tissue of rats of groups C, D and E (fed on different levels of aflatoxin contaminated feed). In group A rats fed on 502 mg kg^{-1} AFB1 and 62 mg kg^{-1} AFB2, the spleen tissue showed highly congested blood sinuses and bizarre arrangement of T & B lymphocytes indicative of the toxic effect of high concentration AFB1 and AFB2 in their diet. The functions of lymphoid follicles being to support antigen-specific immunoglobulin production [27], the dysfunction of lymphoid follicles contributes to mucosal immunosenescence [28].

Kidney: In this study, architecture of the kidney tissue in control groups (groups H, AA & B) showed normal histological feature with a detailed cortical parenchyma and the renal corpuscles as dense rounded structure with the glomerulus surrounded by a narrow Bowman's space (Figure 4a and Figure 4b). The Photomicrographs of the kidney tissue of treated groups A, C, D and E fed with aflatoxin in a dose-dependent manner showed an enlarged Bowman space when compared to control groups. Enlargement of Bowman's space was more pronounced in groups A and E rats which were placed on diet containing 501.99 + 36.19 and 65.37 + 10.38 mg kg^{-1} of AFB1 and AFB2, respectively. Interstitial oedema, necrosis and extraglomerular hypercellularity were also observed in the kidney tissue of groups A and E rats. Aflatoxin induced histopathological change in animal organs is associated with their dysfunction [29]. Li et al., [30] also averred that AFB1 (0.5 mg/kg), AFM1 (3.5 mg^{-1}), and AFB1 (0.5 mg^{-1}) + AFM1 (3.5 mg kg^{-1}) activated oxidative stress and caused renal damage.

Liver: The histopathology of liver tissue in control groups (H, AA & B) revealed hepatocytes arranged in plates around the Central Vein (CV) with sinusoids (arrow) between the plates. From the photomicrographs in Figures 5a and b, no specific lesion was observed in liver tissue in groups C, D, and E. In this study, it was observed the members of group A fed on 501.99 + 36.19 mg kg^{-1} AFB1 and AFB2, respectively and group E fed on 65.37 + 10.38 mg kg^{-1} of AFB1 and AFB2, respectively mg kg^{-1} caused significant histopathological changes in liver than those fed on smaller doses of aflatoxin 18.94 + 2.41, 48.73 + 4.93 mg kg^{-1} of AFB1 + AFB2, respectively. The photomicrographs of their liver tissue were presented with distortion of liver tissue architecture and extrahepatic hypercellularity. The liver is the target organ for AFB1. Ingestion of aflatoxin is known to be capable of inducing poisoning and aflatoxicosis and is known to participate in the development of primary liver cancer [31]. Vascular congestion was also observed in the Central Vein (CV) of members of group A (Figure 5a and Figure 5b). Rotimi et al., [26] reported that AFB1 and AFB2 administered at concentrations lower than the ones used in this study adversely affected the functioning of rat liver.

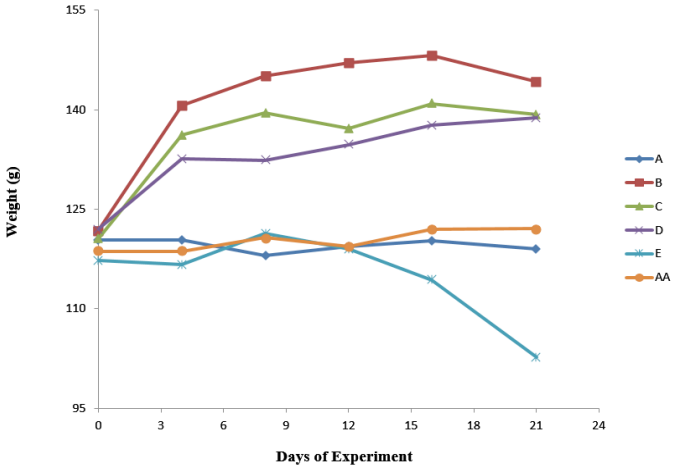

Figure 2: Average Body weight responses of Albino rats during the experiment.

