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Beyond Efficacy: High-Dose Neem Extract Induces Hepatotoxicity in Rats amidst Widespread Aflatoxin Contamination of Nigerian Groundnuts

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Abstract

Aflatoxin B1 (AFB1) contamination in staple crops is a major cause of hepatocellular carcinoma in sub-Saharan Africa. Although plant extracts like *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) are proposed as low-cost detoxificants, their *in vivo* efficacy and safety profiles are inadequately characterized. This study first quantified aflatoxin contamination in 300 stored groundnut samples from Nasarawa State, Nigeria, using High-Performance Liquid Chromatography (HPLC). Concurrently, the detoxification potential and toxicity of aqueous extracts of these botanicals were evaluated in an albino rat model. Rats were divided into groups and treated via oral gavage for 28 days with AFB1 alone or in combination with either *C. longa* or low- (1000 mg/kg) or high-dose (5000 mg/kg) *A. indica* extract. Serum biochemistry was analyzed using standard diagnostic kits.The groundnut analysis revealed widespread contamination, with 51.3 % of samples exceeding the USFDA safety limit (20 µg/kg) and AFB1 levels as high as 1791 µg/kg. In the rat model, high-dose *A. indica* (5000 mg/kg) significantly elevated serum alanine aminotransferase (92.56 vs. 76.23 IU/L in controls, $P < 0.003$) and reduced total protein, indicating hepatotoxicity. *C. longa* showed no significant biochemical alterations. These findings highlight a serious public health risk from aflatoxin exposure and reveal a dose-dependent hepatotoxicity that severely limits the therapeutic use of high-dose neem extract. The promotion of plant-based detoxification requires caution, underscoring the urgent need to improve post-harvest storage infrastructure to mitigate contamination at its source.

Keywords: Azadirachta indica, Curcuma longa, aflatoxins, hepatotoxicity, food safety, Nigeria

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Introduction

Aflatoxins, particularly aflatoxin B1 (AFB1) produced by *Aspergillus flavus* and *A. parasiticus*, are potent hepatocarcinogens that persistently contaminate staple crops such as groundnuts in tropical regions [1]. In sub-Saharan Africa, chronic dietary exposure contributes significantly to the burden of liver disease and poses a severe constraint on food security and trade [2, 3]. While stringent international regulations exist (e.g., EU limit of 4 µg/kg), compliance remains a major challenge in developing nations, largely due to inadequate post-harvest storage practices [4, 5]. In the search for affordable, accessible solutions, plant-derived extracts have garnered considerable interest for their potential to bind or degrade mycotoxins. *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) are particularly prominent in traditional medicinal systems, credited with broad pharmacological properties including antioxidant, anti-inflammatory, and antimicrobial activities [6, 7].

Consequently, they are frequently proposed as natural agents for aflatoxin mitigation. However, most studies focus on *in vitro* efficacy, leaving a critical gap in the literature: a comprehensive *in vivo* evaluation of both their detoxification potential and, more importantly, their safety profiles in animal models. This is a significant oversight, as the complex phytochemistry of plants like neem may confer not only benefits but also dose-dependent toxicities [8]. Therefore, moving beyond simple demonstrations of efficacy to rigorous risk-benefit assessment is essential for translational application.

This study was designed to address this gap through an integrated investigation. First, we quantified the prevalence and levels of aflatoxin contamination in stored groundnuts from a key agricultural state in Nigeria, establishing the context of exposure. Second, and most critically, we conducted a controlled *in vivo* experiment to evaluate the effects of *A. indica* and *C. longa* extracts on growth and hepatic

biochemistry in albino rats, explicitly testing for potential adverse effects. Our objective was not only to assess the promise of these botanicals but to identify any toxicological constraints that could limit their practical use in food safety interventions.

Materials and Methods

Study area and groundnut sample collection

The study was conducted in Nasarawa State, Nigeria, which is divided into three agricultural zones (North, South, West). A total of 300 stored groundnut samples were collected from traditional mud storage barns ('rumbu') across all 13 Local Government Areas (LGAs). The sample size was determined using Yamane's formula [9] based on a registered farmer population of 1,170, with a 5 % margin of error. Sampling was purposive, focusing on storage structures in active use, and was facilitated by local agricultural extension officers.

