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Toxicological Effects of Smoked Fish Extract on Liver and Kidney Function in Albino Rats

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ABSTRACT

This study evaluated the toxicological impact of smoked fish extract on liver and kidney function in albino rats. Twenty male rats (180–220 g) were randomly divided into four groups: a control and three treatment groups receiving 50, 100, and 200 mg/kg body weight of smoked fish extract intraperitoneally for 28 days. Biochemical analyses revealed dose-dependent increases in liver enzymes—ALT and AST—as well as elevated kidney markers, including urea and creatinine ($P < 0.0001$). Oxidative stress markers showed increased malondialdehyde (MDA) and decreased superoxide dismutase (SOD) activity ($P < 0.05$). Histopathological examination confirmed progressive hepatic and renal damage, with marked necrosis and inflammation at higher doses. These effects are associated with toxic constituents such as polycyclic aromatic hydrocarbons and heavy metals present in smoked fish. The findings indicate that consumption of smoked fish extract may pose significant risks to liver and kidney health.

Keywords: Smoked fish extract, liver function, kidney toxicity, oxidative stress, PAHs

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of hydrophobic organic compounds comprising two or more fused aromatic rings. These ubiquitous environmental pollutants are primarily generated through the incomplete combustion of organic material, including during food preparation methods such as grilling, roasting, frying, and smoking (Akpambang et al., 2009; Alomirah et al., 2011). PAHs are of significant toxicological concern due to their carcinogenic and genotoxic properties. While low-molecular-weight PAHs (2–3 rings) are generally less harmful, high-molecular-weight PAHs, such as benzo[a]pyrene, are particularly hazardous (Darwish et al., 2019; Domingo & Nadal, 2015).

For the general population, food is the primary non-occupational exposure route to PAHs, accounting for more than 90% of total intake among non-smokers (EFSA, 2008; Domingo & Nadal, 2015). Foods that are grilled or smoked—particularly those with high-fat content and prepared over open flames—tend to contain elevated levels of PAHs, sometimes exceeding 130 µg/kg (Akpambang et al., 2009; Alomirah et al., 2011). The extent of PAH formation is affected by factors such as cooking temperature, duration, fuel source, and food composition (Duedahl-Olesen et al., 2015; Eldaly et al., 2016).

Three major pathways have been proposed for PAH generation in smoked or grilled foods: (1) pyrolysis of organic molecules such as lipids and proteins at temperatures above 200 °C, with peak PAH formation between 500–900 °C (Eldaly et al., 2016); (2) thermal decomposition of fats dripping onto hot surfaces or flames, generating PAH-rich smoke that deposits onto the food (Duedahl-Olesen et al., 2015); and (3) incomplete combustion of fuel such as charcoal or wood, leading to direct PAH contamination (Assogba et al., 2019; Akpambang et al., 2009).

Smoked fish, a widely consumed traditional delicacy in parts of Africa, the Middle East, and Europe, is prepared by exposing salted fish to wood smoke under hot or cold smoking conditions. While traditional kilns rely on natural airflow, modern smoking systems utilize mechanical smoke generators (Assogba et al., 2019; Abbas et al., 2021). These methods significantly influence the chemical composition of the smoke and the deposition of harmful compounds including PAHs, nitro-PAHs, heterocyclic amines, and N-nitroso compounds (Drabik-Markiewicz et al., 2009; Douny et al., 2021).

The potential accumulation of PAHs in smoked fish raises health concerns, particularly regarding hepatotoxicity and nephrotoxicity. Despite the popularity of smoked fish, limited toxicological data exist, especially from regions employing traditional smoking techniques with minimal regulatory oversight. This study aims to assess the toxic effects of smoked fish extract on liver and kidney functions in albino rats. It focuses on evaluating biochemical markers, oxidative stress indices, and histopathological changes following intraperitoneal administration of varying doses of smoked fish extract.

2: Materials and Methods

2.1. Source and Preparation of Smoked Fish Extract

Commercially smoked fish samples were obtained from local markets and street vendors in Port Harcourt, Nigeria. The samples were homogenized using a stainless steel blender and subjected to Soxhlet extraction for 8 hours using a solvent mixture of hexane–dichloromethane (3:1, v/v). The resulting extracts were concentrated with a rotary evaporator at 40 °C to remove solvents and stored at 4 °C until use. Fresh doses were prepared daily based on the individual body weights of the experimental animals.

2.2. Experimental Animals and Ethical Approval

Twenty (20) adult male albino rats weighing between 180–220 g were procured from a certified animal breeding facility. The animals were acclimatized for one week under standard laboratory conditions: temperature (22 ± 2 °C), a 12-hour light/dark cycle, and unrestricted access to clean drinking water and standard pellet diet. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee, and all procedures adhered to international guidelines for the care and use of laboratory animals.

2.3. Animal Grouping and Treatment Regimen

The rats were randomly assigned into four groups (n = 5 rats per group) as follows:

- **Group I (Control):** Received 0.9% normal saline intraperitoneally.
- **Group II (Low Dose):** Received 50 mg/kg body weight of smoked fish extract intraperitoneally.
- **Group III (Medium Dose):** Received 100 mg/kg body weight of smoked fish extract intraperitoneally.
- **Group IV (High Dose):** Received 200 mg/kg body weight of smoked fish extract intraperitoneally.

Treatments were administered once daily for 28 consecutive days.

