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Aflatoxincontent of Three Condiments and Their Effects on Some Biochemical and Haematological Parameters in WistarRats

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Abstract:*Condiments as spices are widely used in Nigeria, they provide taste and flavour to meals. In the present study, these condiments; dadawa, ogiri, and okpehewere estimated for aflatoxin contents, their phytochemicals and effects on some biochemical and haematological parameters were also carried out. Twenty four Wistar rats of body weight between 130 – 180 g were randomly distributed into four groups of six rats each; Group 1 were control rat which received 0.5 ml of distilled water per day while Groups 2, 3 and 4 received 400 mg/kg bw. aqueousextracts of dadawa, ogiri and okpehe respectively for 21 days. Phytochemical screening reveals the presence of alkaloids, flavonoid, terpenes and steroids while tannins and balsams were not detected in all condiments. Okpehe has the highestaflatoxin content per kg followed by dadawa and ogiri respectively. Aqueous extracts significantly (P<0.05) decrease White bloodcells (WBC) in all treated rats while no significant effects (P>0.05) were observed in monocytes of dadawa and ogiritreatedwhen compared to the control. Total protein was significantly decrease (P<0.05) in all treated groups and in albumin content of Ogiri and Okpehe. Also, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed significant increase (P<0.05) in all treated groups when compared to the control.Removing aflatoxin from contaminated food and foodstuffs remains a major problem, thereforeaGood Manufacturing Practice (GMP) is highly encouraged in production of these condiments aswellas a close monitoring of their storage and shelf life.*

Keywords: *Aflatoxin, dadawa, haematological parameter, ogiri, okpehe, phytochemicals*

I. Introduction

Mycotoxins are secondary metabolite produced by organisms of the fungus kingdom commonly known as moulds [1]. The term is usually reserved for the toxic chemicals produced by fungi that readily colonize crops. Aflatoxins are a major type of mycotoxins produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*[2]. There four types of aflatoxins produced by mycotoxins, which are B1, B2, G1 and G2 [3]. AflatoxinB1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer in the tropics and subtropics[3].

Aflatoxins-producing Aspergilluses are common and widespread in nature. They colonize and contaminate grains before harvest or during storage. Crops which are frequently affected include cereals (maize, sorghum, millet, rice, and wheat), oil seeds (groundnuts, soyabean, sunflower, and cotton), spices (chilli pepper, coconut,Brazil nut), toxins can also be found in milk of animals which arefed with contaminated feed [4]. Favourable conditions for widespread include high moisture content and high temperature [2].

Animal species are not immune to acute toxic effects of aflatoxins including humans; however we, humans have an extraordinary high tolerance for aflatoxin exposure and rarely succumb to acute aflatoxicosis[5]. Acute aflatoxicosis, associated with extremely high doses of aflatoxin, is characterized by hemorrhage, acute liver damage, edema, and death in humans[6]. Some of the other health effects found in animals and humans includeidentifiable diseases or health problems andweakened immune systems [7].

Fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumer tastes and preferences for such foods [8], and several of such condiments are available for consumers' choice. *Dadawa*,*Ogiri*and*Okpehe* are among the common condiments found in North-central Nigeria. Their production process and storage are source of concern, because of the possibility of microorganism growth which can produce mycotoxins. *Parkiabiglobosa*, *Prosopisafricana* and *Citrullusvulgaris*are plants in which their seeds are commonly used for production of condiments. *Dadawa*, *Ogiri*and*Opkehe* are produce from these plantsrespectively. The production process has been described by Achi, [9].The present study was carried out to estimate the aflatoxin content of the three condiments produced by locally fermented-bean and their effects on some Biochemical and Haematological parameters in rats.

II. Material And Methods

2.1 Equipment

The following equipmentwere used in the study; Enzyme-linked ImmunoSorbent Assay (ELISA) reader and Kit (Stat Fax® ELISA Reader, Romer Labs Diagnostics GmbH, Technopark 1, Austria), Vortex mixer (VM-1000 Vortex, Digisystem Laboratory, Instruments Inc., Taiwan), Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China) and other standard laboratory equipment.

2.2 Collection Of Plant Materials

The three locally made condiments (*Dadawa, Ogiri* and *Okpehe*) were purchased in parts from Terminus market, Jos, Plateau state and Kakuri market, Kaduna south, Kaduna state, Nigeria.

