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Dietary smoked fish consumption induces genotoxicity in experimental rats

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Abstract
Smoked fish is an important dietary protein source; however, traditional fuelwood smoking can generate polycyclic aromatic hydrocarbons (PAHs), several of which are genotoxic following metabolic activation. This study investigated the association between dietary PAH exposure from smoked fish and genotoxic responses in experimental rats. Thirty-five male albino rats were randomly assigned to five dietary groups (n = 7), comprising a control diet formulated with oven-dried fish and four diets containing increasing proportions of fuelwood-smoked fish. Experimental feeds were analyzed for PAH concentrations, proximate composition, phytochemicals, and antioxidant capacity, while genotoxicity was assessed using liver and kidney DNA fragmentation and micronucleus frequency. PAH concentrations increased with smoked fish inclusion, with the highest exposure diet recording elevated concentrations of benz(a)anthracene (2.04 ± 0.04 mg/kg), chrysene (1.19 ± 0.02 mg/kg), indeno(1,2,3-cd) pyrene (69.89 ± 1.40 mg/kg), and benzo(ghi)perylene (44.19 ± 0.88 mg/kg). Benzo(a)pyrene was detected only in the two highest exposure diets (0.101 ± 0.00 and 0.098 ± 0.00 mg/kg). The control diet exhibited the highest protein content (25.88 ± 0.05%) and antioxidant activity, including total flavonoids (203.62 ± 5.76 mg/L), FRAP (80.32 ± 4.32), total antioxidant capacity (651.42 ± 25.93), DPPH radical scavenging activity (82.42 ± 2.38%), and total phenolic content (267.98 ± 5.64 mg/L), whereas the highest smoked fish diet showed the weakest antioxidant performance. DNA fragmentation exhibited tissue-specific alterations, while micronucleus frequency increased progressively with smoked fish inclusion, indicating enhanced chromosomal damage. These findings demonstrate that dietary exposure to PAH-contaminated smoked fish induces measurable genotoxicity and highlight the need for improved smoking technologies, routine PAH surveillance, and strengthened food safety measures to minimize human exposure.

Keywords: Smoked fish, polycyclic aromatic hydrocarbons, genotoxicity, DNA fragmentation, micronucleus assay, food toxicology

Introduction

Smoked fish is an important source of high-quality dietary protein and constitutes a staple food in many low- and middle-income countries owing to its extended shelf life, affordability, and desirable sensory properties. Traditional fuelwood smoking remains the predominant preservation method in many regions; however, incomplete combustion of wood generates complex mixtures of polycyclic aromatic hydrocarbons (PAHs) that readily deposit on the surface of smoked foods. Consequently, smoked fish has become a recognized dietary source of PAHs, raising concerns regarding food safety and long-term health risks associated with repeated consumption (Adeyemi & Adeyemi, 2020) [3].

Polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants formed during the incomplete combustion of organic materials and are widely recognized for their mutagenic and carcinogenic properties. Although PAHs are chemically inert, many require metabolic activation before exerting toxic effects. Phase I metabolism, primarily mediated by cytochrome P450 enzymes, converts PAHs into highly reactive electrophilic intermediates, including diol epoxides, capable of covalently binding to DNA and forming stable DNA adducts (Audebert *et al*., 2012; Henkler *et al*., 2012; Koschembahr *et al*., 2019; Maciejczyk *et al*., 2023) [6, 32, 39, 51]. If these lesions escape cellular repair mechanisms, they can initiate mutations, chromosomal abnormalities, and malignant transformation (Feng *et al*., 2025; Hardonnière *et al*., 2017) [20, 30]. In addition to direct DNA adduction, PAH biotransformation generates excessive reactive oxygen species (ROS), resulting in oxidative DNA damage, strand breaks, lipid peroxidation, and impairment of cellular macromolecules (Parrado *et al*., 2019) [61]. Benzo[a]pyrene represents one of the most extensively studied PAHs because its reactive diol-epoxide metabolite exhibits a high affinity for DNA and is strongly implicated in chemical carcinogenesis (Géniès, 2013; Youssef *et al*., 2022) [24, 83]. Furthermore, PAHs activate the aryl hydrocarbon receptor (AhR), leading to induction of CYP1A and CYP1B enzymes, thereby amplifying the formation of reactive metabolites and oxidative injury (Sombiri *et al*., 2024; Miranda-Guevara *et al*., 2023) [54, 73].

Dietary intake represents one of the principal routes of human exposure to PAHs because foods processed by smoking, grilling, roasting, or other high-temperature methods readily accumulate these contaminants. Frequent consumption of PAH-contaminated foods has been associated with increased oxidative stress, genomic instability, and elevated risks of chronic diseases, including cancer, particularly in tissues involved in digestion, metabolism, and detoxification (Iwu *et al*., 2024) [35]. Once metabolically activated, PAHs induce genetic injury through multiple mechanisms, including the formation of bulky DNA adducts, oxidative modification of DNA bases, single- and double-strand DNA breaks, and disruption of chromosomal integrity. Persistent accumulation of these

lesions can overwhelm cellular DNA repair systems, resulting in irreversible mutations and genomic instability (Melki, 2017) [53].