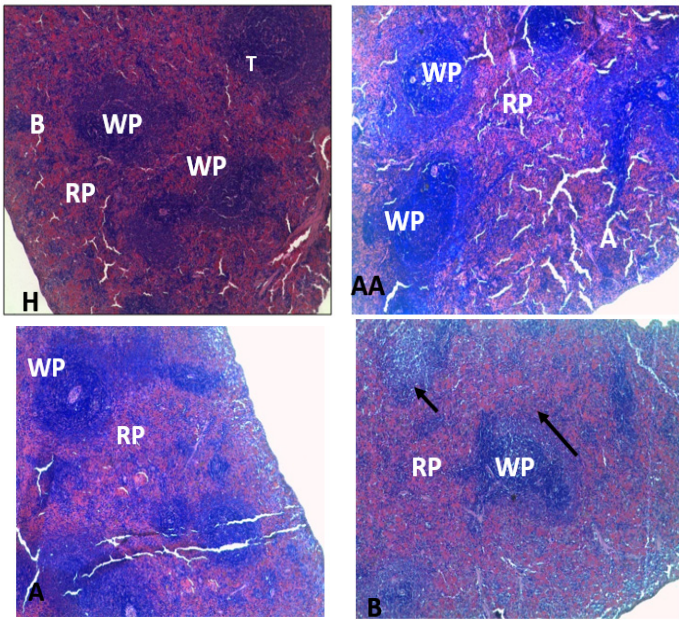

Figure 3a: Photomicrographs showing the spleen tissue of control and treated rats.
AA-(Control) rats fed with protein free diet [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1}; [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.

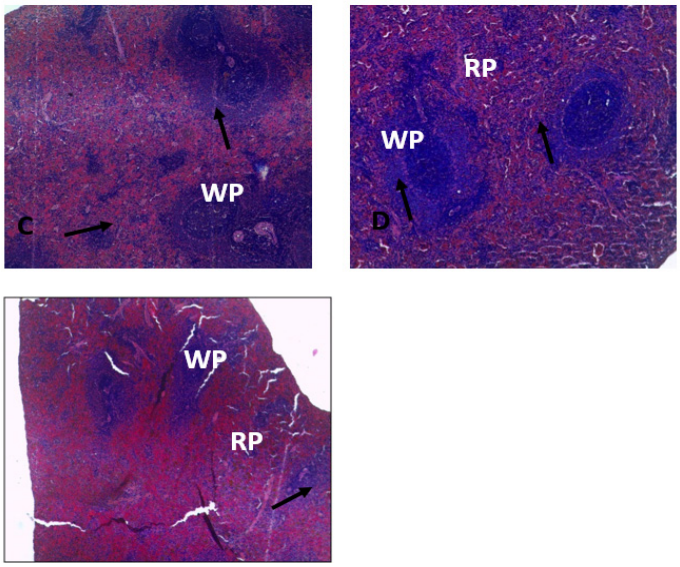

Figure 3b: Photomicrographs showing the spleen tissues of control and treated rats.
AA-(Control) rats fed with protein free diet, [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1}; [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.
RP: Red Pulp; WP: White Pulp.

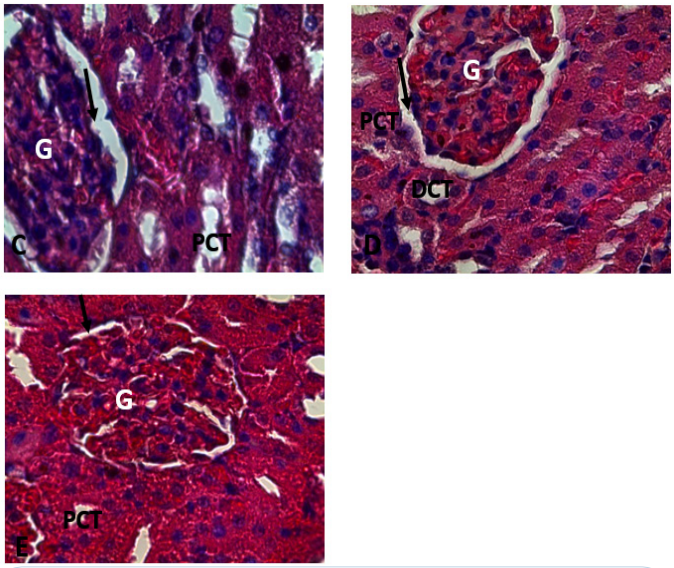

Figure 4b: Photomicrographs showing the kidney tissue of control and treated rats.
AA-(Control) rats fed with protein free diet, [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1}; [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.
G: Glomerulus; DCT: Distal Convoluted Tubule; PCT: Proximal Convoluted Tubule.

