J. BIOL. ENVIRON. SCI.,
2009, 3 (9), 81-90

Anti-Aflatoxigenc Potentials of Two Nigerian Herbs on Albino Rats

Stephen Oyedele Fapohunda[1*], Tosin Akintewe[1], Ayodefi Olarinmoye[2] and Chinbudu Ngozi Ezekiel[1]

[1] Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Nigeria
[2] Department of Agriculture and Industrial Technology, Babcock University Ilishan Remo, Nigeria,

ABSTRACT

The anti-aflatoxigenic activity of methanolic and ethanolic extracts of *Phyllanthus niruri* and *Pseudocedrella kotschyi* were studied in male and female Wister albino rats fed with aflatoxin –contaminated chow (112ppb) for 20 d. Morbidity studies arising from the dietary aflatoxin were carried out. Packed Cell Volume (PCV), fasting blood sugar (FBS), haemoglobin concentration (HB), total white blood cells (WBC), and serum bilirubin (SB) were profiled.. Extracts from both herbs lowered the PCB, blood glucose, Hb, WBC and SB levels in the test male than in control at $P<0.05$ using the ANOVA test for significance. The ethanolic extracts of *Pseudocedrella* and the methanolic extract of *Phyllanthus* were the most effective anti-aflatoxigenic agents in the male as they reduced the blood parameter levels by 50% of the positive control. The results were not so in the females. Post-mortem examination revealed kidney discoloration and paleness, mild to moderate hepatomegaly and enlarged spleen in the positive control while test rats showed normal morphology for these organs. The 2 local herbs are good candidates for the management of aflatoxicosis in mammals.

Keywords: aflatoxin, *Pseudocedrella, Phyllanthus*, albino rats, chow.

INTRODUCTION

Aflatoxins, a group of zootoxic secondary metabolites produced by certain fungal species are known to contaminate items of alimentary importance. These extrolites contaminate, among others, stuff like maize, rice, groundnut, beans, and livestock feed (Wang and Liu 2006;Trung *et al* 2008; Zinedine *et al* 2008) all of which are common in tropical African countries.

Aflatoxin B1, the most toxic of the group of 4 (i. e B1, B2, G1, G2) has been listed as a type 1 carcinogen by the International Agency for the Research on Cancer, an arm of the WHO. When consumed by rodents, expression of pathogenicity can be in the form of toxic abnormalities and organ malfunction including interference with the metabolism of amino acids and vitamin B complex (Gimeno and Martins, 2000). Other noticeable clinical signs on rodents include renal, spleen, liver and pulmonary congestions. Increasing interest in diverse forms of aflatoxicoses arising from dietary aflatoxin has led to a search for possible user-friendly intervention strategies. Various forms of physical, chemical and biological methods have different levels of efficiency even though 2 or more of these steps are sometimes combined in both household and municipal aflatoxin management. Biological control involving " fungus-fight" in which the predator is applied as the biopesticide is fast gaining ground (Yin et al 2008) although the overall economic advantage to an average farmer has always been a subject of concern.

Exploiting herbs to mitigate dietary aflatoxin production and action sometimes become challenging due to regular detection of the toxin in some medicinal herbs (Polp and Che, 2006; Rizzio *et al*, 1998; Arranz *et al* 2006; Macdonald and Cartle 1996) and figs (Bircan, 2009). It is however gratifying that some other herbs have been implicated in effective control of livestock feeds. These include *Scutellaria balcalensis* (Boer *et al*, 2005), *Agave* species (Sanchez *et al* 2004), Thai medicinal plants (Vinithetkunen *et al*, 1999), *Curcuma longa* (Gowda *et al* 2008), *Cinnamomon zeylanicum* bark, *Zingiber officinale* rhizome and *Salvia officinales* leaves (Mohammed and Metwally 2009). *Pseudocedrela kotschyi* has been implicated in inhibiting the growth of *Plasmodium falciparum* (Kassim *et a l* 2009), as an antiprotozoal compound (Hay *et al* 2007) and general antimicrobial compound (Ogundiya et al 2006). Ethanolic extract of Ajuwan (*Trachyspermum ammi*) has been successful in treating ochratoxin contamination in Turkey (Marty *et al*, 2009). All these successful forays into antiaflatoxigenic activity by some plants has led to a search for more readily available and affordable species in tropical countries.

It is recalled that the health scare inherent in the consumption of aflatoxin contamination has forced continental bodies like the European Union to put in place some of the strictest standards for levels in foods and feeds (Wu, 2008).It has become necessary to look inward and identify plant species which can alleviate aflatoxin prevalence in agricultural commodities leading to improved human health and enhanced export value.

* Corresponding author: oystak@yahoo.co.uk

The aim of this the present study was to explore the possibility of applying extracts of 2 Nigerian herbs=*Pseudocedrela kotschyi* and *Phyllanthus niruri* in the management of dietary aflatoxin in animal feed with reference to albino rats.

MATERIALS AND METHODS

Two medicinal herbs (*Pseucocedrela kotschyi* and *Phyllanthus niruri*), were investigated for their antiaflatoxic activity on dietary aflatoxin fed to Wister strain Albino rats.

