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Hepatorenal and Cerebral Toxicity Induced by Consumption of Cow Meat Singed with Scrap Tyres: An Experimental Study in a Rat Model

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ABSTRACT

Background: The practice of singeing cow meat employing various fuel sources such as firewood, liquefied petroleum gas (LPG), and scrap tyres is prevalent across West Africa. This practice raises significant food safety concerns due to the potential for toxic chemical contamination.

Aims: This study aimed to investigate the toxicological effects of consuming cow meat singed with LPG, firewood, or waste tyres on hepatic, renal, and cerebral functions in a controlled rat model.

Methods: A 90-day experimental study was conducted using sixty male Wistar rats, randomly allocated into ten groups (n = 6). The animals were administered diets containing various proportions (5:15, 10:10, 15:5) of singed cow meat and standard laboratory feed. Biochemical assays were performed to assess oxidative stress markers (malondialdehyde [MDA], 4-hydroxynonenal [4-HNE], total antioxidant capacity [TAC], glutathione [GSH]), hepatic function indices (alanine aminotransferase [ALT], aspartate aminotransferase [AST], bilirubin, total protein, albumin), and renal biomarkers (blood urea nitrogen [BUN], creatinine, uric acid). Histopathological analyses of liver, kidney, and brain tissues were also conducted.

Results: Rats administered a diet containing tyre-singed meat exhibited significant ($p < 0.01$) increases in oxidative stress markers (MDA, 4-HNE) and biomarkers indicative of hepatic (ALT, AST, bilirubin) and renal (BUN, creatinine, uric acid) dysfunction compared to those administered LPG- or firewood-singed meat. Antioxidant parameters (GSH, TAC) were markedly depleted. Histopathological examination revealed severe hepatic necrosis, renal tubular degeneration, and neuronal damage in the tyre-singed meat group, whereas LPG-singed meat caused minor alterations.

Conclusions: These findings indicate that consumption of tyre-singed cow meat poses substantial risk of hepatotoxicity, nephrotoxicity, and systemic oxidative stress, raising critical food safety hazard. Strict regulatory enforcement and public awareness campaigns are urgently required to eliminate the use of hazardous materials such as tyres in meat processing.

Keywords: Singed cow meat; Oxidative stress; Nephrotoxicity; Hepatotoxicity; Food safety; Scrap tyres.

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1 INTRODUCTION

Singeing animal carcasses with open flames from various fuel sources is common in West Africa, including Ghana and Nigeria, where cow and goat meat are popular dietary staples (Hill, 2015; Okareh *et al.*, 2021). Singeing is performed primarily to remove hair from the hide and is traditionally done with different fuel types like firewood, scrap automobile tyres, liquefied petroleum gas (LPG), etc. (Ugochukwu *et al.*, 2018). Although slaughtermen in many low- and middle-income countries have been provided some access to LPG for singeing, scrap tyres are used due to their affordability and efficiency (Hill, 2015). While these methods also give some flavor to the meat (Gideon *et al.*, 2024), concerns have been raised about their potential health risks due to the introduction of toxic contaminants (Gbenga *et al.*, 2023; Tay *et al.*, 2022).

The utilization of scrap tyres for singeing has emerged as a significant public health concern. During combustion, tyres release a complex mixture of hazardous chemicals, including polycyclic aromatic hydrocarbons (PAHs), heavy metals, and persistent organic pollutants (POPs), which readily contaminate meat surfaces (Abdulai *et al.*, 2024; Ephraim-Emmanuel & Ordinioha, 2021). Chronic exposure to these toxicants has been clinically associated with severe systemic pathologies, including carcinogenesis, hepatotoxicity, nephrotoxicity, and various metabolic disorders (Vignesh *et al.*, 2024; Bukowska *et al.*, 2022; Barsouk *et al.*, 2021).

While, the incomplete combustion of firewood produces harmful organic compounds (Vicente *et al.*, 2020), LPG is generally regarded as a cleaner alternative. However, even LPG may contribute to the formation of trace combustion-related by-products under certain conditions (Monks *et al.*, 2020), necessitating a comparative toxicological assessment to determine the relative safety.