Plant material and extract preparation

Fresh leaves of *Azadirachta indica* (neem) and rhizomes of *Curcuma longa* (turmeric) were collected from the vicinity of Lafia, Nasarawa State. Voucher specimens were authenticated and deposited at the Herbarium Unit of the Department of Plant Science and Biotechnology, Federal University of Lafia (Voucher numbers: FUL/PLS/010 for *A. indica*, FUL/PLS/011 for *C. longa*). Plant materials were washed, shade-dried, and pulverized. Aqueous extracts were prepared by macerating 100 g of each powder in 500 mL of distilled water for 24 h at 100 rpm. The mixture was filtered sequentially through a No. 60 sieve, Whatman No. 1 filter paper, and a sterile 0.2 µm membrane filter. The filtrate was concentrated using a rotary evaporator (Buchi, Switzerland) and lyophilized to obtain a dry extract, which was stored at 4 °C until use.

Animal experiments

Ethical statement

All experimental protocols involving animals were reviewed and approved by the Research Ethics Committee of the Federal University of Lafia, Nigeria (Approval No. FUL/REC/2025/168). The study was conducted in strict accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals [10].

Experimental design for toxicity and growth assessment

Sixty-three healthy adult albino rats (120-150 g) were acclimatized for one week under standard conditions (23±2 °C, 12 h light/dark cycle) with free access to a standard pellet diet and water. The rats were randomly divided into seven groups (n=9 per group):

- **Group 1:** Control (administered normal saline).
- **Groups 2-4:** Treated with *C. longa* extract at 100 mg/kg (low), 1500 mg/kg (medium), and 5000 mg/kg (high) body weight.
- **Groups 5-7:** Treated with *A. indica* extract at 100 mg/kg (low), 1500 mg/kg (medium), and 5000 mg/kg (high) body weight.

Extracts were administered orally via gavage once daily for 21 days. Body weights were recorded daily. The high dose (5000 mg/kg) was selected to evaluate the safety margin and potential toxicological effects under conditions of exaggerated exposure.

Experimental design for aflatoxin-detoxification assessment

A separate cohort of forty-five rats was used to assess detoxification potential. Rats were allocated into five groups (n=9):

1. **Control:** Received normal saline.
2. **AFB1 Group:** Administered 1 mg/kg body weight of aflatoxin B1 (AFB1, Sigma-Aldrich) intraperitoneally.
3. **AFB1 + Sorafenib Group:** Received AFB1 (1 mg/kg) plus Sorafenib (1 mg/kg, as a reference chemoprotective agent).
4. **AFB1 + *C. longa* Group:** Received AFB1 plus *C. longa* extract (100-300 mg/kg).
5. **AFB1 + *A. indica* Group:** Received AFB1 plus *A. indica* extract (1 g/kg).

Treatments were administered for three weeks following AFB1 induction. At the end of the experimental period (Day 28), all animals were fasted overnight, anesthetized, and sacrificed by cardiac puncture for blood and organ collection.

Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 15 min. to obtain serum. Hepatic function was assessed by measuring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and conjugated bilirubin using standard diagnostic kits (Randox Laboratories Ltd., UK) on an automated biochemistry analyzer (Model: RX9000).

Aflatoxin analysis in groundnuts

Certified reference standards for aflatoxins B1, B2, G1, and G2 were acquired from Sigma-Aldrich (USA). Individual stock solutions were prepared in a toluene-acetonitrile solvent system. All other solvents and chemicals utilized were of the highest available chromatographic or analytical grade. Aflatoxin extraction from groundnut kernels was performed using a chloroform-based protocol adapted from established literature. Representative samples were finely ground, and a 20-gram aliquot was combined with 100 mL of chloroform in an airtight vessel. This mixture was agitated continuously for 30 min to ensure efficient transfer of mycotoxins into the organic solvent. The resulting mixture was subsequently filtered to separate the solid matrix from the liquid extract. The crude chloroform extract underwent a purification process using solid-phase extraction with florisil as the stationary phase. Interfering compounds were removed, and the target aflatoxins were selectively eluted using an acetone-water (99:1 v/v) solution. The collected eluate was then concentrated to complete dryness under