Plant Collection (Phyllanthus niruri and Pseudocedrela kotschyi)
Fresh plants of *Phllanthus niruri* also referred to as *Chanca piedra* (Yor. Ewe olobe) were collected in April 2008 around the School of Science and Technology complex as well as from Rigmor Nyberg and Ogden female hall of residence region in Babcock University. These plants were allowed to air dry for one month before the aerial parts were homogenized to fine powder with a Binatone BLG-401 mechanical blender. They were then stored in airtight containers.

Dried stem cuttings of *Pseudocedrela kotschyi* (Yor. Emi gbegiri) were purchased from Iyana Ipaja in Lagos State, Nigeria. The stem was ground with a mortar and pestle before it was then homogenized into fine powder with a mechanical blender. This was stored in airtight containers till further use.

Solvent Extraction
One hundred grams of the ground each plant materials were extracted with 1000ml of methanol of reagent grade. This was done with the aid of gentle heat using a water bath that was maintained at a temperature of 50-60^{0}C for 4 hours to permit full extraction of the active ingredients. Stirring was done every hour with a glass rod to ensure even distribution of heat.

Filtration was later carried out inside conical flasks using glass wool immersed into funnels. The extracts were then stored in clean bottles at low temperature (Parekh *et al*., 2005).

The extracted solutions were rotary dried using a rotary evaporator Bibbly Rotary evaporator RE100 in order to obtain the concentrate and dried using SB4 Freeze Dryer. Prior to use, the dried samples were dissolved each in 1000ml of distilled water and stored in a refrigerator..

Defatting and Extraction
The dried powdered plant materials (50g each) were extracted first with 250ml each of n-hexane and allowed to stand for ten minutes before filtering out the supernatant. This was done to remove fats. The defatted powders of the two plants were separately macerated in 500ml of 90% ethanol for 48 hours. Filtration was also carried out after to obtain the extracted material. The hydroalcoholic solution was concentrated to dryness under a vacuum using a rotary evaporator and freeze dryer, dissolved in 1000ml distilled water and stored under low temperature (Ngane *et al*., 2000; Bonjar, 2004).

Chow Preparation
The rat chow was specially formulated as shown in table 1

Table 1: Diet Formulation used in the preparation of the rat chow.

Ingredients	Quantity (Kg)
Maize	2.6
Soybean meal	1.24
Groundnut cake	1.01
Sodium chloride	0.07
Bone meal	0.05
Iron tabs	0.005
Layer's premix	0.025

The crop components of the formulation readily constitute substrates of aflatoxin in store and the choice of the chow formula was informed by season, availability, market prices and health of the rodents.

The source of the aflatoxin used on the rat was from aflatoxin-contaminated maize obtained from Babcock university farm house and also from groundnut cake purchased from a feed mill at Ijebu-Ode, Ogun State, Nigeria. The various ingredients were measured in the right proportion, ground and the aflatoxin content analysed. Using the ELISA AgraQuant total Aflatoxin assay 4/40 kit for analysis, the complete ration had a contamination level of 112 parts per billion (ppb) by the time it was fed to the test animals.

Test Animals

Eleven week- old, healthy Wister strain albino rats were obtained from a private breeder in the University of Ibadan, Oyo state, Nigeria and acclimatized in a pathogen free well ventilated room in the animal house of Babcock University for one week. They were housed in special wooden cages with wood shaving beds. Each cage had a roll of four cells such that each cell housed one rat to avoid overcrowding and ensure free space. Supply of food, water and light was regulated to avoid malnutrition during the acclimatization period and the cages were cleaned every two days to avoid any likelihood of infection posed by lice and houseflies.

Treatment Schedule

The rats numbering 30 were randomly allocated to five groups each. The treatment groups were as follows;

- Control – Aflatoxin contaminated chow and water without extract
- Treatment 1 – Aflatoxin contaminated chow with *Phyllanthus niruri* methanolic extract
- Treatment 2 – Aflatoxin contaminated chow with *Phyllanthus niruri* ethanolic extract
- Treatment 3 – Aflatoxin contaminated chow with *Pseudocedrela kotschyi* methanolic extract
- Treatment 4 – Aflatoxin contaminated chow with *Pseudocedrela kotschyi* ethanolic extract

There were replicates for each of the five groups based on sex differences, that is each group had either all male rats or all female rats.

The following abbreviations were adopted for ease of observation

CEM – *(Phyllanthus* **E**thanolic **M**ale), CMM – (*Phyllanthus* **M**ethanolic **M**ale), PEM – (***P****seudocedrela* **E**thanolic **M**ale), PMM – (***P****seudocedrela* **M**ethanolic **M**ale, CEF – (*Phyllanthus* **E**thanolic Female) CMF – (*Phyllanthus* **M**ethanolic **F**emale), PEF – (***P****seudocedrela* **E**thanolic Female), CMM – (*Phyllanthus* **M**ethanolic Female)

After the acclimatization period, the rats in each group were served 10 grams of the aflatoxin contaminated chow daily (five grams in the morning and five grams in the evening) along with 2mls of the plant extract per meal (each treatment group was administered its respective plant extract) with the aid of clean syringes and intubator. Distilled water was supplied *ad libidum*.