Reliable biomarkers are therefore essential for evaluating the genotoxic potential of dietary PAH exposure. DNA fragmentation is a sensitive indicator of oxidative DNA damage and apoptotic cell death, reflecting irreversible disruption of genomic integrity following chemical insult. Similarly, the micronucleus assay is an established and widely accepted biomarker of chromosomal damage because it detects both chromosome breakage (clastogenicity) and whole-chromosome loss (aneugenicity) during cell division (Kuo *et al*., 2022) [43]. The assay has become a cornerstone of genetic toxicology due to its sensitivity, simplicity, and reproducibility, and is extensively applied in toxicological risk assessment, regulatory testing, biomonitoring, and biodosimetry studies (Luzhna *et al*., 2013; Sommer *et al*., 2020; Wills *et al*., 2021) [48, 74]. Furthermore, combining micronucleus assessment with complementary biomarkers such as DNA fragmentation and γH2AX foci provides a more comprehensive evaluation of chemically induced genomic damage, particularly double-strand DNA breaks and chromosomal instability (Takeiri *et al*., 2019; Poblete-Naredo & Albores, 2016) [62, 77]. Consequently, the combined assessment of DNA fragmentation and micronucleus formation offers a robust approach for characterizing the genotoxic consequences of chronic dietary exposure to PAHs.

Despite increasing concerns regarding dietary exposure to PAHs, relatively few studies have simultaneously characterized the contaminant burden of smoked fish diets and examined their nutritional composition, phytochemical constituents, antioxidant capacity, and genotoxic consequences within a single experimental model. Understanding these interrelationships is essential because the biological response to dietary PAHs may be influenced not only by the level of contaminant exposure but also by the antioxidant and phytochemical composition of the diet, which can modulate oxidative stress and cellular defense mechanisms. Consequently, an integrated assessment of dietary composition alongside established biomarkers of genotoxicity provides a more comprehensive understanding of the toxicological risks associated with consumption of traditionally smoked fish.

The present study was therefore designed to evaluate the genotoxic effects of dietary smoked fish consumption in experimental rats by linking the PAH burden of smoked fish-based diets with their proximate composition, phytochemical profile, antioxidant capacity, DNA fragmentation, and micronucleus formation. The findings are expected to provide mechanistic insights into the contribution of dietary PAH exposure to genomic instability and to generate evidence that supports improved fish-smoking practices, routine monitoring of carcinogenic PAHs, and the development of effective food safety strategies aimed at reducing human exposure to these contaminants.

Materials and Methods

Experimental Animals

Thirty-five healthy adult male albino rats weighing 10–180 gwere used for the study. The animals were housed in clean polypropylene cages under standard laboratory conditions (25 ± 2°C, 50–60% relative humidity, and a 12 h light/12 h dark photoperiod) with free access to feed and drinking water. Animals were acclimatized for seven days before the commencement of the experiment. Experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals (National Research Council, 2011) [60].

Preparation of Experimental Diets

Fresh African catfish (*Clarias gariepinus*) were obtained from a commercial fish farm in Delta State, Nigeria. Half of the fish samples were oven-dried at 65°C to produce the control fish meal, while the remaining samples were processed by traditional fuelwood smoking. The processed fish samples were separately milled into fine powder and incorporated into experimental diets.

Five experimental diets were formulated by replacing oven-dried fish meal with increasing proportions of smoked fish meal while maintaining similar quantities of other dietary ingredients. Corn starch (55%), maize cob (5%), granulated sugar (9%), vegetable oil (5%), and vitamin-mineral premix (2%) were kept constant in all diets, whereas smoked fish meal was incorporated at graded concentrations of 0, 6, 12, 18, and 24%.

Experimental Design

Following acclimatization, rats were randomly assigned into five groups (n = 7). Group I received the control diet containing oven-dried fish meal, whereas Groups II–V received diets containing progressively increasing proportions of smoked fish meal. The feeding trial lasted for 28 consecutive days, during which feed and water were supplied *ad libitum*. At the end of the experiment, animals were fasted overnight and humanely sacrificed for sample collection.

Determination of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) were determined in the formulated diets following extraction with an appropriate organic solvent and analysis by gas chromatography coupled with mass spectrometry (GC–MS) according to United States Environmental Protection Agency (USEPA) Method 8270D (USEPA, 2014). The sixteen priority PAHs designated by the USEPA were quantified, and concentrations were expressed as mg/kg of diet.

Proximate Analysis

Moisture, crude protein, crude lipid, crude fibre, ash, and carbohydrate contents of the experimental diets were determined using standard analytical procedures described by the Association of Official Analytical Chemists (AOAC, 2023) [5]. Carbohydrate content was calculated by difference.

Phytochemical Analysis

Total phenolics, flavonoids, tannins, saponins, steroids, and cardiac glycosides were quantified using established spectrophotometric procedures. Total phenolic content was determined using the Folin–Ciocalteu method (Singleton *et al*., 1999), while total flavonoids were estimated using the aluminium chloride colorimetric method (Chang *et al*., 2002) [13]. Tannins, saponins, steroids, and cardiac glycosides were determined according to the methods described by Harborne (1998) [29] and Sofowora (2008) [72].

Determination of Antioxidant Capacity

Methanolic extracts of the experimental diets were prepared for antioxidant analyses. Ferric reducing antioxidant power (FRAP) was determined according to Benzie and Strain (1996) [8]. Free radical scavenging activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Brand-Williams *et al*. (1995). [11] Total antioxidant capacity (TAC) was determined using the phosphomolybdenum method of Prieto *et al*. (1999) [63]. Total phenolic and flavonoid contents were also determined as indices of antioxidant potential.