vacuum at a controlled temperature of 40 °C. To facilitate sensitive detection via HPLC, a chemical derivatization step was employed. The dry residue from the cleanup procedure was reacted with trifluoroacetic acid (TFA) for a period of 15 min. This reaction converts specific aflatoxins (AFB1 and AFG1) into their more fluorescent derivatives. The reaction was then quenched and prepared for injection by dilution with an acetonitrile-water solution. Quantitative analysis was conducted using a High-Performance Liquid Chromatography (HPLC) system fitted with a fluorescence detector. Separation of the four target aflatoxins was achieved on a reversed-phase C18 column maintained at 45 °C. An isocratic mobile phase composed of acetonitrile, methanol, and water was delivered at a constant flow rate. Detection relied on the native fluorescence of the aflatoxins and their derivatives. The detector wavelengths were optimized to 360 nm for excitation and 450 nm for emission. Under these conditions, each aflatoxin eluted at a characteristic retention time, allowing for clear identification and measurement. Quantification was performed using an external standard calibration method. A series of standard solutions containing known concentrations of all four aflatoxins were processed and analyzed identically to the samples. Calibration curves were constructed by plotting the peak area response against the corresponding concentration for each analyte. The method's reliability was confirmed through a validation protocol. This included establishing major performance parameters such as the limit of detection (LOD), limit of quantification (LOQ), and percentage recovery for each aflatoxin by analyzing control samples spiked with known amounts of the standards.

Statistical analysis

Data were analyzed using GraphPad Prism version 9.0 (GraphPad Software, USA). Normality was assessed using the Shapiro-Wilk test. A two-way analysis of variance (ANOVA) was used to analyze rat weight data (factors: treatment and time). Biochemical parameters and aflatoxin levels were compared using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Prevalence data were analyzed using Pearson's Chi-square test. The correlation between moisture content and aflatoxin levels was assessed using Spearman's rank correlation coefficient. Statistical significance was set at $P < 0.05$. Data are presented as mean ± standard deviation (SD).

Results and Discussion

Widespread aflatoxin contamination in stored groundnuts exceeds international safety limits

Analysis of 300 groundnut samples stored in traditional mud barns ('rumbu') across Nasarawa State revealed severe aflatoxin contamination. Over half (51.3 %) of all samples exceeded the 20 µg/kg safety threshold set by the US Food and Drug Administration (FDA) (Table 1). The mean total aflatoxin concentration across the state was 32.62 ± 14.21 µg/kg, with no significant difference between the three agricultural zones ($P > 0.05$). However, extreme contamination hotspots were identified, with individual samples from Lafia and Keana Local Government Areas (LGAs) containing up to 1791 and 2577 µg/kg of total aflatoxins, respectively. Aflatoxin B1 (AFB1) was the predominant and most concerning contaminant, detected in 89.3 % of samples and constituting the primary driver of total toxicity. Alarmingly, only 29.7 % of samples complied with the stricter European Union/NAFDAC limit of ≤4 µg/kg.

The extent of aflatoxin contamination documented here is a serious public health concern. With over 50 % of samples surpassing the USFDA regulatory limit and a mean AFB1 concentration (28.74 µg/kg) far exceeding the EU's 2 µg/kg threshold, the exposure risk for local populations is substantial [11]. The extremely high levels detected in hotspots are consistent with documented failures in post-harvest handling, such as inadequate drying and storage in non-hermetic structures, factors repeatedly linked to toxin proliferation in sub-Saharan Africa [12-14]. The dominance of AFB1 aligns with the prevalence of *Aspergillus flavus* in tropical climates and confirms the primary carcinogenic risk [15]. The lack of correlation between moisture content and aflatoxin levels in our study suggests that, in this context, factors like insect damage and storage duration may be more critical drivers once a basic fungal threshold is crossed, a nuance observed in other studies [16].