Food safety remains a critical public health challenge, particularly in low-resource settings where regulatory oversight of meat processing is inadequate. The contamination of meat with toxicants from singeing fuels poses a significant risk of food-borne toxicity and poisoning, potentially leading to some organ dysfunction (Adenuga & Montowska, 2023). Despite these concerns, limited studies have comprehensively assessed the impact of consuming meats singed with the different fuel sources on organ toxicity and oxidative stress in consumers.

The current study aimed to assess potential health consequences on consuming cow meat singed with the different fuel sources by evaluating the toxicity on the brain, liver, and kidney of Wistar rats to establish their relative safety for meat processing.

Findings from this study will contribute to evidence-based policy recommendations for safer meat processing methods and also enhance public awareness of food safety risks, prompting the adoption of improved regulatory measures to mitigate exposure to harmful contaminants in meat processing.

2 Materials and Methods

2.1 Study Design

A controlled experimental study investigated the toxicological effects of consuming cow meat singed with different fuel sources. The study assessed oxidative stress biomarkers, antioxidant status, and histopathological changes in brain, kidney, and liver tissues following a 90-day dietary exposure in an animal model.

The overall experimental layout and workflow are illustrated in Figure 1, which outlines the collection and preparation of singed meat samples, animal grouping, feeding regimen, sample analysis, and data evaluation process.

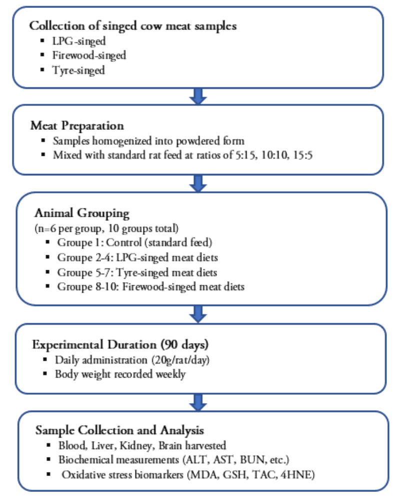


Figure 1. Schematic of the Experimental Design for Evaluating the Toxicological Effects of Cow Meat Singed with Different Fuel Sources in a Rat Model

2.2 Test Material

Cow meat samples singed with LPG, scrap tires, and firewood, all commonly used in abattoirs across Ghana, were used for this research. The meat samples were purchased from Accra, Kumasi, Tamale, Koforidua, and Ho abattoirs. The samples were homogenized into powder, mixed with normal rat feed in different ratios and administered to the rats.

2.3 Animal Experimentation

Sixty male Wistar albino rats (6 ± 1 weeks old) were utilized in this study. The animals were housed in the Animal House Facility of the Kwame Nkrumah University of Science and Technology (KNUST), Ghana, in clean, disinfected plastic cages. The rats were maintained under controlled environmental conditions, including a temperature of 25 ± 2 °C, a relative humidity of 50 ± 15%, and a 12:12 hour light–dark cycle.

The animals were allowed to acclimatize to the laboratory environment for seven days prior to the experiment. Throughout the study, the rats were provided with a standard rat diet (a commercial formulation (Top Feeds®, Nigeria) and water ad libitum, with the exception of a fasting period preceding sacrifice. The diet consisted of approximately 21% crude protein, 4% fat, 6% crude fiber, 8% ash, 1% calcium, and 0.4% phosphorus, with the balance composed of carbohydrates. This formulation provided approximately 3,000 kcal/kg of metabolizable energy.

Ethical approval for the study (Ref: UPH/CEREMAD/REC/MM73/014) was originally granted under a collaborative framework with the University of Port Harcourt, Nigeria, which served as the host ethics board during project inception. Nonetheless, all experimental procedures were carried out at KNUST under institutional oversight and in full compliance with KNUST's ethical and animal welfare regulations.