DNA Fragmentation Assay

DNA fragmentation was determined using the diphenylamine colorimetric assay described by Gercel-Taylor (2005) [25]. Briefly, tissue homogenates were prepared in Tris-EDTA buffer containing Triton X-100 and centrifuged at 27,000 × *g* for 20 min to separate fragmented DNA from intact chromatin. DNA concentrations in both fractions were quantified using diphenylamine reagent, and absorbance was measured at 620 nm. Percentage DNA fragmentation was calculated as

$$\%DNA\ Fragmentation = \frac{\text{Fragmented DNA}}{\text{Fragmented DNA} + \text{Intact DNA}} X100$$

Micronucleus Assay

Micronucleus frequency was evaluated using the method described by Hooftman and de Raat (1982). [34] Peripheral blood smears were prepared immediately after sample collection, air-dried, fixed in absolute methanol, stained with 5% Giemsa solution, and examined under a light microscope at ×1000 magnification. Micronucleated erythrocytes were scored according to established criteria, and micronucleus frequency was expressed as the number of micronucleated cells per total erythrocytes examined.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Differences among treatment groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Values of $p < 0.05$ were considered statistically significant.

Results

PAH Concentrations in Experimental Feed

The PAH profile differed clearly across the five diets (Table 1). Group 1 generally showed the lowest concentrations, while Groups 4 and 5 carried higher levels of high-molecular-weight PAHs. Benzo(a)pyrene was detected only in Groups 4 and 5. The results demonstrate that the smoked fish diets, particularly in Group 5, contained substantially higher concentrations of carcinogenic PAHs such as Benz(a)anthracene, chrysene, benzo(b/k) fluoranthene and benzo(a)pyrene than the control diet. The progressive increases in these PAHs and the appearance of detectable benzo(a)pyrene in Groups 4 and 5 suggest that fuelwood smoking significantly increased the accumulation of carcinogenic PAHs in the experimental diets.

Table 1: Polycyclic Aromatic Hydrocarbon (PAH) Concentrations (mg/kg ± SEM) in Experimental Feed

PAH Compound	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
Naphthalene	6.64 ± 0.13^{d}	14.86 ± 0.30^{b}	11.67±0.23^{c}	11.98±0.24^{c}	13.17±0.26^{b}
Acenaphthylene	0.153± 0.003^{c}	2.335±0.046^{a}	0.078±0.002^{d}	0.548±0.011^{b}	0.580±0.012^{b}
Acenaphthene	ND	ND	ND	2.950 ± 0.06^{a}	2.328 ± 0.05^{b}
Fluorene	0.154± 0.003^{d}	0.126± 0.003^{d}	0.288±0.006^{b}	0.502±0.010^{a}	0.628±0.013^{a}
Phenanthrene	2.24 ± 0.04^{d}	1.53 ± 0.03^{e}	3.70 ± 0.07^{b}	3.84 ± 0.08^{b}	4.28 ± 0.09^{a}
Anthracene	0.257±0.005^{d}	1.98 ± 0.04^{b}	4.65 ± 0.09^{a}	0.815 ± 0.02^{c}	0.878 ± 0.02^{c}
Fluoranthene	0.663 ± 0.01^{d}	0.0648±0.001^{c}	1.15 ± 0.02^{c}	1.32 ± 0.03^{b}	1.27 ± 0.03^{b}
Pyrene	0.786 ± 0.02^{c}	0.005±0.0001^{c}	1.08 ± 0.02^{b}	1.53 ± 0.03^{a}	1.45 ± 0.03^{a}
Benz(a)anthracene	0.297 ± 0.01^{c}	0.000^{d}	0.868 ± 0.02^{b}	0.357 ± 0.01^{c}	2.04 ± 0.04^{a}
Chrysene	0.131± 0.003^{d}	ND	0.479 ± 0.01^{b}	0.168±0.004^{c}	1.19 ± 0.02^{a}
Benzo(b/k) fluoranthene	0.436 ± 0.01^{c}	ND	0.209 ± 0.00^{d}	0.612 ± 0.01^{b}	0.558 ± 0.01^{b}
Benzo(a)pyrene	ND	ND	ND	0.101 ± 0.00^{a}	0.098 ± 0.00^{a}
Indeno(1,2,3-cd) pyrene	34.93 ± 0.70^{d}	61.07 ± 1.22^{b}	46.46 ± 0.93^{c}	53.12 ± 1.06^{c}	69.89 ± 1.40^{a}
Benzo(ghi)perylene	22.07 ± 0.44^{d}	38.82 ± 0.78^{b}	29.55 ± 0.59^{c}	33.67 ± 0.67^{c}	44.19 ± 0.88^{a}

Proximate and phytochemical composition

The diets differed significantly in proximate composition (Table 1). Group 1 had the highest protein and fibre values, whereas Group 4 had the highest carbohydrate content. Group 5 showed lower protein and fibre values, suggesting a shift away from protein- and fibre-rich composition toward a more energy-dense feed matrix. The significant elevation of ash content in Group 2 indicates enhanced mineral loading, likely attributable to increased inclusion of mineral-rich ingredients such as fish meal or inorganic additives. The markedly low ash content in Group 4 suggests dilution of mineral components, possibly due to increased carbohydrate or lipid fractions, which aligns with its high CHO profile.