Preparation Of Extract

The powdered extracts were dissolved in 1Litre volume of distilled water to make a final solution. This was carried out as follows:

The extract for Treatment 1 was prepared by weighing 8grams of the powdered methanolic extract *Phyllanthus niruri* in 1 litre of distilled water giving a final concentration of 0.008 grams per litre.

Treatment 2 included dissolving 2grams of powdered ethanolic *Phyllanthus niruri* extract in 1 litre of distilled water to give a final concentration of 0.002grams per litre.

Treatment 3 included dissolving 11.5 grams of powdered methanolic extract of *Pseudocedrela kotschyi* in 1 litre of distilled water that is 0.0115grams per litre.

Treatment 4 included dissolving 4.5 grams of the powdered ethanolic extract of *Pseudocedrela kotschyi* in 1 litre of distilled water to give a final concentration of 0.0045 grams per litre.

On the 20-day of treatment, the rats were sacrificed through cervical dislocation. Using a sterile surgical blade, the skin of the each rat was carefully removed by cutting right from the posterior end above the external reproductive area and gradually towards the anterior end. For analyses, the following organs were harvested from each group; liver, lungs, heart, kidneys, and spleen and were preserved at low temperatures. Also the blood sample was taken for analysis.

Blood Sample Collection And Analysis

Two mls of blood samples were collected from rats within the various treatment groups and the control group using sterile syringes and needles. The blood was withdrawn with minimum stasis from a suitable vein in the forelimb, or neck (jugular vein or carotid artery), or directly from the heart and was slowly poured into

sample bottles. For each of the groups, blood samples were pooled using either of the two types of anticoagulants. The anticoagulants were lithium heparin and floride oxalate. The rationale behind the use of two anticoagulants being that one is more suitable than the other, that is, the bottle having fluoride oxalate was used for the estimation of blood sugar level while the lithium heparin has been found to be suitable for the determination of the haemogram. Heparin is found in the blood and it acts as an anticoagulant in the body, thus helping to maintain the normal physiology of the blood. This is why it is used as an anticoagulant in sample bottles used for blood collection. Fluoride oxalate is used for blood when glucose level is to be estimated because it helps to halt the process of glycolysis in the blood once it is withdrawn from the organism With this, the correct amount of glucose level can be obtained without any interference.

The haemogram data included the Packed Cell Volume (PCV), Red blood cell (RBC) count, Haemoglobin concentration, White Blood Cell (WBC) count. Blood glucose level and Total bilirubin was also analyzed. The PCV was determined using the haematocrit method by using an automated analyzer (Haematology Autoanalyzer HB 7021on the heparinized blood which also determined the total white blood cell and red blood cell count as well as the haemoglobin concentration.

The total bilirubin in the blood was screened by first centrifuging the blood to separate the serum from the cells. The test was carried out on the serum. The appropriate amounts of reagents, sample blank and samples to be used were carried out according to the Randox Bilirubin (BIL) Manual. These were then mixed and allowed to stand for 20 minutes at a temperature of 20-25^{0}C. The absorbance of the sample was then read against the sample blank at 578nm.

Blood glucose analysis was also carried out on the serum according to the user manual. Samples were then incubated for 10 minutes at 37^{0}C and read at 560nm in a spectrophotometer (UNISPEC 230).

Liver Analysis

The liver of each treatment and control group was analysed for the level of aflatoxin in them and this was carried using the AgranQuant Total Aflatoxin Assay (Romer AgraQuant Total Aflatoxin Assay 4/40 Test Kit) which is a direct competitive enzyme-linked immunosorbent assay (ELISA).

The procedure is carried out by grinding the pooled liver using a mortar and pestle. The desired weight is measured by first weighing the empty container before adding the liver. The initial weight is then subtracted from the final weight to get the liver. The weights for each group used are stated in Table 2.

Table 2: Mean weight of liver from each treatment and control group used for the aflatoxin analysis.

Groups	Mean Weight (g)
Control Female	5.69
Control Male	6.0
CEF	5.17
CEM	5.46
PEM	4.70
PEF	5.38
PMF	6.79
PMM	4.38
CMM	5.05
CMF	4.81

After the required weight of each pooled liver is obtained, 70% methanol was then added to each at the ration of 1:5w/v and allowed to stand for a period of 10 minutes. The extracted sample which is the top layer was filtered in a funnel and the filtrate containing the aflatoxin was collected in a clean jar.

Liver Assay

This involved the use of the AgraQuant Test kit to carry out the analysis of the liver following the instructions on the kit, the components (reagents) of which were maintained at room temperature (18^{0}C-30^{0}C) before use.. A color change from blue to yellow was noted to show that a reaction took place. Finally,

the strips were read with a microwell reader (using a 450nm). The optical density reading for each microwell was recorded.

Statistical analyses were carried out on the blood parameters, liver and weight gain to analyze the differences between the treatment and control groups by means of the ANOVA test of significance at P value ($P>0.05$) level.