2.4 Animal Grouping and Exposure to Test Substance

Adult Wistar rats were randomized into ten groups (n = 6) and administered with powdered singed cow meat in different proportions for ninety (90) consecutive days. The different feed ratios (5 g: 15 g, 10 g: 10 g, and 15 g: 5 g of singed meat to standard rat feed) were designed to simulate low, moderate, and high levels of exposure to singed meat, reflecting potential human consumption patterns. All groups received a fixed daily feed quantity (20 g) to ensure comparable caloric and nutrient intake. Body weights were recorded weekly to monitor growth and overall health status.

The treatment groups were structured as follows:

- *Group 1 (Control):* Normal rat diet (20 g/day).
- *Groups 2, 3, and 4:* LPG-singed meat powder mixed with standard rat meal in ratios of 5 g:15 g, 10 g:10 g, and 15 g:5 g, respectively.
- *Groups 5, 6, and 7:* Tyre-singed meat powder mixed with standard rat meal in ratios of 5 g:15 g, 10 g:10 g, and 15 g:5 g, respectively.
- *Groups 8, 9, and 10:* Firewood-singed meat powder mixed with standard rat meal in ratios of 5 g:15 g, 10 g:10 g, and 15 g:5 g, respectively.

The controlled feeding ensured that differences in biochemical and organ function markers were attributable to contaminants generated during singeing rather than to variations in dietary protein content.

2.5 Feed Intake and Feed Efficiency

Each day, 20 g of feed was provided, and residues were collected and weighed to determine daily feed intake (g/day) according to equation 1.

$$Daily\ Feed\ Intake\ (g/d) = Feed\ offered - Feed\ leftover \quad (1)$$

The feed conversion ratio (FCR) was calculated using equation 2.

$$FCR = \frac{Total\ Feed\ Intake\ (g)}{Overall\ Body\ Weight\ gain\ (g)} \quad (2)$$

$$DCR = 1 + \frac{nx}{1!} + \frac{n(n-1)^2}{2!} + \cdots$$

2.6 Tissue Collection and Organ Weight Assessment

At the end of a 90-day exposure, animals were sacrificed by cervical dislocation after an overnight fast, and blood samples were collected immediately into tubes without anticoagulant. The blood vials were kept at room temperature for clotting, and separation of the serum and stored at -20 ºC for further biochemical analysis. The brain, liver, and kidney tissues were carefully excised, washed in cold phosphate-buffered saline (PBS) at pH 7.4, and weighed immediately. Their relative organ weight (ROW) was calculated using the formula in equation 3.

$$ROW = \frac{Organ\ weight\ (g)}{Final\ body\ weight\ (g)} X\ 100 \quad (3)$$

2.7 Biochemical Analysis

Kidney and liver function tests

Kidney function test was evaluated by measuring serum creatinine, blood urea nitrogen (BUN), and Uric Acid employing the procedure as described by Hassan *et al.* (2022) and Ganesan *et al.* (2020).

Liver function was assessed by determining alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein. ALT and AST were quantified using the Reitman-Frankel method (George-Opuda & Adegoke, 2024), ALP via the p-nitrophenyl phosphate (pNPP) method (Qi *et al.*, 2020), bilirubin by the Jendrassik-Grof method, and total protein using the Biuret method (George-Opuda & Adegoke, 2024). All absorbance readings were obtained spectrophotometrically, with results expressed in standard clinical units.

Oxidative stress analysis

Total Antioxidant Capacity (TAC) was determined via a Fenton-type reaction assay, in which the ability of serum antioxidants to neutralize reactive oxygen species was measured spectrophotometrically and compared against a Trolox standard curve. Results were expressed as millimoles of Trolox equivalents per liter (mmol Trolox eq/L).

Malondialdehyde (MDA) levels, a lipid peroxidation marker, were measured using the thiobarbituric acid (TBA) method (Alvarez-Mon *et al.*, 2022). Tissue homogenates were incubated with TBA and trichloroacetic acid, with absorbance recorded at 532 nm. Results were expressed as µM MDA/g tissue.