Table 2: Proximate analysis (%) of Experimental Feed

Group	Moisture (Mean ± SEM)	Ash (Mean ± SEM)	Lipid (Mean ± SEM)	Protein (Mean ± SEM)	Fibre (Mean ± SEM)	CHO (Mean ± SEM)
1	19.93 ± 0.018^{d}	4.92 ± 0.012^{e}	24.37 ±0.029^{b}	25.88 ± 0.050^{a}	3.12 ± 0.015^{a}	25.02 ± .163^{e}
2	18.94 ± 0.021^{c}	7.03 ± 0.020^{e}	25.34 ± .101^{b}	19.20 ± 0.075^{d}	2.74 ± 0.007^{b}	28.98 ± .462^{d}
3	13.05 ± 0.029^{a}	4.83 ± 0.044^{b}	23.68 ± .083^{c}	24.46 ± 0.394^{b}	2.58 ± 0.015^{b}	34.62 ± .280^{b}
4	13.81 ± 0.056^{b}	2.69 ± 0.098^{a}	24.65 ± .082^{b}	23.25 ± 0.020^{c}	2.48 ± 0.033^{b}	35.51 ± .162^{a}
5	18.96 ± 0.023^{c}	6.53 ± 0.033^{d}	22.74 ± .060^{d}	18.95 ± 0.192^{e}	1.73 ± 0.142^{c}	33.22 ± .456^{c}

Phytochemical composition also varied by diet (Table 3). Groups 4 and 5 were richer in bioactive constituents, particularly tannins, phenols, flavonoids and cardiac glycosides, while Groups 2 and 3 generally showed lower phytochemical density.

Table 3: Phytochemical Composition of Experimental Feed (Mean ± SEM)

Group	Saponins (mg/100 g)	Tannins (mg/100g)	Phenols (mg/100g GAE)	Flavonoids (mg/100g QE)	Steroids (mg/100g)	Cardiac Glycosides (mg/100g)
1	18.20± 1.25^{c}	5.47 ± 0.82^{b}	6.60 ± 0.81^{c}	10.20 ± 0.57^{c}	6.50 ± 0.79^{b}	9.20 ± 0.88^{b}
2	14.40± 1.20^{d}	3.02 ± 0.17^{c}	4.59 ± 0.30^{d}	7.47 ± 0.84^{d}	4.55 ± 0.54^{c}	7.75 ± 0.70^{b}
3	12.60± 1.03^{d}	2.50 ± 0.12^{c}	3.47 ± 0.20^{d}	5.42 ± 0.64^{d}	3.70 ± 0.31^{c}	5.52 ± 0.60^{c}
4	26.40± 1.56^{a}	10.10 ± .01^{a}	11.40± 0.90^{a}	18.38± 1.43^{a}	10.50 ± .96^{a}	13.1 ± 1.26^{a}
5	20.50± 1.46^{b}	10.57 ± .95^{a}	9.43 ± 0.78^{b}	14.93± 1.24^{b}	7.40 ± 0.88^{b}	11.50± 0.99^{a}

Antioxidant Capacity of Feed Extracts

Table 4 demonstrates significant ($p < 0.05$) concentration-dependent variations in total flavonoid content among the experimental feeds, reflecting differences in their antioxidant potential. Group 1 consistently recorded the highest flavonoid content across all extract concentrations (100–300 mg/L), while Group 5 exhibited the lowest values throughout the study. The flavonoid content increased with increasing extract concentration in all groups, the magnitude of this increase varied, with Group 1 showing the greatest enhancement and Group 5 the least.

Table 4: Total Flavonoid Content (mg/L) of Experimental Feed Extracts at Differential Concentrations (100, 200 and 300 mg/L)

Group	Total Flavonoids (100 mg/L)	Total Flavonoids (200 mg/L)	Total Flavonoids (300 mg/L)
1	181.52 ± 44.76^{d}	188.25 ± 25.97^{d}	203.62 ± 5.76^{d}
2	104.63 ± 1.14^{b}	118.08 ± 6.03^{b}	120.72 ± 1.56^{b}
3	164.37 ± 44.54^{c}	171.07 ± 27.75^{c}	178.80 ± 17.46^{c}
4	143.77 ± 28.29^{c}	150.20 ± 32.13^{c}	158.30 ± 37.41^{c}
5	73.80 ± 4.97^{a}	81.17 ± 5.87^{a}	90.43 ± 3.58^{a}

Table 5 demonstrates significant concentration-dependent differences ($p < 0.05$) in the ferric reducing antioxidant power (FRAP) of the experimental feed extracts, indicating marked variations in antioxidant capacity among the feed formulations. FRAP values increased with extract concentration across all groups, although the magnitude of the increase differed significantly. Group 1 consistently exhibited the highest reducing power at all concentrations, reflecting the greatest abundance of electron-donating phytochemicals, whereas Group 5 recorded the lowest antioxidant activity throughout the study. The antioxidant potential followed the order: Group 1 > Groups 3 and 4 > Group 2 > Group 5.

Table 5: Ferric Reducing Antioxidant Power (FRAP) of Experimental Feed Extracts at 100, 200, and 300 mg/L Concentrations (Mean ± SEM)

Group	FRAP 100 mg/L (Mean ± SEM)	FRAP 200 mg/L (Mean ± SEM)	FRAP 300 mg/L (Mean ± SEM)
1	64.87 ± 5.32^{e}	71.98 ± 3.38^{e}	80.32 ± 4.32^{e}
2	40.95 ± 4.30^{c}	44.07 ± 7.58^{d}	59.03 ± 2.06^{c}
3	46.56 ± 4.42^{d}	55.34 ± 4.91^{c}	64.83 ± 1.46^{d}
4	44.28 ± 1.28^{c}	55.73 ± 3.88^{e}	64.83 ± 1.46^{d}
5	35.14 ± 2.83^{a}	45.20 ± 2.47^{d}	55.81 ± 3.77^{b}

Table 6 shows statistically significant ($p < 0.05$) differences in the total antioxidant capacity (TAC) of the experimental feed extracts, reflecting marked variation in their overall antioxidant potential. Group 1 consistently exhibited the highest TAC values across all concentrations, indicating the richest antioxidant composition, while Group 5 recorded the lowest values throughout the study. Group 3 ranked second, displaying moderate concentration-dependent increases in TAC, whereas Groups 2 and 4 showed intermediate but less consistent antioxidant capacities. Although TAC generally increased with extract concentration, the response varied among groups, with some formulations showing only slight fluctuations, suggesting differences in antioxidant extractability and composition. Overall, the antioxidant capacity followed the order Group 1 > Group 3 > Groups 2 and 4 > Group 5, indicating substantial differences in the redox buffering potential of the feed formulations and suggesting that antioxidant-rich diets may offer greater protection against oxidative stress.