RESULTS AND DISCUSSION

Throughout the period of the experiment, the average weight of the rats in each experimental and control group increased as shown in Fig 1. This was agreement with the result of Al -Habib (2007) who reported that weight gain was attributed to fluid retention which is a direct follow-up to renal damage. Profound anaemia was expressed among the control group as compared with the groups exposed to extracts. At sacrifice, the organs (spleen, liver, kidneys, and lungs) from the control group looked pale and congested also resulting in significant increase in weight (Gimeno and Martins 2000). There were also unique abnormalities in the lungs as some appeared very pale with discrete areas of pin-point and paint brush haemorrhages. Other expressions were emphysema with bullae formation in the dorsal and cordo-ventral aspect of the lateral and medial surfaces in the apical cardiac and diaphragmatic lobes. Apart from that, they were also uniform congestion indicating possible inflammation of the lungs. The spleens appeared darkened and swollen with blunt edge.

In Fig 2 and 3 PCV and glucose level were observed. Fig. 4 expressed the haemoglobin profile in the animals, while the total white blood cell counts are presented in Fig 5. In Fig. 6 Total bilirubin level for the males and females respectively are shown. Figures 7 (a and b) and 8 (a and b) also show a physical observation of some of the spleen and lungs.

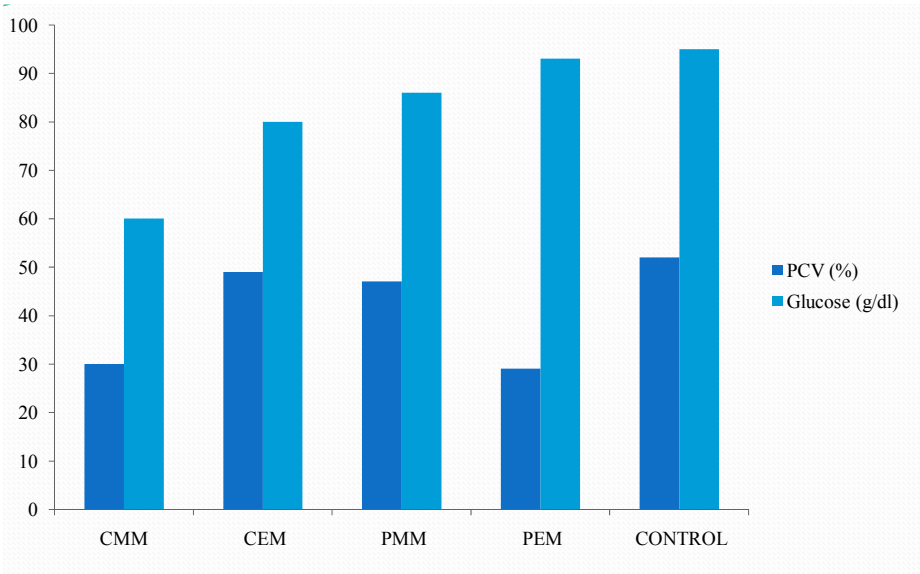

Figure 1: PCV and Blood glucose level in male rats

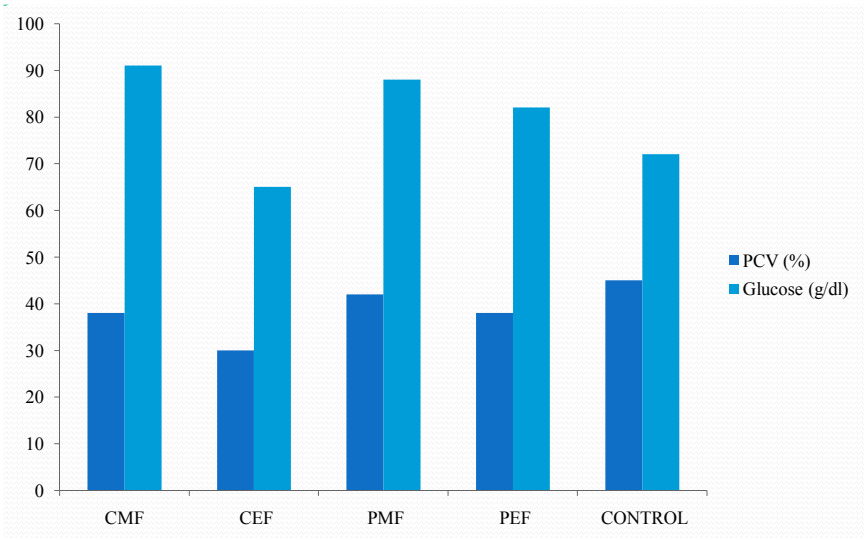

Figure 2: PCV and Blood glucose level in female rats