2.3 Experimental rats

The experiment animals (*Rattus norvegicus*) numbering 24 where obtained from the animal house of University of Jos, Nigeria. Rats are of body weight between 130 – 180 g. The animals were maintained under standard environmental conditions, had free access to food (Grand Cereal Products, Jos, Nigeria) and water *ad libitum.* Four groups of six rats each were randomly distributed in cages and acclimatized for 7 days.

2.4 Experimental design

Group 1: Control
Group 2: *Dadawa*extract
Group 3: *Ogiri*extract
Group 4: *Okpehe* extract
Each group consist of six animals (n = 6).

2.5 Treatment of experimental animals

Group 1 received 0.5 ml of distilled water per day, while others (Groups 2 – 4) received orally 400 mg/kg bw.of the condiments as stated for 21 days.

2.6 Collection of samples

At 22nd day, the rats were anesthetized at the time of sacrifice by been placed in a seal cotton wool soaked in diethyl ether inhalation jar. Blood samples were collected from the animals in batches. 3 animals' blood samples were separately collected into a clean, dry tube and allowed to cloth for 45 minute and spun at 3000 rpm for 5 minutes before the serum was collected for biochemical assay. Blood sample from the last 3 were separatelycollected into an anti-coagulant and were used for Haematological assay.

2.7 Phytochemicals

Phytochemical tests were carried out using standard procedures by Harborne,1973 [10], Trease& Evans,1989[11] and Sofowora,1993 [12].

2.8 Determination of Aflatoxin

Ram *et al*., 1986 [13] method was used in the determination of total aflatoxin by ELISA. Briefly; Aflatoxins were extracted from a grounded sample of each condiment with 70% methanol. The extracts sample and enzyme-conjugated aflatoxin are then mixed and added to the antibody-coated microwell. Aflatoxins in sample and standard are allowed to compete with enzyme-conjugated aflatoxin for antibody binding site. After a wash step, an enzymesubstrate is added and a blue colour develops. The intensity of the colour is inversely proportional to the concentration of aflatoxin in the sample and standard. The microwells are measured using a microwell reader with an absorbance filter of 450 nm and a different filter of 630 nm. The optical densities of the samples are compared to the optical density of the standard.

2.9 Assay of biochemical parameters

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase(AST) were assayedbythemethodofReitman&Frankel, 1957 [14]. Total proteins were assayed by Gornell*et al*., 1949 as modified by Plummer 1978[15] while Albumin was estimated by the methods of Doumas*et al*., 1971 [16].

2.10 Haematological parameters

Haematological parameters were determined using Mindray Haematology Analyser.

2.11 Statistical analysis

Data were presented as Mean ± SD and wereanalyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $P<0.05$ were considered significant.

III. Results

Total aflatoxin concentration of all condiments as detected by the method of Ram *et al*., 1989 [13], revel different concentrations with *Okpehe* having the highest followed by *Dadawa*and*Ogiri* respectively (TABLE I).

Phytochemical screening of the three condiments revel the presence of alkaloids, flavonoids,terpenes and steroids and phenols while tannins and balsams were not detected in all condiments. However, saponnins were not detected in aqueous*Dadawa* and in both aqueous and ethanolicextracts of *Ogiri*, while resin was absent in aqueous extracts of *Ogiri*(TABLE II).

Total protein significantly decrease ($P<0.05$) in all treated groups and in Albumin content of *Ogiri* and *Okpehe*. Also, ALT and AST showed significant increase ($P<0.05$) in all treated groups (TABLE III).

Varying effects of condiments were obtained in the haematological parameters assayed. There was significant decrease ($P<0.05$) in WBC of *Dadawa*, *Ogiri* and *Okpehe*treated rats. Significant increases ($P<0.05$) were observed in Monocytes and granulocytes of *Ogiri* treated rats when compared with the control. Significant increases ($P<0.05$) were also observed in haemoglobin content of *Okpehe*treated rats when compared to the control. Significant decrease ($P<0.05$) where observed in PCV of *Dadawa* and *Ogiri* treated when compared to the control and RBC of *Dadawa* treated rats. In all assayed parameters, no significant effects ($P>0.05$) were observed in monocytes of *Dadawa* and *Okpehe*, PCV of *Okpehe*, % granulocytes of *Dadawa*, *Ogiri* and *Okpehe* and RBC of *Ogiri* and *Okpehe* when compared to the control (TABLE IV).