Table 6: Total Antioxidant Capacity (TAC) of Experimental Feed Extracts at 100, 200, and 300 mg/L Concentrations (Mean ± SEM)

Group	TAC 100 mg/L (Mean ± SEM)	TAC 200 mg/L (Mean ± SEM)	TAC 300 mg/L (Mean ± SEM)
1	665.69 ± 17.56^{e}	644.17 ± 11.96^{e}	651.42 ± 25.93^{e}
2	444.31 ± 13.28^{c}	353.71 ± 82.73^{c}	458.36 ± 14.42^{c}
3	562.51 ± 33.60^{d}	572.44 ± 20.80^{d}	587.23 ± 9.37^{d}
4	465.72 ± 47.15^{c}	440.51 ± 51.17^{c}	450.99 ± 27.50^{c}
5	302.62 ± 7.28^{a}	309.38 ± 7.57^{a}	322.95 ± 12.01^{b}

Table 7 demonstrates significant ($p < 0.05$) differences in DPPH radical scavenging activity among the experimental feed extracts, indicating marked variation in their free radical neutralizing capacity. Group 3 exhibited the highest antioxidant activity, particularly at lower concentrations, while Groups 1 and 4 also showed strong and concentration-dependent radical scavenging abilities. In contrast, Group 5 consistently recorded the lowest DPPH activity, reflecting the weakest antioxidant potential, whereas Group 2 displayed moderate but comparatively lower scavenging capacity. Although most groups showed increased activity with higher extract concentrations, Group 3 exhibited a slight decline at the highest concentration, suggesting possible antioxidant saturation or degradation. The antioxidant activity followed the order Group 3 > Group 1 > Group 4 > Group 2 > Group 5, indicating considerable differences in the hydrogen-donating antioxidant composition of the feed formulations and suggesting that feeds with stronger DPPH activity may provide greater protection against oxidative stress.

Table 7: DPPH Radical Scavenging Activity of Experimental Feed Extracts at 100, 200, and 300 mg/L (Mean ± SEM)

Group	DPPH 100 mg/L (Mean ± SEM)	DPPH 200 mg/L (Mean ±SEM)	DPPH 300 mg/L (Mean ± SEM)
1	63.33 ± 2.58^{d}	70.32 ± 3.94^{d}	82.42 ± 2.38^{c}
2	46.66 ± 11.08^{c}	51.04 ± 5.47^{c}	53.27 ± 1.15^{c}
3	73.21 ± 1.07^{e}	73.97 ± 3.38^{e}	66.65 ± 17.69^{d}
4	66.47 ± 2.92^{d}	66.73 ± 1.53^{d}	74.71 ± 2.34^{d}
5	34.32 ± 6.11^{a}	39.18 ± 4.20^{a}	42.31 ± 0.56^{b}

Table 8 shows statistically significant ($p < 0.05$) differences in the total phenolic content of the experimental feed extracts, indicating substantial variation in their phenolic antioxidant composition. Group 1 consistently recorded the highest phenolic content across all concentrations, reflecting the richest source of phenol-based antioxidants, whereas Group 5 exhibited the lowest values throughout the study. Phenolic content generally increased with extract concentration, the magnitude of increase varied among groups, suggesting differences in phenolic composition and extractability. The phenolic content followed the order Group 1 > Group 3 > Group 4 ≈ Group 2 > Group 5, indicating that feeds with higher phenolic levels possess greater antioxidant potential and are likely to provide enhanced protection against oxidative stress.

Table 8: Total Phenolic Content of Experimental Feed Extracts at 100, 200, and 300 mg/L Concentrations (Mean ± SEM)

Group	Total Phenols 100 mg/L (Mean ± SEM)	Total Phenols 200 mg/L (Mean ± SEM)	Total Phenols 300 mg/L (Mean ± SEM)
1	250.37 ± 5.86^{c}	255.13 ± 8.67^{c}	267.98 ± 5.64^{c}
2	180.78 ± 7.50^{c}	184.73 ± 9.85^{c}	202.60 ± 7.97^{c}
3	224.77 ± 18.91^{d}	235.10 ± 14.58^{d}	241.27 ± 13.71^{d}
4	209.40 ± 5.38^{d}	215.03 ± 3.05^{d}	215.63 ± 1.63^{c}
5	148.13 ± 4.26^{a}	153.95 ± 5.90^{a}	159.10 ± 6.17^{a}

DNA Fragmentation and Micronucleus Formation

DNA fragmentation differed between tissues and among treatment groups (Figure 1). In the liver, DNA fragmentation was higher in Groups 1 and 3 and lowest in Group 4. In the kidney, fragmentation was relatively higher in Groups 1, 3 and 5 and lower in Groups 2 and 4.