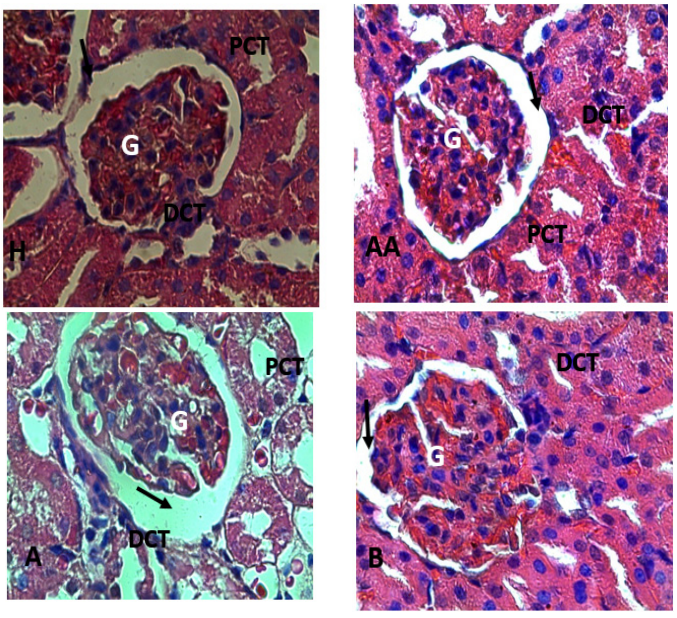

Figure 4a: Photomicrographs showing the kidney tissue of control and treated rats.
AA-(Control) rats fed with protein free diet, [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1}; [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.
G: Glomerulus; DCT: Distal Convoluted Tubule; PCT: Proximal Convoluted Tubule.

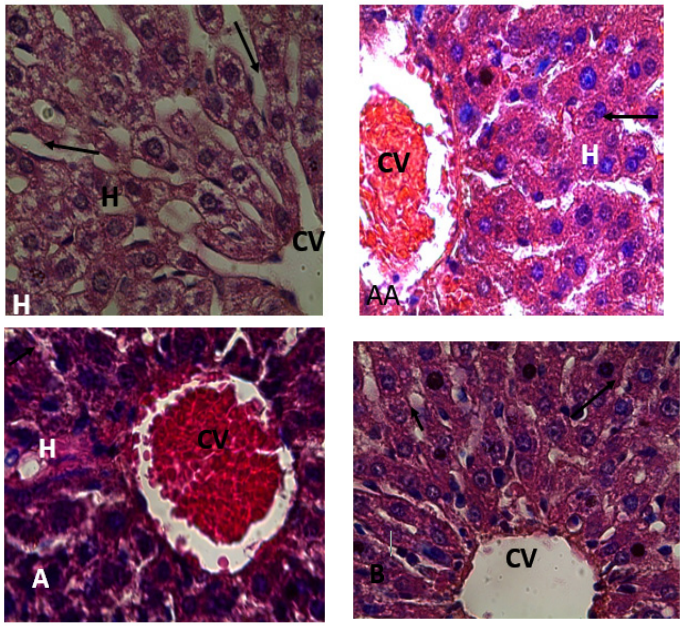

Figure 5a: Photomicrographs showing the liver tissue of control and treated rats.
AA-(Control) rats fed with protein free diet, [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1}; [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.
CV: Central Vein; H: Hepatocytes.