Table I: Total Aflatoxin Concentration of Condiments

Sample	Amount (g)	Concentration (µg/kg)
Dadawa	5.0	3.45 ± 0.21^{a}
Ogiri	5.0	1.95 ± 0.07^{b}
Okpehe	5.0	4.65 ± 0.21^{c}

NOTE: Concentrations are Mean of 2 replicate ± SD

Values with different superscript are significantly different ($P<0.05$) down the column

Table II: Phytochemical Screening of Condiments

Phytochemicals	Methods	*Dadawa*		*Ogiri*		*Okpehe*	
		A	E	A	E	A	E
Alkaloids	Wagner test	+	+	+	+	+	+
Flavonoids	Lead acetate test	+	+	+	+	+	+
Tannins	Ferric chloride test	-	-	-	-	-	-
Saponins	Froth test	-	+	-	-	+	+
Balsams	Ferric chloride test	-	-	-	-	-	-
Glycosides	Lieberman-Burchard's test	+	+	+	+	+	+
Terpenes and Steroids	Liebermann-Burchard's test	+	+	+	+	+	+
Resins	Acetic anhydride test + conc. HS_2O_4	+	+	-	+	+	+
Phenols	Ferric Chloride test	+	+	+	+	+	+

NOTE: A = Aqueous extract + = Detected

E = Ethanolic extract - = Not detected

Table III: Effect of Condiments on Some Biochemical Parameters

Groups	Total protein	Albumin	ALT	AST
	(g/L)		(U/L)	
Control	98.83 ± 0.06^{a}	37.23 ± 0.15^{a}	18.00 ± 1.73^{a}	60.33 ± 1.53^{a}
Dadawa	88.77 ± 0.12^{b}	36.33 ± 0.12^{a}	20.67 ± 0.58^{b}	88.67 ± 1.53^{b}
Ogiri	89.67 ± 0.20^{c}	32.17 ± 0.15^{b}	22.00 ± 1.73^{bc}	105.00 ± 1.00^{c}
Okpehe	91.13 ± 0.15^{d}	31.23 ± 0.15^{b}	23.67 ± 1.52^{c}	125.67 ± 2.08^{d}

NOTE: Values are Mean of 3 replicates ± SD

Values with different superscript are significantly different ($P<0.05$) down the column

Table IV: Effects of Condiments on Haematological Parameters

Parameter	Control	*Dadawa*	*Ogiri*	*Okpehe*
WBC (10^9)/L	78.83±0.58[a]	70.77±0.06[b]	65.73±0.06[c]	69.43±0.25[b]
Monocytes ($\times10^9$)/L	1.80±0.02[a]	1.80±0.01[a]	2.10±0.10[b]	1.73±0.06[a]
Granulocytes($\times10^9$)/L	66.67±0.42[a]	63.27±0.21[b]	71.47±0.40[c]	61.90±0.10[d]
Haemoglobin (g/L)	170.33±1.53[a]	156.63±0.06[b]	156.30±0.10[b]	175.33±1.15[c]
PCV (%)	51.13±1.03[a]	47.03±0.06[b]	46.93±0.06[b]	52.50±0.36[a]
Platelet /L	218.00±2.00[a]	218.0±1.73[a]	91.33±0.58[b]	137.33±1.53[c]
Lymphocytes (%)	8.51±0.02[a]	8.03±0.15[b]	8.78±0.14[c]	8.54±0.05[a]
Monocytes (%)	2.40±0.01[a]	2.63±0.06[b]	2.60±0.01[b]	2.50±0.20[ab]
Granulocytes (%)	89.07±0.40[ab]	89.37±0.15[a]	88.57±0.31[b]	89.17±0.06[a]
Lymphocytes($\times10^9$)/L	6.28±0.15[a]	5.70±0.01[b]	7.07±0.12[c]	5.77±0.15[b]
RBC ($\times10^{12}$/L)	8.19±0.02[a]	7.74±0.02[b]	8.06±0.16[a]	8.19±0.02[a]

NOTE: Values are mean of 3 replicates ± SD

Values with different superscript are significantly different ($P<0.05$) across the row

IV. Discussion

Dangerousmycotoxins are naturally present in food, feeds and our environments [17], making it necessary to evaluate food spices for possible contamination with mycotoxins. *Dadawa*, *Ogiri* and *Okpehe* are condiments marketed by women around the city metropolis and markets. Storage conditions and moisture availability are likely to improve the growth of fungi. Result from this study indicated the presence of aflatoxins in varying concentrations with *Okpehe* having the highest followed by *Dadawa* and *Ogiri* respectively (TABLE I).