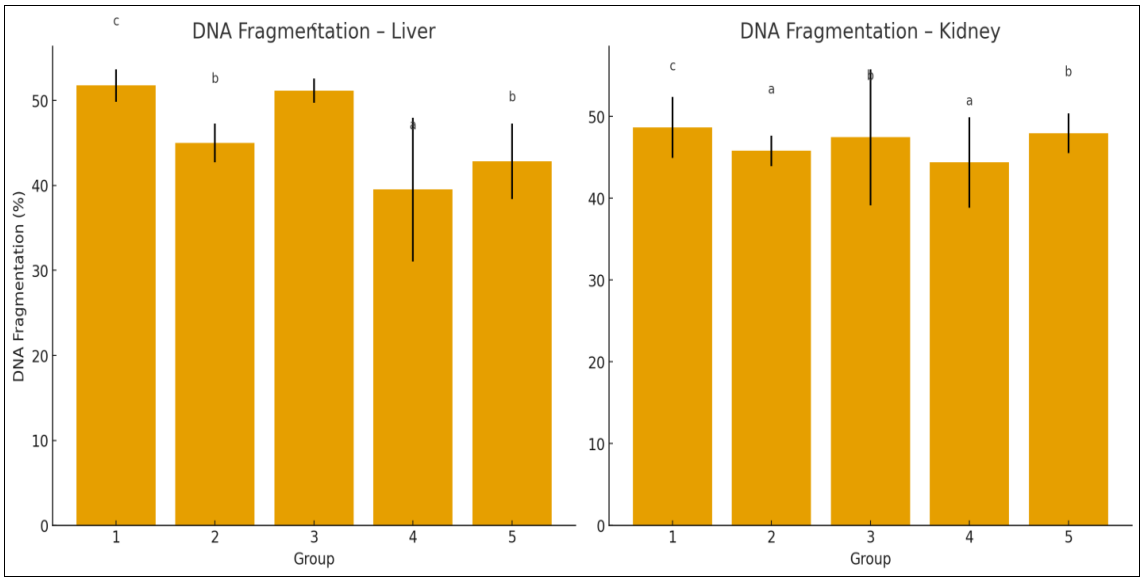

Fig 1: DNA fragmentation (%) in liver and kidney across experimental groups. Bars represent mean ± SEM; lowercase letters indicate significant group differences at $p < 0.05$

Micronucleus frequency increased across the experimental groups (Figure 2). The control group had the lowest frequency, Groups 2 and 3 showed intermediate responses, and Group 5 had the highest values, indicating stronger chromosomal damage at the higher treatment levels.

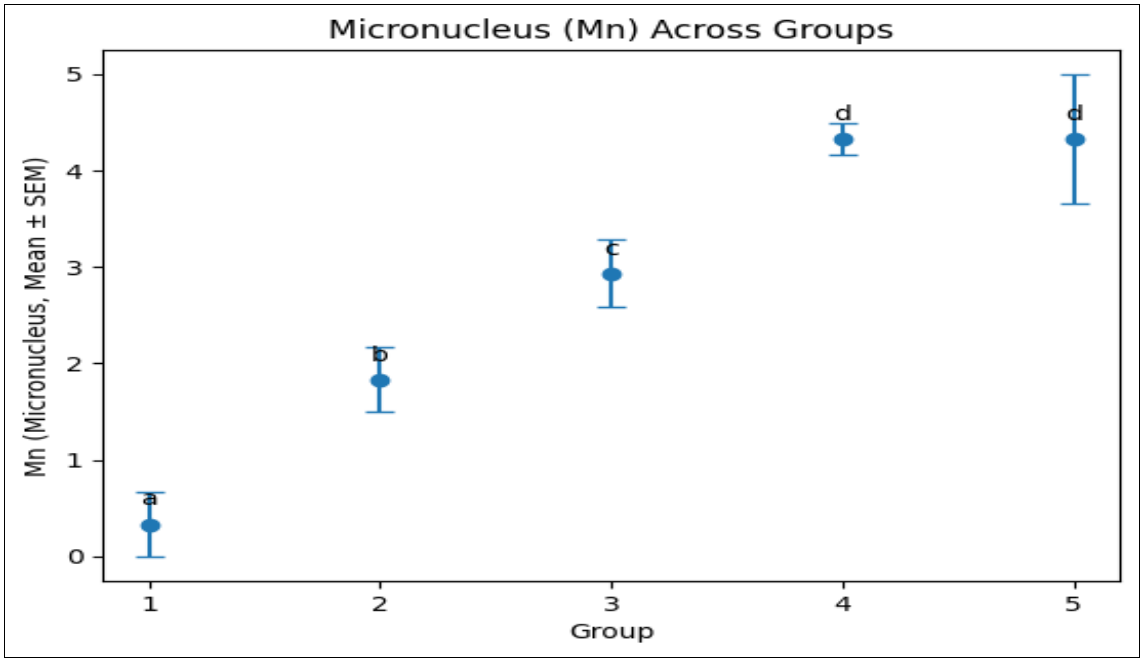

Fig 2: Micronucleus frequency across experimental groups. Points represent mean ± SEM; lowercase letters indicate significant group differences at $p < 0.05$

Discussion

The present study demonstrates that dietary exposure to traditionally smoked fish induces dose-dependent genotoxic effects in experimental rats, evidenced by increased DNA fragmentation and micronucleus formation, alongside progressive increases in dietary polycyclic aromatic hydrocarbon (PAH) concentrations. These findings suggest that traditional fish smoking can introduce genotoxic contaminants into food, thereby posing potential health risks following chronic dietary exposure.

