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Research Article

Transferase of mycotoxins in wistar rats treated with aqueous extract of pap consumed in Enugu, Nigeria

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Abstract

Food contamination by mycotoxins is a widely recognized global public health issue, particularly in impoverished nations. The objective of this work was to quantify the levels of Aflatoxin and Ochratoxin in a sample of pap consumed in Enugu, Nigeria, and to identify the mycotoxins in the blood of wistar rats that were administered an ageous extract of pap. A total of twenty male Wistar rats were separated into four groups, with five rats each group being administered varying doses of the extract for a duration of two weeks. Concentrations of mycotoxins were determined in both the digested pap and ageous extract using the Enzyme Linked Immunosorbent Assay. Data analysis was conducted using Graphpad Prism Version 8.0 and presented as the mean + standard deviation, with a significance level of $p < 0.05$. The pap samples contained amounts of Aflatoxin and Ochratoxin of 16.7 + 0.20 and 11.9 + 0.56 ppm, respectively. The serum concentrations of the mycotoxins Aflatoxin (Group A = 0.00 + 0.00, Group B = 5.2 + 1.16, Group C = 5.7 + 2.1, Group D = 8.4 + 2.6 ppm) and Ochratoxin (Group A = 0.00 + 0.00, Group B = 1.7 + 0.58, Group C = 3.5 + 1.9, Group D = 6.1 + 4.3 ppm) for the treated rats increased in a dose-dependent manner. The observed results fell under the acceptable thresholds of mycotoxins in food. The present results indicate that the consumption of pap in Enugu is generally safe. However, it is necessary to remove mycotoxins from it.

1. Introduction

Pap, also referred to as Ogi by the Yoruba, Akassa by the Hausa, and Akamu by the Igbo tribes of Nigeria, is a fermented cereal-derived product processed from maize (zea mays), sorghum (sorghum bicolor), and millet (pennisetumm glaucum). It is a significant dietary staple for the middle and low-income individuals in Nigeria. According to [1–3], It is commonly used as the initial food introduced to infants during weaning to complement breast milk and as a primary morning meal for both children and adults. [3] One significant obstacle that restricts the food safety of pap is the presence of mycotoxins. Reference: [4] Mycotoxins are secondary metabolites of low molecular weight

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(MSw 2700 Da) that are mostly synthesized by the fungus species Aspergillus, Penicillum, and Fusarium. The references cited are [1, 5, 6]. Ingestion of contaminated foods, inhalation, contact with skin, and bioaccumulation are all pathways by which mycotoxins induce acute and chronic health consequences in people. [7] Food sources contain five distinct kinds of mycotoxins, namely Aflatoxin (AF), Ochratoxin (OTA), Patulin (PT), Fumonisins (FN), and Sterigmatocystins (STC). References: [8, 9] The objective of this work was to evaluate the occurrence of mycotoxins, namely Aflatoxin and Ochratoxin, in the pap and serum of wistar rats that were administered the pap extract.

2. Materials and Methods

Area of Study

Southeast Nigeria's Enugu State served as the study's location. Enugu, the state's capital and largest metropolis, is where the state gets its name. With a population of 3,267837 and an area of 7,161 km^2, it is primarily home to the Igbo tribe from southeast Nigeria. It is located between latitudes 5o15'N and 7o15'N and longitudes 6o 30'E and 6o 55'E. Three senatorial divisions make up this group: Enugu East, Enugu North, and Enugu West. Igwe and [10].

Sample of Pap

The sample of pap used for the study was that prepared by the traditional method of fermentation which was purchased from the popular Ogbete Main Market in Enugu.

Digestion of the Pap Sample

After mixing, 50g of the sample were weighed with a chemical weighing scale and transferred into a beaker. To achieve homogeneity, a wet extraction powder (1 packet for a 50g sample) was mixed with the liquid sample in the beaker for two minutes using a mixer. A filtrate was subsequently obtained by filtering the combination via Whatman No. 1 filter paper, which was utilised to estimate the levels of aflatoxin and ochratoxin. Once more, 900 µl of buffer (1:10 dilution) was combined with 100 µl of the filtrate to create a diluted extract.

Extraction of Pap Sample

A chemical balance was used to weigh 10 g of the material, which was then combined with 100 ml of water. What man No. 1 filter paper was used to filter the mixture, producing an concentrated filtrate.

Wistar Rats

Male specimens of the rattus nervigicus species, weighing between 150-250g, were obtained from the Physiology Department of the University of Nigeria in Enugu, Nigeria. They were maintained under standard conditions of light (12/24 hours) and temperatures ($25 - 29°C$) in an aluminum wire gauze cage and fed with standard growers pellet (Pfizer, Nigeria, Ltd) and allowed free access to water for a two weeks of acclimatization prior to the experimental protocol.

Ethical Considerations

Ethical Clearance was obtained from the Animal Research Ethics Committee of the Faculty of Health and Allied Sciences Enugu State University of Science and Technology with assigned number : FHAS/EC/2024/001

Sample Size

The sample size was calculated using the minimum sample size relation according to One Way Analysis of Variance.
• Minimum Sample Size (n) = maximum DF/k + 1 Where.
• DF = the maximum between subject error
• k = the number of groups
• n = minimum number of rats per group
The minimum sample size (n) per group obtained by the relation was 4.