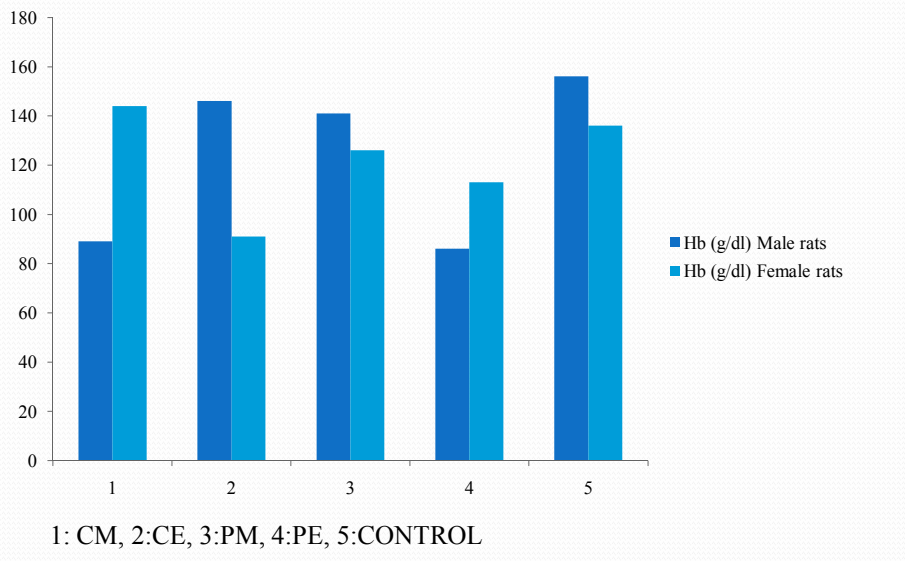

Figure 3: Hb concentration in male and female rats

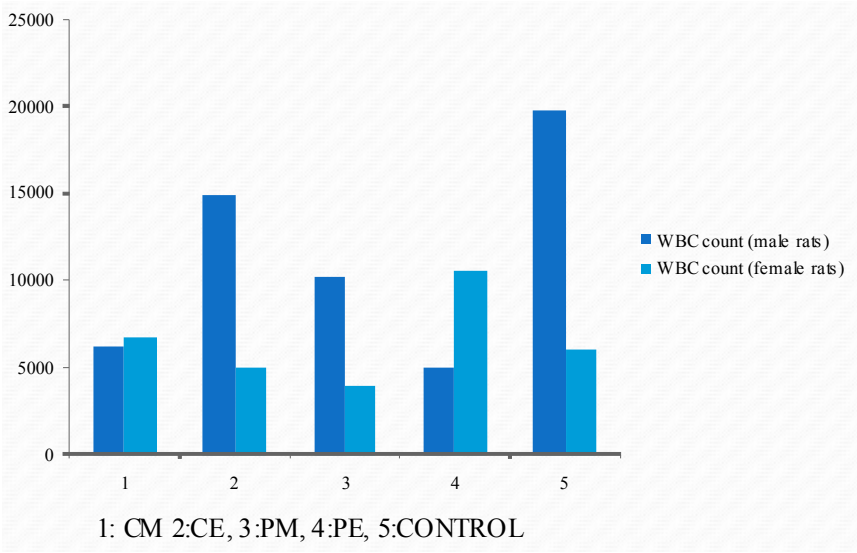

Figure 4: WBC count in male and female rats

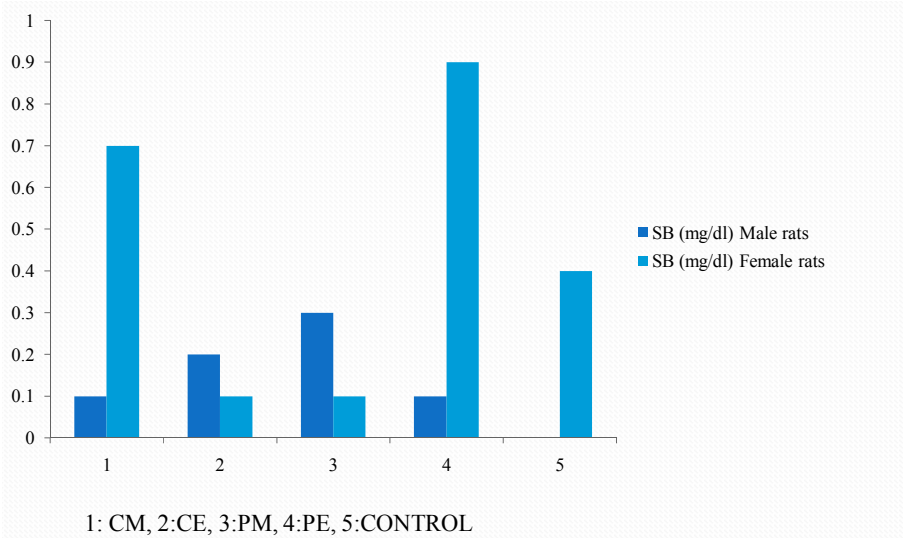

Figure 5: SB Concentration in maleand female rats

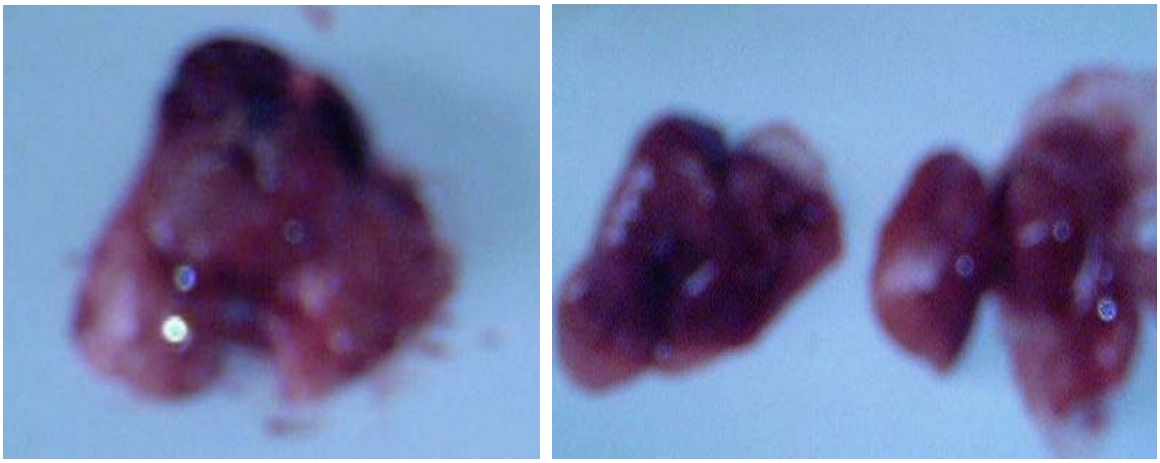

Figure 6 a and b. Decolorized lungs with paint brush haemorrhages

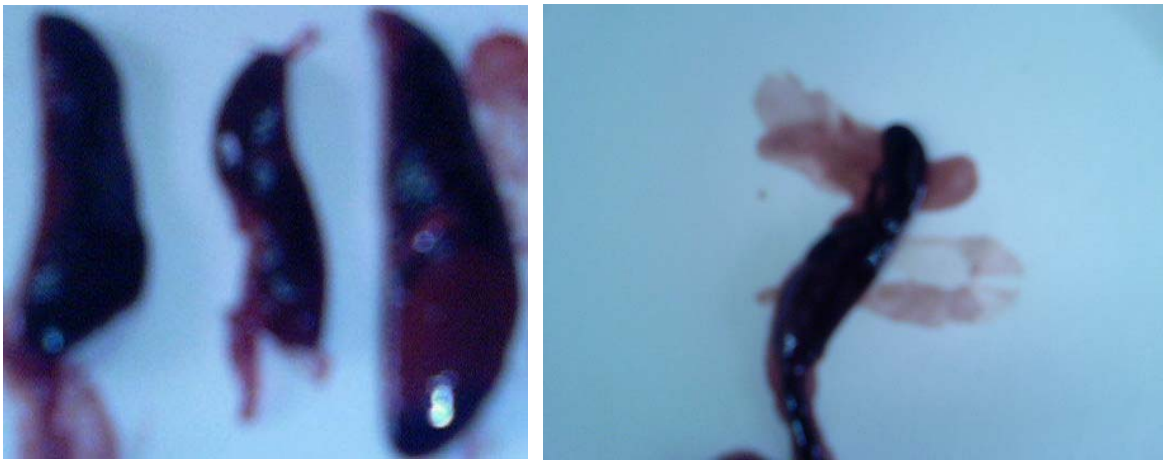

Figure 7 a and b. Darkened and swollen spleen indicating congestion.

Table 3: Aflatoxin levels in parts per billion (ppb) in the liver of the different treatment and control groups in both male and female rats.

Sample	Optical Density (nm)	Aflatoxin level (ppb)
Control male	2.26	1.291
Control Female	2.367	0.833
CMM	1.986	2.61
CEM	2.29	1.25
PMM	2.132	1.876
PEM	2.251	1.296
CMF	2.134	1.875
CEF	2.353	0.838
PMF	1.977	2.625
PEF	1.86	3.3

Based on the ANOVA test of significance at P value ($P<0.05$), it can be shown that among the males, considering their PCV, there was a significant difference in PEM, PMM and CMM when compared to the control. The PCV in the control rat when compared with the other treatment groups for instance is very high, presumably because of the intake of the toxin. PCV is a measure of the relative mass of erythrocytes present in a sample of whole blood. Also for the haemoglobin concentration and WBC count, there was a significant

difference in all the treatment groups. Corn one of the ingredients for the chow is probably the commodity of greatest worldwide concern because it is grown in climates that are likely to have perennial contamination with aflatoxin (Frazier & Westhoff, 1998).

