https://doi.org/10.48047/AFJBS.7.6.2025.264-274

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Quantitative Assessment of Postmortem Mycotoxin Levels in Brain Tissue for Forensic Analysis

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Volume 7, Issue 6, Jun 2025

Received: 15 April2025

Accepted: 05 May 2025

Published: 09 Jun 2025

doi:10.48047/AFJBS.7.6.2025.264-274

Abstract Background: Mycotoxins, toxic secondary metabolites from certain fungi, present significant health risks to humans and animals. Although their effects on living organisms have been widely studied, their presence in postmortem tissues, especially the brain, is underexplored in forensic analysis. Understanding the quantitative assessment of mycotoxin levels in postmortem brain tissue is vital due to the brain's critical role and its susceptibility to mycotoxin-induced damage. **Objective**: This study aimed to develop an analytical method for quantitatively evaluating mycotoxin levels in postmortem brain tissue and investigating their forensic implications.

Method: An observational cross-sectional design was used to analyze postmortem brain tissue samples from forensic autopsies for mycotoxin content via high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Standardized protocols and quality control measures ensured accurate and reliable mycotoxin measurements.

Results: Detectable levels of aflatoxins, ochratoxins, and trichothecenes were found in postmortem brain tissue samples. Mycotoxin levels varied across demographic groups and brain regions, with higher levels observed in individuals diagnosed with neurological disorders compared to healthy controls. **Conclusion**: This study highlights mycotoxin exposure's prevalence and potential forensic implications in postmortem brain tissue, underscoring the need for further research on mycotoxin-induced neurotoxicity and standardized analytical protocols.

Keywords: Mycotoxins; Postmortem; Brain; Forensic; Quantitat

1. Introduction

Certain fungi create toxic secondary metabolites called mycotoxins, which are extremely dangerous to both human and animal health; these substances can infect a variety of food products, which, when consumed, can have harmful health effects (Awuchi et al., 2021). Mycotoxins' effects on live things have been thoroughly researched, but their existence and impact in postmortem tissues, especially the brain, have gotten less attention because of their complicated structure and critical role; the brain is especially vulnerable to the harmful effects of mycotoxins (Kępińska-Pacelik & Biel, 2021). Forensic studies seeking to ascertain the cause and mode of death must thus comprehend the quantities and distribution of mycotoxins in postmortem brain tissue, mainly when mycotoxin poisoning is suspected; a variety of substances are included in the category of mycotoxins, such as fumonisins, trichothecenes, aflatoxins, and ochratoxins (C. G. Awuchi et al., 2022). Some fungi that are commonly found in food crops, stored grains, and indoor environments, like Aspergillus, Penicillium, and Fusarium, produce toxic poisons; it is a primary global food safety concern that can affect the health of humans and animals when they are present in food and feed (Adeyeye, Ashaolu, & Idowu-Adebayo, 2022).

Mycotoxin types vary in their harmful effects on the body, causing everything from immunosuppression and neurotoxicity to mutagenic and carcinogenic qualities. Because of its high metabolic activity and rich lipid content, the brain is highly susceptible to injury from mycotoxin-induced deterioration; it can penetrate the blood-brain barrier and build up in different parts of the brain, where it causes oxidative stress, neuroinflammation, and disrupt neurotransmitter function, numerous neurological conditions, such as encephalopathy, cognitive decline, and neurodegenerative illnesses, might result from these impacts (Janik et al., 2020). Mycotoxins are known to be poisonous to living things, but little is known about how they behave and persist in postmortem tissues; the amount of mycotoxins in cadaveric tissues, especially the brain, and their possible significance for forensic pathology have yet to be thoroughly studied; examining the presence and distribution of mycotoxins in postmortem brain tissue is essential to understanding their forensic significance and how they contribute to the cause of death (Omotayo et al., 2019).

The complexity of biological matrices and the possibility of toxin degradation over time present several difficulties in the analysis of mycotoxin levels in postmortem tissues; low concentrations of mycotoxins in tissue samples and the existence of interfering substances can impede the efficacy of conventional diagnostic techniques like mass spectrometry (MS) and high-performance liquid chromatography (HPLC) (Alshannaq & Yu, 2017). The absence of established protocols for mycotoxin extraction and quantification in postmortem tissues further complicates the analytical method; the postmortem delay, the cause of death, and the premortem exposure to mycotoxin-contaminated foods or settings must all be carefully taken into account when interpreting the mycotoxin levels in postmortem tissues (Singh & Mehta, 2020). In order to establish trustworthy correlations between mycotoxin exposure and neurological effects in forensic cases, it is also necessary to consider the variability in mycotoxin distribution within the brain and its correlation with specific neuropathological abnormalities (Arce-López et al., 2020).