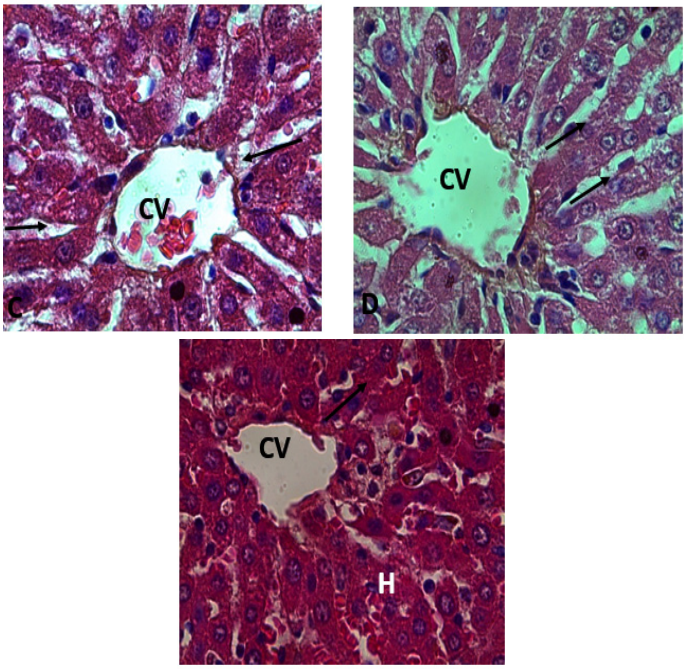

Figure 5b: Photomicrographs showing liver tissue of control and treated rats.
AA-(Control) rats fed with protein free diet, [A]-experimental rats fed with aflatoxin contaminated groundnut with AFB1 502 + AFB2 36.19 mg kg^{-1} [B]-Rats fed with aflatoxin free *kulikuli*; [C]-Rats fed with *kulikuli* containing 18.94 and 2.41 mg kg^{-1} of AFB1 and AFB2; [D]-Rats fed with *kulikuli* containing 48.73 and 4.93 mg kg^{-1} of AFB1 and AFB2; [E]-Rats fed with *kulikuli* containing AFB1 65.37 and 10.38 mg kg^{-1} of AFB1 and AFB2.
CV: Central Vein; H: Hepatocytes.

Table 1: Results of aflatoxin concentration (mg kg^{-1}) of *kulikuli* produced from uncontaminated groundnuts samples.

Temp/time	B1	B2	G1	G2
Control	138.17	24.34	0.00	0.00
120/30	0.00	0.00	0.00	0.00
120/50	0.00	0.00	0.00	0.00
120/70	0.00	0.00	0.00	0.00
120/90	0.00	0.00	0.00	0.00
120/110	0.00	0.00	0.00	0.00
120/130	0.00	0.00	0.00	0.00
140/30	0.00	0.00	0.00	0.00
140/50	0.00	0.00	0.00	0.00
140/70	0.00	0.00	0.00	0.00
140/90	0.00	0.00	0.00	0.00
140/110	0.00	0.00	0.00	0.00
160/20	0.00	0.00	0.00	0.00
160/30	0.00	0.00	0.00	0.00
160/40	0.00	0.00	0.00	0.00
180/10	0.00	0.00	0.00	0.00
180/20	0.00	0.00	0.00	0.00

Each value represents the mean of ten replicates ± standard error. Mean on the same column followed by the same superscript are not significantly different at 5% level of significance. B1: Aflatoxin B1; B2: Aflatoxin B2; G1: Aflatoxin G1; G2: Aflatoxin G2.

Table 2: Concentration (mg kg^{-1}) of aflatoxin in groundnut and *kulikuli* produced from groundnut roasted at 120°C.

Amount in raw seed			Whole seed		Dehulled		*Kulikuli*	
B1	B2	Time (min.)	AFB1	AFB2	AFB1	AFB2	AFB1	AFB2
501.99	36.19	30	361.66 ± 0.83^{a}	30.02 ± 0.46^{a}	275.44 ± 1.61^{a}	20.19 ± 1.81^{a}	188.15 ± 1.15^{a}	13.74 ± 0.16^{a}
501.99	36.19	50	309.68 ± 1.36^{ab}	26.08 ± 5.86^{ab}	248.07 ± 2.71^{ab}	18.71 ± 1.4^{ab}	154.31 ± 1.92^{ab}	13.15 ± 0.53^{a}
501.99	36.19	70	293.67 ± 1.46^{ab}	22.98 ± 0.07^{bc}	242.86 ± 1.49^{ab}	14.52 ± 0.46^{c}	125.18 ± 1.01^{b}	9.43 ± 0.45^{b}
501.99	36.19	90	282.97 ± 2.092^{ab}	21.10 ± 1.99^{bc}	221.01 ± 5.21^{b}	11.48 ± 423^{d}	71.43 ± 1.58^{c}	7.91 ± 0.48^{c}
501.99	36.19	110	232.64 ± 1.32^{ab}	20.25 ± 1.07^{b}	139.2 ± 0.3^{c}	9.58 ± 0.29^{c}	64.83 ± 1.48^{d}	6.56 ± 1.23^{d}
501.99	36.19	130	219.74 ± 2.98^{b}	19.18 ± 1.26^{ab}	111.35 ± 3.49^{d}	4.94 ± 1.54^{d}	56.57 ± 2.08^{e}	4.98 ± 0.17^{e}