Aflatoxins are potent natural carcinogenic substances linked to higher prevalence of hepatocellular cancer in Africa [18], also it has been revealed that high risk occur with hepatitis B and hepatitis C carriers in developing liver cancer when exposed to aflatoxins[5]. Other studies have also linked aflatoxins to immunosuppression and stunted growth in children [19, 20]. Liver is the principal organ affected by aflatoxins, in the liver, aflatoxin may be transformed by certain P_{450} enzymes (CYP1A2, 3A4, 3A5, 3A7) to its DNA-reactive form aflatoxin-8,9-epoxide. This molecule may bind to liver proteins and lead to their failure, potentially resulting in acute aflatoxicosis[6].

Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenes and steroids, and phenols while tannins and balsams were not detected in all condiments. The toxic effects of tannins have been eliminated in this study due to their absence in the phytochemical screening conducted. Tannins in diets have been reported to have anti-nutritional, antihaemorrhagic and toxic effects including reduced fed intake, growth and net metabolism energy [21, 22]. Saponins were absent in 3 of the 6 extracts, in aqueous*Dadawa*, aqueous and methanolic*Ogiri*. Saponins have also been demonstrated to have antifungal properties [23],their presence would have assisted in preventing the proliferation of fungi.

Biochemical and haematological parameters have been reported to change in toxic effects of aflatoxins, these changes are said to occur before clinical symptoms develop [24, 25]. Several haematological changes where observed in rats treated with the 3 condiments with significant ($P<0.05$) changes in all parameters in *ogiri*treated except for RBC, and monocytes, platelets and % granulocytes in *dadawa* treated while *okpehe* showed no significant effect ($P>0.05$) on monocytes, PCV, lymphocytes and RBC.

The changes in haematological parameters maybe due to many factors which have been describe to include inhibition of protein synthesis [26] as seen in low albumin in *ogiri* and *okpehe* treated groups, decrease in total iron binding capacity [27] and in haemopoietic cellular defects of aflatoxin contamination [28]. Reductions in PCV and haemoglobin observed in *dadawa* and *ogiri* may suggest anaemia which may result from impaired red blood cell production [29, 30]. However, *okpehe* treated groups express the opposite.

Significant decrease ($P<0.05$) in WBC was observed in all treated groups indicating that the immune system was not activated during the period of treatment, normally a decrease in WBC shows the suppression of leucocytes and their production from bone marrow [31], viscosity of the blood of *ogiri* treated group maybe adverselyaffected due to increase in monocytes and granulocytes.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the study were significantly ($P<0.05$) increased in all treated groups when compared to the control. These transaminases are two enzymes which are associated with hepatocellular damage and are used as markers for predicting possible toxicity [32]. ALT and AST are elevated in presence of toxic substances [33], these values suggest liver damage by the extracts and resulting increase in ALT and AST in serum of experimental rats.

Condiments when used as spices involve other cooking process after preparation; this can be boiling, steaming or frying. These processes which also include nixtamalization and extrusion cooking can reduce some of the aflatoxin content of the condiments [34], Bankole*et al*. [35]however, reported that some aflatoxin still remain at high concentration even after such preparations.

V. Conclusion

The study reveals the presence of aflatoxins in varying concentrations in *dadawa*, *ogiri*and*okpehe*. These condiments when fed to experimental rats increased significantly ($P<0.05$) serum ALT and AST indicating their possible toxicity. Even after the different solvents used in extraction, a quit number of phytochemicals were indicated in the extracts. Aflatoxicosis, adverse haemopeotic activities and effects on marker enzymes should encourage a Good Manufacturing Practice to be enforced on the preparation of these condiments and also close monitoring of their storage and shelf life.

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