This study aims to create a thorough analytical method for the quantitative evaluation of mycotoxin levels in brain tissue after death, offering crucial new information on the forensic consequences of mycotoxin exposure and its possible role in the cause of death.

This study aimed to develop an analytical method for quantitatively evaluating mycotoxin levels in postmortem brain tissue and investigating their forensic implications.

2. Methodology

2.1. Study Design

An observational cross-sectional design was employed in this research, wherein postmortem brain tissue samples obtained from forensic autopsies were analysed for mycotoxin content. This design facilitated the assessment of mycotoxin levels in a diverse population of deceased individuals, providing insights into the prevalence and distribution of mycotoxin exposure across different demographic and geographic backgrounds.

2.2. Sample Size

A total of 50 postmortem brain tissue samples were included in the study. This sample size was determined to ensure robust statistical analysis and enhance the generalizability of findings. Including samples from diverse demographic and geographic backgrounds enabled a comprehensive examination of mycotoxin levels and their potential variations among different populations.

2.3. Sampling Procedure

Brain tissue samples were collected during routine forensic autopsies conducted. Samples were obtained according to standardised procedures to minimise contamination and preserve mycotoxin integrity. Proper sample collection and handling protocol documentation was maintained to ensure traceability and data reliability.

2.4. Mycotoxin Analysis

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) was employed to detect and quantify mycotoxin levels in the brain tissue samples. This analytical technique offered high sensitivity, specificity, and resolution, enabling accurate identification and quantification of various mycotoxins in complex matrices like brain tissue. Standardised protocols and calibration procedures were followed to ensure the precision and accuracy of mycotoxin measurements.

2.5. Quality Control

Internal standards and quality control samples were included in each analysis batch to maintain the results' reliability. These control measures helped assess instrument performance, validate analytical procedures, and monitor for potential sources of error or variability. Additionally, calibration curves and spiked samples were utilised to verify the linearity and recovery of mycotoxin quantification methods.

2.6. Ethical Consideration

Informed consent was obtained from the authorised representatives of deceased individuals, ensuring respect for autonomy and confidentiality in handling sensitive biological materials.

3. Results

The results of this study provide quantitative data on the presence and concentration of various mycotoxins in postmortem brain tissue samples. Preliminary analyses indicate the detection of several common mycotoxins, including aflatoxins, ochratoxins, and trichothecenes, in the brain tissue samples. The findings are presented below using descriptive statistics to illustrate mycotoxin levels across the sample population.

Table 1 presents the mean concentration, standard deviation, minimum, and maximum levels of aflatoxins, ochratoxins, and trichothecenes detected in postmortem brain tissue samples. The results indicate varying levels of mycotoxins in the brain tissue, with aflatoxins exhibiting the highest mean concentration, followed by ochratoxins and trichothecenes.

Table 1: Mycotoxin Levels in Postmortem Brain Tissue Samples

Mycotoxin	Mean Concentration (ng/g)	Standard Deviation (ng/g)	Minimum (ng/g)	Maximum (ng/g)
Aflatoxins	15.3	5.7	8.2	23.1
Ochratoxins	12.6	4.2	7.1	18.9
Trichothecenes	9.8	3.5	5.3	14.2

Table 2 presents the mean concentration, standard deviation, minimum, and maximum levels of aflatoxins, ochratoxins, and trichothecenes detected in postmortem brain tissue samples. The results indicate varying levels of mycotoxins in the brain tissue, with aflatoxins exhibiting the highest mean concentration, followed by ochratoxins and trichothecenes.

Table 2: Distribution of Mycotoxin Levels by Demographic Characteristics

Demographic Characteristic	Aflatoxins (ng/g)	Ochratoxins (ng/g)	Trichothecenes (ng/g)
Age Group			
- 18-30 years	14.2	10.5	8.9
- 31-50 years	16.7	13.2	9.4
- >50 years	12.8	11.9	10.1
Gender			
- Male	15.6	12.4	9.8
- Female	14.8	11.8	9.6

Table 3 presents the distribution of mycotoxin levels in postmortem brain tissue samples based on demographic characteristics such as age group and gender. There are slight variations in mycotoxin levels across different age groups and genders, with no significant differences observed.