4-Hydroxy-2-Nonenal (4-HNE), a lipid peroxidation biomarker, was quantified in serum via ELISA using 4-HNE-

BSA conjugate standards. Absorbance was measured at 450 nm, and concentrations were expressed in nM (Qiao *et al.*, 2023).

Glutathione (GSH) levels, indicative of antioxidant defense, were measured in the homogenates using the DTNB method. Absorbance was recorded at 412 nm and expressed as µM GSH/mg tissue protein (Kumar *et al.*, 2024).

Histopathological examination of brain, kidney, and liver tissue

Excised brain, kidney, and liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5µm, and stained with hematoxylin and eosin (H&E). Tissue sections were examined under a light microscope at X400 magnification. Micrographs of histopathological findings were analyzed and interpreted as described by El-Desoky *et al.* (2022).

2.8 Data Analysis

All data were analyzed using GraphPad Prism software. Statistical comparisons were performed using one-way ANOVA, followed by Tukey's post hoc test. Results were expressed as mean ± standard error of the mean (SEM), and significance was set at $p < 0.05$.

3 Results

3.1 Growth Performance and Feed Utilization in Rats Administered Cow Meat Singed with Various Fuel Sources

The effects of consuming cow meat singed with different fuel sources on growth performance, feed intake, and feed conversion efficiency in rats are presented in Table1.

The initial body weight of the rats varied from 102.7 g to 135.1 g across treatment groups due to differences at procurement. However, animals were randomized into groups to achieve comparable mean body weights, and feeding ratios were standardized. This minimized the potential influence of initial weight variation on physiological and biochemical outcomes. At the end of animal experimentation, significant differences were observed in the final body weights of rats across the different treatment groups (F = 8.82, p = 0.0001). Rats administered with 5 g firewood-singed meat and 15 g normal rat feed recorded the highest final body weight (171.60 ± 3.54 g), followed by those administered 10 g LPG-singed meat and 10 g normal rat feed (166.33 ± 12.66 g). In contrast, the lowest final body weight was observed in the group administered 15 g tyre-singed meat and 5 g normal rat feed (111.60 ± 1.20 g).

Body weight gain differed significantly among the treatment groups (F = 7.5856, p = 0.0002). The highest weight gain was recorded in the 10 g LPG-singed meat and 10 g normal feed group (62.83 g), while the lowest was observed in the 15 g tyre-singed meat and 5 g normal rat feed group (8.90 g).

Overall feed intake did not differ significantly across the groups (F = 2.3411, p = 0.0626), with values ranging from 1453.33 g to 1490.67 g. However, feed conversion efficiency, as measured by the feed conversion ratio (FCR), varied significantly among the treatment groups (F = 3.7604, p = 0.0091). The lowest FCR (best feed efficiency) was recorded in the 10 g LPG-singed meat group (23.51), whereas the highest FCR (poorest feed efficiency) was observed in the 15 g tyre-singed meat group (163.33), indicating impaired feed utilization.

Figure 2 (a-d) illustrates a significant increase in the relative organ weight (ROW) of the liver (p = 0.0348), brain (p = 0.0029), and right kidney (p = 0.0187) in rats exposed to cow meat singed with tyres at 10:10 and 15:5 ratios compared to those exposed to LPG- and firewood-singed meat.

Table 1. Effect of Consuming Cow Meat Singed with Various Fuel Sources on Growth Performance and Feed Efficiency in Rats