Statistical analysis could not be carried out on the bilirubin level of the males. This was due to insufficient blood recorded in their system which was aggravated by clot (Bababunmi and Bassir 1969). The present result showed that the methanolic and ethanolic extracts of vthe tested herbal plants had a positive effect on in reducing the aflatoxic activity in the blood and l;iver with some level of significance when compared with the control. Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. Bilirubin metabolism begins with the breakdown of red blood cells. Red blood cells contain hemoglobin, which is broken down to heme and globin. Heme is converted to bilirubin, which is then carried by albumin in the blood to the liver. In the liver, most of the bilirubin is chemically attached to another molecule, making them water soluble before it is released in the bile. Conjugated bilirubin is released into the bile by the liver and stored in the gallbladder, or transferred directly to the small intestines. Bilirubin is further broken down by bacteria in the intestines, and those breakdown products contribute to the color of the feces. A small percentage of these breakdown compounds are taken in again by the body, and eventually appear in the urine. An increase in bilirubin concentration in the serum or tissue is an indication of liver problem which results into a condition called jaundice. Evident liver damage in untreated rats was an expression of increased cellular activity of the hepatic enzymes (Tripathi and Mishra 2009). Mehta *et al* (1993) had earlier reported an acute hepatic response in rats fed with aflatoxin B –laden feed.