Curcuma longa and *Azadirachta indica* extracts exhibit distinct effects on rat growth and hepatic biochemistry

To evaluate the safety and potential detoxifying efficacy of the botanicals, we conducted a parallel *in vivo* study in albino rats.

Table 1: Aflatoxin contamination profile in stored groundnuts from Nasarawa State, Nigeria

Agricultural Zone	Samples (n)	Total Aflatoxin (µg/kg) Mean ± SD	AFB1 (µg/kg) Mean ± SD	% Samples > USFDA Limit (20 µg/kg)	% Samples > EU Limit (4 µg/kg)	Maximum AFB1 Detected (µg/kg) (LGA)
Nasarawa North (Nasarawa Eggon)	69	37.93 ± 16.61	32.40 ± 14.64	65.2 %	89.7 %	1595
Nasarawa South (Lafia)	116	37.75 ± 13.66	32.97 ± 12.05	56.0 %	80.0 %	1791
Nasarawa West (Nasarawa LGA)	115	25.19 ± 11.13	21.86 ± 9.86	39.1 %	81.7 %	1570
Overall	300	32.62 ± 14.21	28.74 ± 12.85	51.3 %	83.0 %	1791

LGA = Local Government Area; USFDA = United States Food & Drug Administration; EU = European Union

Table 2: Effects of *Curcuma longa* and *Azadirachta indica* on final body weight and weight gain in albino rats

Treatment Group	Dose (mg/kg)	Initial Weight (g) Mean ± SD	Final Weight (g) Mean ± SD	Weight Gain (g) Mean ± SD
Control	–	153.00 ± 2.64	187.00 ± 1.53	34.00 ± 2.1
C. longa Low	100	154.00 ± 2.37	187.33 ± 1.21	33.33 ± 1.9
C. longa Medium	1500	155.50 ± 5.00	191.33 ± 4.27	35.83 ± 3.5
C. longa High	5000	157.67 ± 4.03	188.33 ± 3.00	30.66 ± 3.2
A. indica Low	100	159.33 ± 1.97	197.85 ± 1.20	38.52 ± 2.1
A. indica Medium	1500	161.17 ± 2.60	200.66 ± 2.50	39.49 ± 2.8
A. indica High	5000	160.55 ± 3.17	200.22 ± 1.10	39.67 ± 3.5

Weight gain was calculated as Final – Initial; A two-way ANOVA revealed a significant effect of treatment and time (P<0.001); but no significant interaction for *C. longa* (P=0.494)

Body weight changes

All animal groups gained weight over the 21-day experimental period. Administration of *Curcuma longa* (turmeric) extract across a wide dose range (100–5000 mg/kg) did not significantly affect final body weight or weight gain compared to the control group (Table 2). Treatment with *Azadirachta indica* (neem) extract resulted in a non-significant trend of higher final weight, particularly at medium and high doses (1500 and 5000 mg/kg) (Table 2).

Dose-dependent hepatotoxicity of *A. indica*

Biochemical analysis of serum revealed a critical, dose-dependent hepatotoxic effect for *A. indica* (Table 3). While low and medium doses showed no adverse effects, administration of the high dose (5000 mg/kg) significantly elevated the liver enzyme alanine aminotransferase (ALT) by 21 % compared to controls (92.56 ± 1.03 vs. 76.23 ± 5.53 IU/L; P < 0.003). Concurrently, the same high-dose group exhibited a significant 9 % reduction in total serum protein (62.88 ± 1.62 vs. 68.93 ± 1.15 g/L; P < 0.019). In stark contrast, *C. longa* administration, even at the highest dose of 5000 mg/kg, did not induce any significant alterations in hepatic biochemical markers, including ALT, AST, total protein, or albumin (Table 3).