Each value represents the mean of three replicates ± standard error. Mean on the same column followed by the same superscript are not significantly different at 5% level of significance. AFLB1: Aflatoxin B1; AFLB2: Aflatoxin B2.

Table 3: Concentration (mg kg^{-1}) of aflatoxin in groundnut and groundnut cake *kulikuli* produced from groundnut roasted at 140°C.

Amount in raw seed			Whole seed		Dehulled		*Kulikuli*	
B1	B2	Time (min.)	AFB1	AFB2	AFB1	AFB2	AFB1	AFB2
501.99	36.19	30	309.62 ± 1.39^{a}	25.66 ± 2.40^{a}	271.05 ± 2.39^{a}	19.00 ± 1.00^{a}	183.94 ± 1.77^{a}	13.42 ± 2.4^{a}
501.99	36.19	50	100.8 ± 2.82^{b}	21.32 ± 3.30b	87.78 ± 0.20^{b}	16.23 ± 0.54^{b}	65.37 ± 3.40^{b}	10.38 ± 0.25^{b}
501.99	36.19	70	109.62 ± 2.5^{bc}	19.85 ± 2.50^{c}	73.84 ± 3.17^{c}	9.92 ± 0.80^{c}	37.23 ± 2.23^{c}	5.64 ± 0.54^{c}
501.99	36.19	90	39.81 ± 1.31^{c}	17.61 ± 2.15^{d}	37.93 ± 0.82^{d}	5.44 ± 0.11^{d}	29.97 ± 0.54^{d}	4.49 ± 0.48^{d}
501.99	36.19	110	ND	ND	ND	ND	ND	ND
501.99	36.19	130	ND	ND	ND	ND	ND	ND

Each value represents the mean of three replicates ± standard error. Mean on the same column followed by the same superscript are not significantly different at 5% level of significance. ND: Not determined; AFLB1: Aflatoxin B1; AFLB2: Aflatoxin B.

Table 4: Concentration (mg kg^{-1}) of aflatoxin in groundnut and *kulikuli* produced from groundnut roasted at 160°C.

Amount in raw seed			Whole seed		Dehulled		*Kulikuli*	
B1	B2	Time (min.)	AFB1	AFB2	AFB1	AFB2	AFB1	AFB2
501.99	36.19	10	215.97 ± 0.7^{a}	21.53 ± 0.96^{a}	107.52±1.41^{a}	17.43±1.9^{a}	77.19±1.2^{a}	6.88±0.16^{a}
501.99	36.19	20	153.62 ± 4.33^{b}	18.80 ± 1.75^{b}	99.69±0.35^{b}	5.41±0.75^{b}	48.73±1.08^{b}	4.93±0.07^{b}
501.99	36.19	30	33.24 ± 0.55^{c}	7.23 ± 0.16^{c}	18.82 ± 0.31^{c}	3.87 ± 0.34^{c}	10.31 ± 0.42^{c}	0^{c}
501.99	36.19	50	ND	ND	ND	ND	ND	ND
501.99	36.19	70	ND	ND	ND	ND	ND	ND
501.99	36.19	90	ND	ND	ND	ND	ND	ND
501.99	36.19	110	ND	ND	ND	ND	ND	ND
501.99	36.19	130	ND	ND	ND	ND	ND	ND

Each value represents the mean of three replicates ± standard error. Mean on the same column followed by the same superscript are not significantly different at 5% level of significance. ND: Not determined; AFLB1: Aflatoxin B1; AFLB2: Aflatoxin B2.

Table 5: Concentration (mg kg^{-1}) of aflatoxin in groundnut and *kulikuli* produced from groundnut roasted at 180°C.