Table 3: Correlation Analysis of Mycotoxin Levels in Postmortem Brain Tissue

	Aflatoxins	Ochratoxins	Trichothecenes
Aflatoxins	1.00	0.82	0.67
Ochratoxins	0.82	1.00	0.75

Trichothecenes	0.67	0.75	1.00

Table 4 displays the correlation coefficients between mycotoxin levels in postmortem brain tissue samples. Moderate to strong positive correlations are observed between aflatoxins and ochratoxins, aflatoxins and trichothecenes, and ochratoxins and trichothecenes.

Table 4: Comparison of Mycotoxin Levels in Postmortem Brain Tissue Among Different Regions

Region	Aflatoxins (ng/g)	Ochratoxins (ng/g)	Trichothecenes (ng/g)
Frontal Lobe	16.5	11.2	9.4
Temporal Lobe	14.8	12.6	10.1
Occipital Lobe	13.2	10.8	9.8

Table 5 presents the mycotoxin levels in postmortem brain tissue samples obtained from individuals diagnosed with Alzheimer's disease, Parkinson's disease, and healthy controls. Compared to healthy controls, elevated levels of aflatoxins, ochratoxins, and trichothecenes are observed in individuals with neurological disorders, suggesting a potential association between mycotoxin exposure and neurological conditions.

Table 5: Mycotoxin Levels in Postmortem Brain Tissue of Individuals with Neurological Disorders

Neurological Disorder	Aflatoxins (ng/g)	Ochratoxins (ng/g)	Trichothecenes (ng/g)
Alzheimer's Disease	18.2	13.5	11.2
Parkinson's Disease	17.6	12.8	10.6
Healthy Controls	12.4	10.2	8.5

4. Discussion

This study shed light on the quantitative assessment of postmortem mycotoxin levels in brain tissue, providing valuable insights into the forensic implications of mycotoxin exposure. Several key findings have emerged through comprehensive analyses, necessitating careful consideration and comparison with existing literature to elucidate their significance.

One crucial aspect to consider in discussing postmortem mycotoxin levels in brain tissue is the potential sources of exposure and accumulation pathways. Mycotoxins can enter the human body through various routes, including ingestion of contaminated food and beverages, inhalation of fungal spores or mycotoxin-contaminated dust, and dermal contact with contaminated surfaces (C. G. Awuchi et al., 2022). Understanding the primary sources of mycotoxin exposure and their interactions with physiological systems is essential for elucidating their forensic significance and health implications. The metabolism and biotransformation of mycotoxins within the body can influence their distribution, persistence, and toxicological effects. While mycotoxins are primarily eliminated through hepatic metabolism and renal excretion, some compounds may undergo enterohepatic circulation or metabolic activation, forming toxic metabolites with enhanced bioactivity (Okoye et al., 2022). Therefore, investigating the metabolism of mycotoxins in postmortem tissues is crucial for understanding their fate and potential contributions to the cause of death.

The interaction between mycotoxins and other environmental toxins or predisposing factors may exacerbate their neurotoxic effects and contribute to the development of neurological disorders. For instance, synergistic interactions between mycotoxins and heavy metals, pesticides, or neuroinflammatory mediators could amplify neurotoxicity and increase the risk of neurodegenerative diseases (X. Pei et al., 2021). Therefore, comprehensive toxicological assessments should consider potential co-exposures and their additive or synergistic effects on neurological health.

In addition to neurodegenerative diseases, mycotoxin exposure has been implicated in the pathogenesis of psychiatric disorders, such as depression, anxiety, and schizophrenia. Emerging evidence suggests that mycotoxins, particularly trichothecenes and fumonisins, may disrupt neurotransmitter balance, alter neuronal signalling pathways, and induce neuroinflammation, contributing to the onset or exacerbation of psychiatric symptoms (Nguyen et al., 2022). Therefore, exploring the relationship between mycotoxin exposure and psychiatric morbidity in postmortem brain tissue could provide valuable insights into the aetiology and pathophysiology of mental health disorders. The impact of genetic predispositions and individual susceptibilities on mycotoxin toxicity and neurotoxicity warrants investigation. Genetic polymorphisms in detoxification enzymes, transporters, or target receptors may influence an individual's susceptibility to mycotoxin-induced neurotoxicity and modify their risk of developing neurological diseases (Xingyao Pei et al., 2021). Therefore, integrating genetic analyses into forensic studies of mycotoxin exposure could enhance our understanding of interindividual variability in toxin metabolism and toxicity profiles. Another important consideration is the potential contribution of chronic mycotoxin exposure to neurodevelopmental disorders, particularly in vulnerable populations such as infants, children, and pregnant women. Prenatal or early-life exposure to mycotoxins, either through maternal ingestion or environmental contamination, may disrupt neurodevelopmental processes, impair cognitive function, and increase the risk of developmental disorders, including autism spectrum disorders and attention deficit hyperactivity disorder (ADHD) (Adaku Chilaka & Mally, 2020). Therefore, assessing mycotoxin levels in postmortem brain tissue from individuals with neurodevelopmental disorders could elucidate their role in disease pathogenesis and inform early intervention and prevention strategies.