Treatments	Initial body weight (g)	Final average body weight (g)	Weight Gain (g)	Overall Feed Intake (g)	Overall Feed conversion ratio
Control CM	104.80 ± 5.17	150.50 ± 14.82	45.70	1465.67	32.07
5 g LPG-singed meat: 15 g normal rat feed	105.60 ± 13.79	158.75 ± 13.79	53.15	1471.33	27.68
10 g LPG-singed meat: 10 g normal rat feed	103.50 ± 5.20	166.33 ± 12.66	62.83	1477.33	23.51
15 g LPG-singed meat: 5 g normal rat feed	120.50 ± 5.62	163.40 ± 3.45	42.90	1453.33	33.88
5 g Firewood-singed meat: 15 g normal rat feed	135.10 ± 3.54	171.60 ± 3.54	36.50	1490.67	40.84
10 g Firewood-singed meat: 10 g normal rat feed	126.60 ± 3.62	169.40 ± 5.57	42.80	1486.67	34.74
15 g Firewood-singed meat: 5 g normal rat feed	105.00 ± 7.99	148.7 ± 18.48	43.70	1479.67	33.86
5 g Tyre-singed meat: 15 g normal rat feed	120.50 ± 6.26	145.90 ± 9.81	25.40	1473.67	58.02
10 g Tyre-singed meat: 10 g normal rat feed	102.90 ± 1.96	130.50 ± 0.25	27.60	1462.67	53.00
15 g Tyre-singed meat: 5 g normal rat feed	102.70 ± 1.20	111.60 ± 1.20	8.90	1453.67	163.33
F-value	8.82	7.5856	-	2.3411	-
p-value	0.0001	0.0002	-	0.0626	-

Data are presented as Mean ± Standard Error of Mean (SEM)

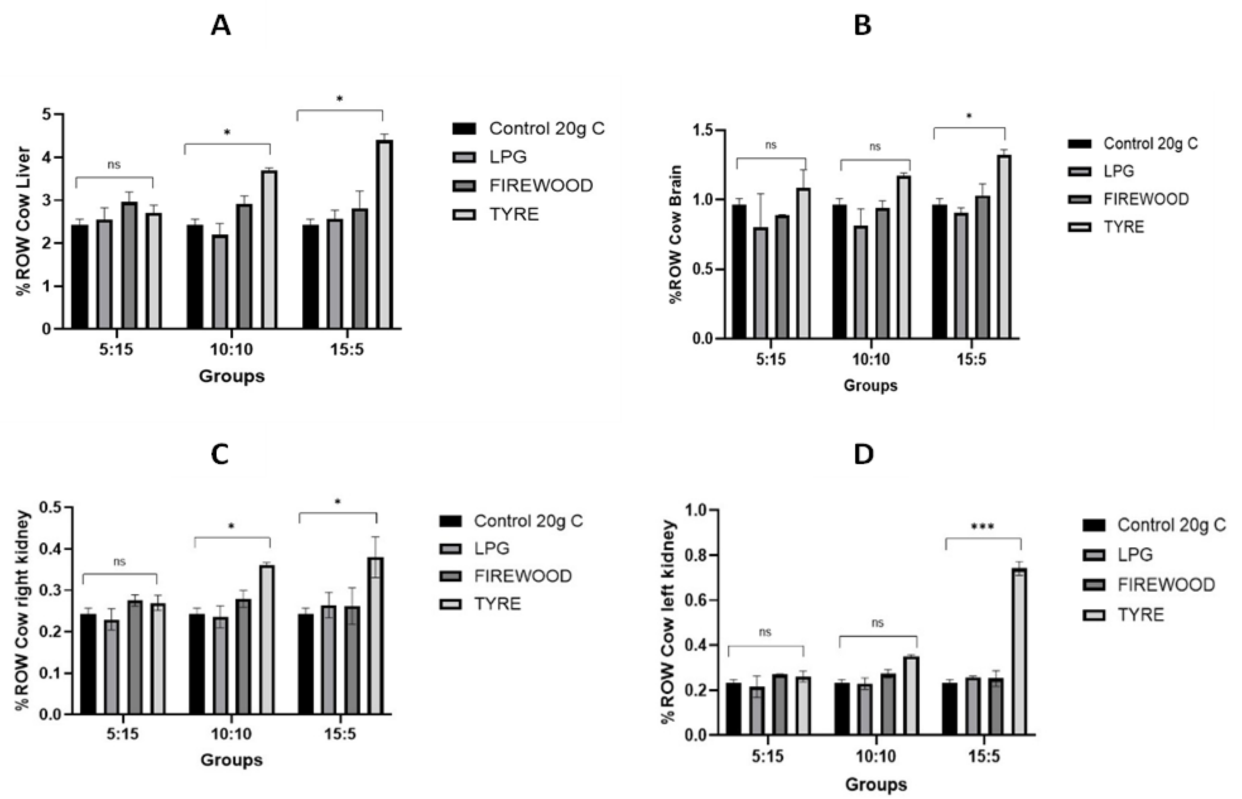


Figure 2. Body Organ Index of Experimental Rats Exposed to Singed Cow Meat (A = liver, B = brain, C = right kidney, D = left kidney) ** Significantly different from control at p < 0.05; ** significantly different from control at p < 0.01; *** significantly different from control at p < 0.001*