Amount in raw seed			Whole seed		Dehulled		*Kulikuli*	
B1	B2	Time (min.)	AFB1	AFB2	AFB1	AFB2	AFB1	AFB2
501.99	36.19	10	72.43 ± 9.36^{a}	6.33 ± 0.90^{a}	43.86 ± 2.70^{a}	4.7 ± 0.16^{a}	18.94 ± 0.02^{a}	2.41 ± 1.31^{a}
501.99	36.19	20	31.53 ± 0.41^{b}	6.23 ± 0.13^{b}	18.02 ± 0.33^{b}	3.35 ± 0.35^{b}	9.6 ± 0.42^{b}	0^{b}
501.99	36.19	30	ND	ND	ND	ND	ND	ND
501.99	36.19	50	ND	ND	ND	ND	ND	ND
501.99	36.19	70	ND	ND	ND	ND	ND	ND
501.99	36.19	90	ND	ND	ND	ND	ND	ND
501.99	36.19	110	ND	ND	ND	ND	ND	ND
501.99	36.19	130	ND	ND	ND	ND	ND	ND

Each value represents the mean of three replicates ± standard error. Mean on the same column followed by the same superscript are not significantly different at 5% level of significance. ND; Not Determined; AFLB1: Aflatoxin B1; AFLB2: Aflatoxin B2.

Conclusion

The study has established that unit operations such as dehulling, roasting of groundnuts at temperatures of 120, 130, 140 and 160 °C for 30 to 130 minutes and processing into *kulikuli* drastically reduced aflatoxin content of *kulikuli* product. It was also shown that the *kulikuli* contaminated with aflatoxin also affected weight gain by the animals as the animals fed with the aflatoxin laden product did not gain weight after 21 days of the study. Furthermore, the effect of consumption of contaminated feed on the vital organs of experimental animals were also elucidated as the architecture of the spleen, kidney and liver of rats exposed to highest level of AFB1 and AFB2 were distorted depicting the potentials of aflatoxins B1 and B2 as cancer causing agents. However, the tissues of vital organs of rats fed on aflatoxin-free diet and diet treated with lower level of AFB1 and AFB2 were majorly without distortions.

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References

1. ICRISAT. International Crop Research Institute for Semi-arid Tropics. 2009 Annual Report. Patancheru, India. 2009; 76.
2. Adeeko KA, Ajibola OO. Processing factors affecting yield and quality of mechanically expressed groundnut oil. J Agric Eng Res. 1990: 45: 31-43.
3. Bonku R, Yu J. Health aspects of Peanuts as an outcome of its Chemical Composition. Food Sci Hum Wellness. 2020; 9: 21-30.
4. Bankole SA, Ogunsanwo BM, Eseigbe DA. Aflatoxins in Nigerian dry-roasted groundnuts. Food Chem. 2005; 89: 503-506.
5. Okello KD, Biruma M, Deom CM. Overview of Groundnuts Research in Uganda: Past, Present and Future. Afr J Biotechnol. 2010; 9: 6448-6459.
6. Asibuo JY, Akromah R, Safo- Kantanka O, Osei Adu-Dapaah OS, Hans K, et al. Chemical Composition of Groundnut, Arachis hypogea (L) land races. Afr J Biotechnol. 2008; 7: 2203- 2208.
7. Ezekiel CN, Sulyok M, Warth B, Odebode AC, Krska R. Natural Occurrence of Mycotoxins in peanut cake from Nigeria. Food Control. 2012; 27: 338-342.
8. Ezekiel CN, Anokwuru CP, Fari A, Olorunfemi MF, Fadairo, et al. Microbiological Quality and Proximate Composition of peanut cake (Kulikuli) in Nigerian markets. Academ Arena. 2011; 3: 103-111.
9. Florkowski WJ, Shashidhara K. Aflatoxin control strategies in the groundnut value chain in Ghana. International Food Policy Research Institute Strategy Support Program. IFPRI Discussion Paper. 2014; 01369.

10. Rodrigues P, Soares C, Kozakiewicz Z, Paterson RRM, Lima N, et al. Identification and Characterization of Aspergillus flavus and aflatoxins in winemaking grapes. In: Mendes-Vilas A (ed.) Microbiology Book Series Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 2007; 2: 527-534.