Investigating the spatial distribution of mycotoxins within the brain and their association with specific neuropathological changes could provide valuable insights into their neurotoxic mechanisms and regional vulnerability. Different brain regions may exhibit varying levels of mycotoxin accumulation due to differences in blood-brain barrier permeability, metabolic activity, and neurotransmitter distribution (Chinaza Godseill Awuchi et al., 2022). Therefore, conducting histopathological analyses alongside mycotoxin quantification in postmortem brain tissue could facilitate correlations between toxin deposition patterns and neuronal damage, shedding light on the underlying mechanisms of mycotoxin-induced neurotoxicity. Exploring the temporal dynamics of mycotoxin accumulation and clearance in postmortem tissues could enhance our understanding of postmortem interval (PMI) estimation and forensic toxicology. Mycotoxins may undergo degradation or redistribution over time, influenced by temperature, humidity, and microbial activity (Bonicelli et al., 2022). Therefore, characterising the kinetics of mycotoxin degradation in cadaveric tissues and its implications for PMI estimation could improve the accuracy of forensic investigations and enhance postmortem interval determinations.

Developing standardised protocols and reference materials for mycotoxin analysis in postmortem tissues ensures data reliability, reproducibility, and comparability across studies. Harmonising analytical methodologies, validation procedures, and quality control measures will facilitate cross-validation of results and meta-analyses to assess global trends in mycotoxin exposure and toxicity (Chatterjee et al., 2023). Collaborative efforts among forensic laboratories, regulatory agencies, and academic institutions are needed to establish consensus guidelines and proficiency testing programs for mycotoxin analysis in postmortem tissues. Integrating advanced analytical techniques, such as mass spectrometry imaging (MSI) and high-resolution microscopy, could provide spatially resolved information on mycotoxin distribution, cellular localisation, and tissue-specific toxicity profiles (Righetti et al., 2022). By combining molecular imaging with quantitative mycotoxin analysis, researchers can elucidate the subcellular mechanisms of mycotoxin-induced neurotoxicity and identify potential biomarkers of exposure and toxicity in postmortem brain tissue.

Furthermore, conducting longitudinal studies to track mycotoxin exposure and neurobehavioral outcomes in human populations could provide valuable insights into the long-term health effects of chronic mycotoxin exposure and inform preventive interventions and regulatory policies. By prospectively monitoring mycotoxin levels in biological samples, such as blood, urine, or cerebrospinal fluid, researchers can assess temporal trends in exposure, identify highrisk populations, and evaluate the effectiveness of mitigation strategies (Omotayo et al., 2019).

The strengths of this study are its comprehensive quantitative analysis of postmortem mycotoxin levels in brain tissue, enhancing understanding of forensic implications. However, reliance on observational cross-sectional design may limit causal inference and generalizability of findings.

5. Conclusion

In conclusion, this study highlights the importance of quantitatively assessing postmortem mycotoxin levels in brain tissue for forensic analysis. It reveals mycotoxin exposure's prevalence, distribution, and potential forensic implications. The study found detectable levels of aflatoxins, ochratoxins, and trichothecenes across diverse demographic groups and brain regions. The findings suggest a potential link between mycotoxin exposure and neurobehavioral outcomes. Further research is needed to understand the mechanisms underlying mycotoxin-induced neurotoxicity and their contribution to neurological diseases. Collaborative efforts among forensic laboratories, regulatory agencies, and academic institutions are needed for standardised analytical protocols and proficiency testing programs.

Conflict of Interest

The author declares no conflict of interest.

Informed Consent

Informed consent was obtained from the participants.

Funding

No external funding was granted for this study.

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