The kidney function test results, presented in Figures 3 (A to C), indicate significant alterations in blood urea nitrogen (BUN), creatinine, and uric acid levels among rats administered meat singed with different fuel sources.

BUN levels varied significantly across treatments (p = 0.0015), with the highest levels observed in rats administered tyre-singed meat, particularly at the 15 g tyre-singed meat: 5 g normal rat feed ratio (48.75 ± 0.64 mg/dL). This was followed by rats administered with 10g tyre-singed meat (43.05 ± 2.84 mg/dL). In contrast, the control and the LPG groups had the lowest BUN levels (Figures 3A).

Creatinine levels also showed statistically significant differences (p = 0.022), with the highest value recorded in rats administered 15 g tyre-singed meat (0.45 ± 0.04 mg/dL), followed by 10g tyre-singed meat (0.36 ± 0.02 mg/dL). The lowest creatinine level was observed in the 10g LPG-singed meat group (0.24 ± 0.03 mg/dL), compared to the control group (0.29 ± 0.02 mg/dL) (Figure 3B).

Similarly, uric acid levels were significantly (p = 0.0013) elevated in rats that consumed tyre-singed meat, reaching 4.59 ± 0.46 mg/dL in the 15 g tyre-singed meat group, while the control group recorded 1.95 ± 0.21 mg/dL (Figure 3C).

3.2 Relationship Between Kidney Weight, Final Body Weight, and Renal Function Biomarkers

The relationship between kidney weight, final body weight, and renal function biomarkers (blood urea nitrogen [BUN], creatinine, and uric acid) was analyzed using Pearson correlation (Table 2). No significant correlation was observed between right or left kidney weight and the renal function biomarkers. The Pearson correlation values for right kidney weight ranged from 0.207 to 0.445, while those for left kidney weight ranged from 0.22 to 0.437, with all p-values > 0.05, indicating no statistically significant association.

In contrast, final body weight showed a significant negative correlation with all renal biomarkers. BUN (r = -0.801, p = 0.005), creatinine (r = -0.670, p = 0.034), and uric acid (r = -0.875, p = 0.001) were inversely related to body weight.

Table 2. Correlation between Kidney Weight, Final Body Weight, and Renal Function Biomarkers in Rats Fed with Singed Cow Meat

Parameter		BUN (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
Right kidney weight	Pearson Correlation	0.269	0.445	0.207
	p-value	0.451	0.198	0.566
Left kidney weight	Pearson Correlation	0.280	0.437	0.220
	p-value	0.434	0.206	0.542
Final body weight	Pearson Correlation	-0.801*	-0.670*	-0.875*
	p-value	0.005	0.034	0.001