Experimental Design

Twenty male Wistar rats were randomly allocated into four groups (A, B, C, and D) each consisting of five rats. Group A was provided with a water and rat pellet diet alone, and thus functioned as the control group. Group B received a diet consisting of water, rat pellet, and a minimum recommended dosage (100mg/kg body weight) of pap. Group C received a diet consisting of water, a pellet, and a moderate amount (200 mg/kg b.wt) of the pap extract administered orally for a duration of 2 weeks. Following the administration of the extract, blood samples were collected by retro-orbital puncture for the estimation of serum Aflatoxin and Ochratoxin concentrations and the animal sacrificed under light chloroform anaesthesis.

Estimation of Mycotoxin

The Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique was used to quantify the amounts of Aflatoxin and Ochratoxin in the pap extract and the serum of rats. Absorbance measurements for Aflatoxin and Ochratoxin were conducted using kits from Sulong

Diagnostic Ltd (Catalog number CR8496B and Mk 1137GH, China) and a Microplate Reader (Mindray 96A, Shenzhen, China) at 450 nm. The kits had analytical ranges of 0-40 ppm and 0-30 ppm for Aflatoxin and Ochratoxin, respectively.

Data Analysis

Graph Pad Prism version 8.0 was used to analyze the data, and the mean plus standard deviation of the findings was given. Turkey's test was used to compare the means at the 0.05 level after the data were analyzed using One Way Analysis of Variance.

3. Result

The mean values of Aflatoxin and Ochratoxin in the sample of pap were 16.7 ± 0.20 ppm and 11.9 ± 0.56 ppm respectively. These were below the tolerable limit of 20ppm and 18 ppm for the respective mycotoxins in food Table 1 The mycotoxins were not detected in the serum of the control rats (Group A) while a dose-dependent increase in the mycotoxins concentrations was observed in the serum of the rats treated with pap extract (Groups B, C and D) as shown in Table 2.

Table 1: The Permissible limits for mycotoxins in food compared to the concentrations in pap consumed in Enugu.

Mycotoxin	**Concentration (ppm)**	**Permissible concentration** (ppm) [11]
Aflatoxin	17.7 ±0.20	0 – 20
Ochratoxin	11.9 ±0.56	0 – 18

Values are expressed as means $\pm SD for three measurements$, unit of measurement is parts per million (ppm) (lppm = 1mg/L = 0.001ug/L)

Table 2: Serum concentration of mycotoxins in rats treated with pap extract.

	Treatment Groups				
Mycotoxin	A(control)	B(100mg/kg b.wt)	C(200mg/kg b.wt)	C(300mg/kgb.wt)	
Aflatoxin	0.00±0.00	5.2 ±1.16	5.7 ±2.1	8.4 ±2.6	0.081
Ochratoxin	0.00±0.00	1.7 ±0.58	3.5±1.9	6.1 ±4.3	0.134

Values are expressed as means $\pm SD for three measurements$, unit of measurement is parts per millions (ppm) (lppm = lmg/L = 0.001

4. Discussion

A significant portion of the population in Sub-Saharan Africa is unaware of the dangers posed by mycotoxin contamination of food [12]. There is little knowledge about mycotoxin contamination and exposure to humans and animals, according to a new Tanzanian study. [13] Mycotoxins are known to be immunosuppressive, mutagenic, nephrotoxic, hepatotoxic, carcinogenic, and to cause growth retardation in both humans and animals. [1, 4] Aflatoxin and ochratoxins can cause acute stages that include high fever, quickly progressing jaundice, limb oedema, pain, vomiting, and swollen limbs. On the other hand, chronic toxicity, which arises from prolonged exposure at sub-lethal doses, is characterised by a lack of symptoms and may include nutritional and immune suppression status [13].

Although the detected concentrations of the mycotoxins in the present study are below the established tolerable limits in food, chronic consumption of pap contaminated with mycotoxins can pose a toxicological risk to health. The detection of Aflatoxin and Ochratoxin in the serum of the treated rats is an evidence that a life-long consumption of pap contaminated with mycotoxins could pose a toxicological risk to health. Moreover, the high concentrations of the mycotoxins recorded in the rats which were treated with high doses of the pap extract is an evidence that mycotoxins can bio- accumulate thereby posing a toxicological risk to health if chronically consumed. Mycotoxins co-exist in food and food products and may have synergistic toxicological effect. Cases of synergistic interaction exhibited by combination of mycotoxins resulting to growth depression in different animal models has been reported. In [1] Research has also shown that mycotoxin contamination of food is more prevalent in underdeveloped nations. [5] There are no studies in the literature that describe the association between the concentration of mycotoxins in pap and the serum concentration in rats treated with papa extract. This demonstrates the uniqueness of the current investigation and our contribution to the body of knowledge on the toxicological evaluation of mycotoxins in a biological system as a result of pap eating.

5. Conclusion

The result of the present study suggests that pap sold in common markets in Enugu are suitable for consumption. However, the current findings calls for education and control strategies to combat mycotoxin contamination of stable foods in Nigeria.

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