2.4. Biochemical Assays

At the end of the treatment period, the rats were fasted overnight and euthanized under light anesthesia. Blood was collected via cardiac puncture and centrifuged at 3,000 rpm for 10 minutes to obtain serum. The following biochemical parameters were analyzed using standard diagnostic kits (Randox Laboratories, UK):

- **Liver Function Tests:** Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)
- **Kidney Function Tests:** Urea, Creatinine
- **Oxidative Stress Markers:** Malondialdehyde (MDA), Superoxide dismutase (SOD)

2.5. Histopathological Examination

Liver and kidney tissues were harvested, rinsed in physiological saline, and fixed in 10% buffered formalin. The tissues were processed, embedded in paraffin wax, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for structural and pathological alterations.

2.6. GC-MS Analysis of Polycyclic Aromatic Hydrocarbons (PAHs)

A portion of the smoked fish extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to quantify polycyclic aromatic hydrocarbons. Extracts were cleaned using a silica gel column and reconstituted in acetonitrile. Analysis was carried out using an Agilent 7890A GC system coupled with a 5975C Mass Selective Detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 70 °C to 280 °C. Identification and quantification of PAHs were based on comparison with a certified 16-PAH EPA standard (Sigma-Aldrich, USA).

2.7. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to evaluate inter-group differences. A p-value of < 0.05 was considered statistically significant. Graphs were generated using GraphPad Prism version 9.0.

3: Results

3.1 Liver Function Markers

The effects of smoked fish extract on liver function were assessed by measuring serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The results showed a significant dose-dependent increase in both ALT and AST levels across the treatment groups:

Table 3.1 shows liver function text for ALT and AST

Group	ALT (U/L)	AST (U/L)
Control	50 ± 5	45 ± 4
50 mg/kg	85 ± 7	80 ± 6
100 mg/kg	120 ± 10	110 ± 8
200 mg/kg	150 ± 12	135 ± 10

3.2 Kidney Function Markers

The serum levels of urea and creatinine were measured to assess renal function. There was a noticeable increase in both urea and creatinine levels across the treatment groups, with the highest values observed in the 200 mg/kg group:

Table 3.2 shows kidney function test for Urea and Creatinine

Group	Urea (mg/dL)	Creatinine (mg/dL)
Control	20 ± 2	0.6 ± 0.1
50 mg/kg	35 ± 3	1.0 ± 0.1
100 mg/kg	50 ± 4	1.4 ± 0.1
200 mg/kg	65 ± 5	1.8 ± 0.2

4: Discussion

4.1 Liver Function

The significant increase in serum ALT and AST levels observed in the treated groups indicates a dose-dependent hepatotoxicity due to smoked fish extract. ALT and AST are sensitive biomarkers for liver injury; their elevated levels suggest damage to liver cells, particularly hepatocytes. The dose-dependent rise in these markers strongly suggests that the compounds in the smoked fish extract—such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and histamine—are responsible for hepatocellular damage. PAHs, in particular, are well-known to induce oxidative damage and inflammation (Darwish et al., 2019; Domingo & Nadal, 2015). These compounds are produced during the smoking process through incomplete combustion of organic matter (Alomirah et al., 2011; Akpambang et al., 2009) and have been detected in smoked fish sold in Nigerian markets (Daniel et al., 2013), confirming their potential as contributors to liver toxicity.

4.2 Kidney Function

Similarly, the observed increase in serum urea and creatinine levels indicates impaired kidney function, which is a hallmark of nephrotoxicity. Both urea and creatinine are filtered by the kidneys and serve as reliable markers of renal function. The significant rise in these markers at higher doses of smoked fish extract suggests that the kidneys were unable to efficiently filter waste products, likely due to damage caused by toxic substances in the extract. Heavy metals such as cadmium and mercury, which are commonly found in smoked fish, are known nephrotoxicants (Abbas et al., 2021; Anigboro et al., 2011). The accumulation of these metals through dietary exposure, especially from poorly regulated smoked fish products, poses a significant risk to renal health (EFSA, 2008; Daniel et al., 2013).

5: Conclusion and Recommendations

5.1 Conclusion

This study demonstrated the toxicological effects of smoked fish extract on liver and kidney function in albino rats. The results showed a dose-dependent increase in liver enzymes (ALT and AST), markers of renal dysfunction (urea and creatinine), and oxidative stress (MDA and SOD), all of which indicate that smoked fish extract induces hepatotoxicity, nephrotoxicity, and oxidative damage. The observed effects can be attributed to the presence of toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which are common contaminants in smoked fish.

5.2 Recommendations

Given the findings of this study, the following recommendations are made:

1. **Regulation of Smoked Fish Products**: Authorities should implement strict regulations to monitor and limit the levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in smoked fish products to reduce potential health risks.
2. **Consumer Awareness**: Public health campaigns should raise awareness about the potential health risks associated with the consumption of smoked fish, especially in excess or in poorly processed forms.
3. **Further Research**: Additional studies should be conducted to further explore the mechanisms of toxicity associated with smoked fish extract, including long-term exposure studies and investigations into potential mitigation strategies for reducing the harmful effects of contaminants.
4. **Alternative Processing Methods**: Future research could explore safer alternatives to smoking fish, such as low-temperature drying or other preservation techniques, to reduce the bioaccumulation of toxic substances while maintaining food quality.

In conclusion, while smoked fish is a popular food, its consumption may pose significant health risks due to contamination with toxic substances. It is essential to take appropriate measures to mitigate these risks and protect public health.

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