Table 3: Hepatic biochemical parameters in rats treated with *C. longa* and *A. indica*

Biochemical Parameter	Control	*C. longa* (5000 mg/kg)	*A. indica* (5000 mg/kg)	P-value (*A. indica* vs Control)
Alanine Aminotransferase (ALT) (IU/L)	76.23 ± 5.53	71.76 ± 1.05	92.56 ± 1.03	< 0.003
Aspartate Aminotransferase (AST) (IU/L)	106.69 ± 2.73	115.83 ± 3.39	130.21 ± 2.89	0.335
Total Protein (g/L)	68.93 ± 1.15	67.76 ± 9.96	62.88 ± 1.62	< 0.019
Albumin (g/L)	34.45 ± 5.88	31.46 ± 3.51	29.11 ± 3.82	0.393
Alkaline Phosphatase (ALP) (IU/L)	283.10 ± 2.78	288.18 ± 4.94	285.33 ± 3.73	1.000

Data presented as Mean ± SD; Only the high-dose groups are shown to highlight the toxicological effect; P-values result from one-way ANOVA with Tukey's post-hoc test comparing the *A. indica* high-dose group to the control

No Association between moisture content and aflatoxin levels

The moisture content of the stored groundnut samples varied across LGAs, with an overall mean of 4.72 ± 4.12 % (Table 4). Statistical analysis found no significant correlation between sample moisture content and the level of total aflatoxin contamination (P > 0.05), suggesting that other factors, such as insect damage, microbial load, or storage duration, were more critical drivers of toxin proliferation in this study context.

Table 4: Moisture content of stored groundnuts across agricultural zones

Agricultural Zone	Mean Moisture Content (%) ± SD
Nasarawa North	5.34 ± 2.85
Nasarawa South	4.74 ± 5.41
Nasarawa West	4.08 ± 3.08
Overall Mean	**4.72 ± 4.12**

No significant correlation was found between moisture content and total aflatoxin concentration (Spearman's rho = 0.15, P = 0.21)

***Azadirachta indica*: A double-edged sword with a narrow therapeutic window**

The most significant finding of this work is the unambiguous hepatotoxicity induced by high-dose *A. indica* extract. The significant elevation of serum ALT, a specific marker of hepatocellular injury, coupled with reduced total protein, provides clear biochemical evidence of liver stress [17]. This effect was dose-specific, absent at lower doses, underscoring a narrow therapeutic window. While neem is celebrated for its broad pharmacological properties [6], its complex phytochemistry can exert pro-oxidant and cytotoxic effects at high concentrations [8]. Our data establish a crucial toxicological checkpoint: the dosage required to potentially mitigate aflatoxin effects (5000 mg/kg) concurrently induces significant hepatic damage. This creates a paradoxical scenario where intervention could exacerbate organ stress in aflatoxin-exposed subjects, a risk seldom considered in ethnopharmacological proposals.

The contrasting safety profile of *Curcuma longa* and implications for detoxification strategies

In contrast to neem, *C. longa* extract exhibited a notably benign safety profile, causing no adverse effects on growth or liver biochemistry even at the highest dose. This aligns with the extensive literature on curcumin's hepatoprotective properties [18]. However, its lack of overt toxicity in this model was not paired with evidence of significant aflatoxin detoxification under the studied parameters. This dichotomy between safety and efficacy highlights a key research gap: the need to identify botanicals or formulations that combine a favourable safety profile with potent aflatoxin-counteracting capability.

Conclusion

In conclusion, our integrated assessment reveals that the scale of aflatoxin contamination in Nigerian groundnuts demands urgent intervention. However, the solution is not as simple as applying high doses of readily available botanicals like neem**.** We demonstrate that the hepatotoxic risk of high-dose neem extract critically offsets its purported therapeutic benefit, serving as a cautionary case study. This finding mandates a paradigm shift from seeking solely "effective" plant-based mitigants to rigorously evaluating their safety thresholds *in vivo*. Ultimately, the most reliable public health strategy must prioritize preventing contamination at its source through investment in and farmer education on affordable, evidence-based post-harvest technologies, such as hermetic storage.

Conflict of interest: The authors declare no conflict of interest.

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