The results show that the smoked fish diets especially Groups 4 and 5 were the most contaminated with high molecular weight PAHs. This pattern is consistent with the lipophilic and persistent nature of PAHs, which allows them to accumulate in organic-rich foods and feeds during smoking or other heat-based processing (Bertotti *et al*., 2025; Kristensen *et al*., 2024) [9, 40]. The relatively low values in the control diet support its use as the baseline group, while the progressive changes in the treated diets suggest that formulation and processing conditions shaped the PAH burden. The rise in low molecular weight PAHs such as naphthalene and acenaphthylene in Groups 2, 4 and 5 may reflect volatilisation and re-condensation during smoking. Naphthalene is more volatile and is often enriched when organic material is exposed to incomplete combustion or smoke (Bertotti *et al*., 2025; Liu *et al*., 2024) [9, 45]. The appearance of acenaphthene only in Groups 4 and 5, together with the increase in fluorene and phenanthrene, points to stronger thermal inputs and a shift toward more stable PAH products (Töpperwien *et al*., 2024; Gómez *et al*., 2025; Ramanathan *et al*., 2025) [26, 64, 80]. The peak anthracene value in Group 3 may indicate that intermediate heating conditions favoured its formation before further transformation into heavier PAH fractions (Wenzel *et al*., 2024). The toxicological concerns are greater for the heavier PAHs such as Benz(a)anthracene, chrysene, benzo(b/k) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd) pyrene and benzo(ghi)perylene that were more prominent in Groups 4 and 5. Benzo(a)pyrene is especially important because it is widely used as an indicator of carcinogenic PAH exposure (Hörchner *et al*., 2025). Its detection only in Groups 4 and 5 suggests that the higher treatment diets reached a contamination threshold at which more hazardous PAHs became measurable. The increase in indeno(1,2,3-cd) pyrene and benzo(ghi)perylene also supports a combustion-related origin (Zuo *et al*., 2024) [86]. The PAH pattern suggests movement from lower PAHs toward pyrogenic, high molecular weight compounds as treatment intensity increased (Dhara & Dutta, 2024) [14]. This raises food-safety concerns because chronic PAH exposure has been linked with oxidative stress, endocrine disruption and carcinogenesis in experimental models (Abubakar & Fatoki, 2026; Beygisangchin *et al*., 2026) [2, 10].

The proximate composition data add an important nutritional context. The higher moisture in Group 1 may reflect a less dehydrated or less intensely processed feed matrix, while the lower moisture in Groups 3 and 4 may indicate improved shelf stability but greater exposure to processing conditions that can favour thermogenic contaminants (Garcia *et al*., 2026; Nascimento *et al*., 2024; Li *et al*., 2025; Liu *et al*., 2025) [23, 44, 59]. The high ash content in Group 2 suggests a greater mineral residue, whereas the lower ash value in Group 4 is consistent with dilution by carbohydrate-rich components (Narcy *et al*., 2024; Gadzama *et al*., 2025; Saejiem *et al*., 2025) [21, 58, 69]. Lipid values were only moderately different, but lipid-rich feeds are more susceptible to oxidative deterioration under heat and oxygen exposure (López *et al*., 2026; López-Hernández *et al*., 2026) [33, 46, 47]. Group 1 had the strongest protein and fibre profile, both of which may support normal

tissue repair, gut function and toxin binding (Abdollahi *et al*., 2025; Mangenya *et al*., 2024) [1]. By contrast, Groups 2 and 5 had lower protein values, while fibre declined progressively from Group 1 to Group 5. A fibre-rich diet can reduce the bioavailability of lipophilic toxicants through adsorption in the gut, while reduced fibre may weaken this protective effect (Duncan *et al*., 2025; Ruiz-Saavedra *et al*., 2023) [16, 66]. The high carbohydrate level in Group 4 suggests a shift toward energy-dense formulation, although excessive reliance on carbohydrate at the expense of protein and fibre may alter metabolic pathways (Hall *et al*., 2024) [28]. This study revealed inverse relationship between ash and carbohydrate content which has been widely reported in formulated feeds, where energy-dense diets often exhibit reduced mineral density (Gadzama *et al*., 2025; Saejiem *et al*., 2025) [21, 69].

The phytochemical profile suggests that the diets differed not only in contaminants and nutrients, but also in bioactive constituents that could modify toxicity. Groups 4 and 5 had higher levels of several phytochemicals, indicating stronger potential to influence redox balance, xenobiotic metabolism and cell signalling (Anitha *et al*., 2025; Kumari *et al*., 2025) [4, 42]. Elevated saponins may affect membrane permeability and cholesterol binding, although high concentrations can interfere with nutrient utilisation (Rathod *et al*., 2024) [65]. Tannins were especially high in Groups 4 and 5. They can bind proteins and digestive enzymes, but they may also provide antioxidant protection through radical scavenging and metal chelation (Taha *et al*., 2025) [76]. The higher phenolic and flavonoid values in Groups 4 and 5 point to a potentially protective antioxidant component. Phenolics act as hydrogen donors and metal chelators, while flavonoids can modulate pathways such as Nrf2 and inflammatory signalling (Panche *et al*., 2016). These compounds may reduce PAH-induced oxidative stress by improving phase II detoxification, including glutathione-S-transferase activity (Zhang *et al*., 2025) [85]. Phytosterols and cardiac glycosides also varied across the diets, and their biological effects may be beneficial or harmful depending on dose and exposure duration (Gao *et al*., 2024; Fender *et al*., 2024) [19, 22]. For this reason, the enriched phytochemical profile of Groups 4 and 5 should be interpreted as a double-edged feature: it may buffer oxidative injury, but it may also introduce antinutritional or toxicodynamic effects (Elshaer *et al*., 2024; Beigh, 2024; Gürbüz *et al*., 2025) [7, 17, 27].