11. Bennett JW, Klich M. Mycotoxins-A Review. J Clin Microbiol. 2003; 16: 497-516.

12. Yazdanpanah H, Mohammadi T, Abouhossain G, Cheraghali AM. Effect of Roasting on Degradation of Aflatoxins in Contaminated Pistachio nuts. Food Chem Toxicol. 2005; 43: 1135-1139.

13. Probst C, Bandyopadhyay A, Coty PJ. Diversity of Aflatoxin-producing Fungi and their impact on Food safety in sub-Saharan Africa. Int J Food Microbiol. 2014; 174:113-122

14. Cotty PJ. Comparison of Four Media for the isolation of Aspergillus flavus group fungi. Mycopathologia. 1994; 125: 157-162.

15. Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. Food and Indoor Fungi, (2nd ed.). Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. 2019.

16. Ogunsanwo BM, Faboya OOP, Idowu OR, Lawal OS, Bankole SA. Effect of Roasting on Aflatoxin content of Nigeria Peanut seeds. Afr J Biotechnol. 2004; 3: 451-455.

17. Adjoul E, Boniface S, Yehouenou C, Sossou M, Mohamed M, et al. Occurrence of Mycotoxins and associated Mycoflora in Peanut cake product (kulikuli) marketed in Benin. Afr J Biotechnol. 2012; 11: 14354-1436.

18. Garber RK, Cotty PJ. Formation of Sclerotia and Aflatoxins in developing cotton bolls infected by the S strain of Aspergillus flavus and potential for biocontrol with and atoxigenic strain. Phytopathology. 1997; 87: 940-945.

19. Krinke JG. The Laboratory Rat. Krinke, G.J. (ed). Academic Press, Tokyo pp. 2000; 253-283.

20. Drury RAB, Wallington EA. Carleton's Histological Technique. 5th ed. Oxford University Press, Oxford, UK. 1980.

21. Hasan K, Tamanna MN, Haque A. Biochemical and Histopathological profiling of Wistar rat treated with Brassica napus as a supplementary feed. Food Sci Hum Wellness 2018; 7: 77-82.

22. USDA (United States Department of Agriculture). Grain Fungal Diseases and Mycotoxin Reference Editor. United States Department of Agriculture. 2006; 1-51

23. EFSA (European Food Safety) : Opinion of the Scientific panel on contaminants in the food chain on a request from the Commission related to the Potential Health risk of the consumer by a possible increase of the existing maximum levels of aflatoxins in almonds, hazelnuts, pistachios and related products. EFSA J. 2007; 446: 1-127.

24. Hamada AS, Megalla SE: Effect of Malting and Roasting on reduction of aflatoxin in contaminated soybeans. Mycopathology. 1982; 79: 36.

25. Gong Y, Cardwell K, Hounsa A, Egal S, Turner PC. Dietary Aflatoxin Exposure and Impaired Growth in Young Children from Benin and Togo: a cross-sectional study. BMJ. 2002; 325: 20-21.

26. Rotimi OA, Rotimi SO, Duru CU, Ebebeinwe OJ, Abiodun AO, et al. Acute Aflatoxin B1 Induced Hepatoxicity alters Gene expression and disrupts Lipid and Lipoprotein Metabolism in Rats. Toxicol Rep. 2017; 4: 408-418.

27. Lorenz RG, Newberry RD. Isolated Lymphoid Follicles can function as sites for induction of Mucosal immune responses. Ann NY Acad Sci. 2004; 1029: 44-57.

28. McDonald KG, Leach MR, Huang C, Wang C, Newberry RD. Aging Impacts isolated Lymphoid follicle Development and Function. Comm and Aging Res. 2011; 8: 1-1.

29. Abdalla A, Hesham S, El-Deep S, Abdel-Raheim M, Salah S. Study of protective effect of date and Nigella sativa on aflatoxin B1 toxicity. Health Sci J. 2008; 2: 26-44.

30. Li H, Xing L, Zhang M, Wang J, Zheng N. The Toxic effects of aflatoxin B1 and Aflatoxin M1 on kidney through Regulating L proline and Downstream apoptosis. Biomed Res Int. 2018; 1-11.

31. Neal GE. Genetic Implications in the Metabolism and Toxicity of Mycotoxins. Toxicol Lett. 1995; 82: 861-867.