The methanolic and ethanolic extracts of the tested herbal plants had a positive effect on the level of aflatoxin in the blood and liver. The effect of these extracts can be seen in those that showed a level of significance when compared with the control. It is noted however that at least one of the extracts gave a positive result in some cases while in others, both forms of the extracts worked. Therefore, exposure of such rats for such longer periods can lead to a greater rise in these parameters beyond their critical values and finally, subsequent death of the rats.

The observations reported on rise in these blood parameters (as seen in the control) are in accordance with previous reports on aflatoxin-mediated sperm and blood cell abnormalities in mice fed with contaminated corn (Fapohunda *et al*., 2008).

The blood glucose was high without plant extracts except one treatment group (in the males). When the blood glucose rose above the set point, more insulin was secreted from the pancreas but there cannot be a breakdown in this system like any other system. The pancreas either cannot secrete insulin if the target cells lost their responsiveness to insulin, then blood glucose reaches dangerously high levels in the control as compared to the other treatment groups. *Chanca* was reported to assist in reducing the level of blood glucose in man (Muller 2009) and could therefore be instrumental in curtailing experimental diabetes in rats.

The total bilirubin in the control is also high as compared to the treatment groups particularly in the females. This probably implies the adverse effect of the aflatoxin in the spleen and liver as noted in their darkened and swollen appearance signaling possible congestion. The observation was instructive as it has earlier established a link between aflatoxin B1, rise in alkaline phosphatase activity and bilirubin concentration (Clifford and Rees 1967). The white blood cell count shows a statistically significant increase in the control than the treatment groups. WBC is responsible for both specific and non-specific immunity. These cells increase when there is an infection, they cooperate with each other to recognize and destroy the pathogen. An increase of the WBC in the control group signified the recognition of dietary aflatoxin as a foreign body thus enhancing it s production and invasion of the antigen.

This study is considered significant in Nigeria and other countries with similar climate and agro-focus, where consumption of foods like corn, and peanuts is very high and storage facilities are grossly inadequate together with other factors which favour the growth of the toxigenic organisms. It is also relevant to human health because the toxin is not only capable of inducing liver cancer but also other health problems as the target of the aflatoxin is the DNA (Deoxyribonucleic acid) which exists in all cellular forms. A window of research opportunity is open in the pharmaceutical exploitation of these 2 readily accessible and affordable plant materials in alleviating contamination in stored food and feed moreso when extracts of other African herbs like *Nigella sativa* and *Syzygium aromaticum* have proven effective in protecting against aflatoxicosis (Abdel- Wahab and Aly, 2005). The present products may also be handy in treating cases of aflatoxicosis.

It is hoped that the research will enhance advancement in the new paradigm shift as expressed at the 8th International Mycological Congress (IMC 8 Caines, Australia) where 2 critical issues were addressed: the utility and importance of secondary metabolites and their genes in polyphasic taxonomy, phylogeny and evolutionary history of kingdom Fungi and two, the genetic processes regulating secondary metabolites synthesis (Stadler and Keller, 2008)

REFERENCES

Abdel Wahab M A, Nada S A and Khalid F A (2002). Physiological and toxicological responses in rats fed aflatoxin contaminated diet with or without sorbent materials. Animal Feed Science and Tech 97 (3-4) 209-219.

Abdel Wahab M A and Aly S E (2005). Anti oxidant property of *Nigella sativa* (black cumin) and *Syzygium aromaticum (*clove) in rats during aflatoxicosis J Appl Toxicol 25 (3) 218-228.

Al Habib M F, Jaffar A A and Abdul Ameer H H (2007). Aflatoxin B1-induced kidney damage in rats. J Fac Med. Baghdad. 49 (1) 147-149.

Arranz I, Sizoo E, van Egmond H, Kroeger K, Legarda T M, Burdaspal P Reif K and Stroka J (2006) Determination of Aflatoxin B1 in medicinal herbs: inter-laboratory study. Journal of AOAC International 89 (3) 595-605.

Bababunmi, EA and Bassir 0., (1969). The effects of aflatoxin on blood clotting in the rat. Brit. J. Pharmacol. 37, 497-500.

Bircan C (2009). Incidence of ochratoxin A in dried fruits and co-occurrence with aflatoxins in dried figs Food Chem Toxicol 47 (8):1996-2001.

Boer J G, Quiney B, Walter P B, Thomas C, Hogson K I, Murah S J and Saxena P K (2005). Protection against aflatoxin B1- induced liver mutagenesis by *Scutellaria balcalensis* Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 578 (1-2) 15-22.

Bonjar GH (2004). New Approaches in Screening for Antibacterials in Plants. *Asian Journal of Plant Sciences*. 3 (1): 55-60.

Clifford J I and Rees K (1967). The action of aflatoxin B1 on the rat liver. Bioch. J. 102 (1) 65-75.