The antioxidant assays helped clarify the functional meaning of the phytochemical data. Total flavonoid content increased with extract concentration but differed widely among groups. Group 1 had the highest flavonoid yield, which may reflect a matrix that preserved or released polyphenols more effectively (Kristiani *et al*., 2024; Saeed *et al*., 2024) [41, 68]. The weak flavonoid response in Group 5 may result from lower intrinsic flavonoid content, thermal degradation or stronger binding of phenolics within the feed matrix (Myo *et al*., 2025) [57]. Groups 3 and 4 showed intermediate values, suggesting moderate enrichment and extractability (Sheikh *et al*., 2024) [71], while Group 2 appeared to reach a plateau earlier, possibly because it contained a smaller pool of readily extractable flavonoids (Motiejauskaitė *et al*., 2025; Sabran *et al*., 2025) [56, 67]. Functionally, the higher flavonoid values in Group 1 may strengthen antioxidant defence and influence PAH biotransformation through interactions with cytochrome P450 and phase II enzymes, (Ormosia, 2025; Hu *et al*., 2025).

The FRAP results followed a similar pattern. Group 1 consistently showed the highest reducing power, indicating a richer pool of electron-donating compounds (Kabouche *et al*., 2025; Salhi *et al*., 2024) [38]. This is important because PAH metabolism can generate ROS, and stronger reducing capacity may help limit oxidative stress. Group 5 had the weakest FRAP response, supporting the view that it had lower antioxidant reserve and therefore greater vulnerability to oxidative injury (Sain *et al*., 2025). Groups 3 and 4 had moderate responses, with evidence of concentration-dependent extraction of redox-active compounds (Myo, 2025,) [57]. Group 2 remained modest, suggesting fewer or less potent reducing constituents (Zahari *et al*., 2025) [84]. The TAC, DPPH and total phenolic data provide a broader view of antioxidant behaviour. TAC captures cumulative redox potential, while DPPH reflects hydrogen-donating radical scavenging activity (Knez *et al*., 2025; Türkmen *et al*., 2024; HDT, 2023; Joshi *et al*., 2024) [31, 37, 81]. The total phenolic results support these assertions' because phenolics are central to electron donation, hydrogen transfer, metal chelation and cellular antioxidant signalling (Camarda *et al*., 2026; Mauro *et al*., 2023) [12, 52]. Phytochemical assays suggest that the control diet had stronger inherent antioxidant buffering, whereas Group 5 had weaker functional antioxidant protection despite carrying the highest PAH burden.

The DNA fragmentation results provide a direct biological endpoint linking diet composition to genomic injury. DNA fragmentation is a marker of oxidative DNA damage and apoptosis, often arising from ROS-induced strand breaks and activation of endonuclease pathways during cellular stress (Jewell *et al*., 2024). The relatively high fragmentation observed in Group 1 was unexpected despite its stronger antioxidant profile, this suggests that antioxidant capacity alone does not fully explain tissue-specific DNA damage. Factors such as tissue metabolism, baseline physiological variation or unmeasured toxicants, may have contributed. ROS-mediated lipid peroxidation, mitochondrial dysfunction and caspase-dependent DNA cleavage remain plausible mechanisms (Thomès, 2020) [78]. Antioxidant compounds can interrupt these processes by neutralising ROS and supporting endogenous enzymes such as glutathione-related systems and superoxide dismutase (Farhan, 2024; Korkmaz *et al*., 2024) [18]. The micronucleus data provide clearer evidence of treatment-related chromosomal damage that may arise when chromosome fragments or whole chromosomes fail to enter daughter nuclei during cell division, thus making the assay a useful marker of clastogenicity, aneugenicity and genome instability (Duan *et al*., 2026; MacDonald, 2024) [15, 50]. The progressive increase from Group 1 to Groups 4 and 5 shows a dose-related cytogenetic response. PAH metabolites can form DNA adducts, promote strand breaks and interfere with mitotic spindle function, all of which can increase micronucleus formation (Schuster *et al*., 2024) [70]. The higher micronucleus formation in groups with weaker antioxidant indices suggest that inadequate neutralisation of reactive intermediates may have contributed to chromosomal damage in the tissues (Lv *et al*., 2024; Mohammed *et al*., 2023) [49], 55. The apparent plateau at the highest exposure levels may indicate a threshold beyond which cytotoxicity limits cell proliferation, reducing the number of dividing cells available for micronucleus detection. Persistent oxidative stress can further damage

DNA, proteins and membrane lipids, reinforcing genome instability and raising long-term toxicological concern (Fenech, 2020; Jia *et al*., 2025) [36].

Conclusion

The present study demonstrates that dietary consumption of traditionally smoked fish is associated with increased exposure to polycyclic aromatic hydrocarbons, resulting in measurable genotoxic effects in experimental rats. Progressive increases in PAH concentrations were accompanied by diminished antioxidant capacity and significant elevations in DNA fragmentation and micronucleus frequency, indicating that the protective effects of naturally occurring phytochemicals were insufficient to counteract the adverse effects of PAH-rich diets. These findings highlight the potential health risks associated with prolonged consumption of traditionally smoked fish and underscore the need for improved smoking technologies and stricter monitoring of PAH contamination in smoked fish products to minimize dietary exposure to genotoxic contaminants.

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