Fapohunda SO, Ezekiel CN, Alabi A, Omole A. and Chioma, S. 2008. Aflatoxin-mediated Sperm and Blood Cell Abnormalities in Mice Fed with Contaminated Corn. *Mycobiology*. 36 (4):255-259.

Gimeno A and Martins M L (2006). Mycotoxin and Mycotoxicosis in animals and humans special Nutrients Inc Miami FL 127pp.

Gowda NKS, Ledoux DR, Rottinghaus GE, Bermudez AJ and Chen YC (2008). Efficacy of Turmeric (*Curcuma longa*) containing a known level of curcummin and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks poultry Science 87: 1125-1130.

Hajare SS, Hajare SN and Sharma A (2005). Aflatoxin inactivation using aqueous extracts of Ajowan (*Trachyspermum ammi*) seeds J. Fd Sc.

Hay AE, Iaset JR Ahua KM, Diallo D, Brun R and Hostettman K (2007). Limonoid orthoacetates and antiprotozoal compounds from the roots of *Pseudocedrela kotschyi.* J Nat Prod 70 (1) 9-13.

Kassim OO, Loyevsky M, Amonoo H, Lashley L, Ako- Nai KA and Gordeuk VR (2009). Inhibition of *in vitro* growth of *Plasmodium falciparum* by *Pseudocedrela kotschyi* extract alone and in combination with *Fagara zanthyloides* extract. Trans. R. Soc. Trop. Med. Hyg. 103 (7) 698-702.

McDonalds S and Castle L (1996). A UK retail survey of aflatoxins in herbs and spices and their fate during cooking. Food Additives Contaminants 13 (1) 121-128.

Mehta R, Campbell JS, Layer GW, Stapley R and Mueller R (1993). Acute hepatic response to aflatoxin B1 in rats fed a methyl-deficient, amino acid defined diet Cancer Letters 69: 93-106.

Mohammed AM and Metwally NS (2009). Antiaflatoxigenic activities of some plant aqueous extracts against aflatoxin B1 induces renal and cardiac damage. J Pharm Toxicol 4 (1) 1-16.

Muller V 2009 breakstone, Chanca piedra promotes optimal liver, kidney and gall bladder functions www.wholeworlbotanicals.com/herbs_breakstone.

Murthy PS, Borse BB, Khanum H and Srinivas P (2009). Inhibitory effects of Ajowan (*Trachyspermum ammi*) ethanolic extracts on *A. ochraceous* growth and ochratoxin production. Turk Biol 33: 1-7.

Musaiger A, Al-Jedah JH and D' souza R (2008). Occurrence of contaminants in foods commonly consumed in Bahrain. Food Control:19 (9) 854-861.

Ngane NN, Biyiti L, Zollo PH & Bouchet PH (2000). Evaluation of antifungal activity ofextracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Guill. et Perr. and *Zanthoxylum xanthoxyloides* Waterm. *Journal of Ethnopharmacology*. 70: 335-342.

Ogundiya MO, Okunade MB & Kolapo AL (2006). Antimicrobial Activities of Some Nigerian Chewing Sticks. *Ethnobotanical Leaflets*. 10: 265-271.

Parekh J, Jadeja D and Chanda S (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *Turkish Journal of Biology*. 29: 203-210.

Polp S and Che CT (2006). Determination of aflatoxins in Chinese medicinal herbs by high performance liquid chromatography using immuno- affinity column clean-up: Improvement of recovery. J. Chrom A 1135 (2) 241-244.

Rizzio I, Varsavsky E, Vedoya G, Haiokowski M, Frasde H and Chiale C (1998). Fungal and aflatoxin contamination of medicinal herbs Mycotoxin Research 14 (2) 46-53.

Sanchez E, Heredia N and Garcia S (2004). Inhibition of growth and mycotoxin production of *Aspergillus flavus* and *Aspergillus parasiticus* by extracts of Agave species Int. J Fd Microb. 98 (30 271-79.

Stadler M and Keller NP (2008). Paradigm shift in fungal secondary metabolite research. Editorial to special issue. Mycol. Research.112: 251-270.

Tripathi S and Mishra HN (2009). Effect of feeding enzymatically detoxified aflatoxin B diet as liver function test of Wistar rats. Nutition and Food Science 39 (3) 235-248.

Trung TS, Tabuc C, Baily S, Querin A, Guerre P and Bailly JD (2008). Fungal flora and contamination of maize from Vietnam with aflatoxin B1 and Fumonisin B1 World Mycotoxin Journal 1 (1) 87-94.

Vinitketkurnuen U, Chewonarin T, Dhumtanon P, Lertprasertsuk N and Wild C (1999). Aflatoxin-albumin adduct formation after single and multiple doses of aflatoxin B1 in rats treated with Thai medicinal plants. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 428 (1-2) 345-351.

Wu F (2008). A tale of two commodities: how EU mycotoxin regulations have affected US tree nut industries. World Mycotoxin Journal 1 (1) 95-102.

Yin Y, Yan L, Jiang J and Ma Z (2008). Biological control of aflatoxin contamination of crops. J Zheijang Univ Science B 9 (10) 787-792.