*Note: * shows significant correlation with final body weight*

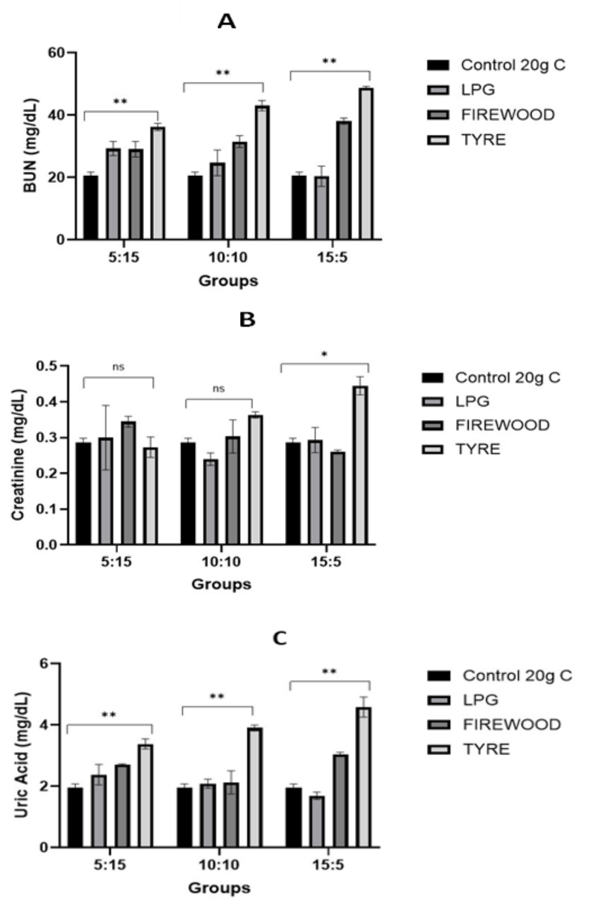


Figure 3. Kidney Function Status of the Experimental Rats Exposed to Singed Cow Meat (A = BUN, B = Creatinine, C = Uric acid) ** Significantly different from control at p < 0.05; ** significantly different from control at p < 0.01*

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) varied significantly among the treatment groups (p = 0.0004, 0.0003, respectively) (Figures 4A and B). Rats administered tyre-singed cow meat exhibited the highest ALT levels, with a dose-dependent increase observed from 239.28 ± 30.90 U/L (5 g meat) to 418.29 ± 53.66 U/L (15 g meat). A similar trend was observed in AST levels, where the highest concentration (588.42 ± 20.90 U/L) was recorded in the 15 g tyre-singed cow meat group. Rats administered firewood-singed cow meat also showed significantly elevated ALT and AST levels compared to other treatments, albeit lower than those in the tyre-singed meat group. Conversely, LPG-singed cow meat did not induce significant changes in ALT and AST levels relative to the control.

Total bilirubin levels were significantly (p = 0.0001) elevated in rats exposed to firewood- and tyre-singed cow meat, with the highest levels observed in the 15 g tyre-singed meat group (0.80 ± 0.04 mg/dL) (Figure 4C). Similarly, direct and indirect bilirubin levels showed a dose-dependent increase in rats exposed to firewood- and tyre-singed cow meat, with the highest values recorded in rats administered tyre-singed cow meat (Figures 4D and E). However, bilirubin levels remained relatively unchanged in rats exposed to LPG-singed meat, compared to the control group.

Total protein levels were significantly (p = 0.0001) lower in rats administered tyre-singed meat compared to the control, with a marked reduction (3.50 ± 0.07 g/dL) observed in the 15: 5 g ratio group (Figure 4F). Similarly, albumin levels declined significantly (p = 0.0001) in the tyre-singed meat group, with the lowest value recorded in the 15: 5 g ratio group (1.78 ± 0.67 g/dL) (Figure 4G). Feeding of firewood-singed meat to rats also resulted in a reduction in total protein and albumin levels, albeit less pronounced than in the tyre-singed meat group.

3.3 Correlation Between Liver and Body Weight with Liver Function Parameters

The Pearson correlation analysis revealed significant associations between final body weight and various liver function parameters (Table 3). Final body weight showed strong negative correlations with alanine aminotransferase (ALT) (r = -0.817, p = 0.004), aspartate aminotransferase (AST) (r = -0.875, p = 0.001), total bilirubin (r = -0.830, p = 0.003), and direct bilirubin (r = -0.900, p = 0.000). Additionally, it exhibited positive correlations with total protein (r = 0.789, p = 0.007) and albumin (r = 0.772, p =

Table 3. Correlation of Liver and Body Weight with Liver Function Parameters

Parameter		ALT	AST	Total bilirubin	Direct bilirubin	Indirect bilirubin	Total protein	Albumin
Liver weight	Pearson Correlation	0.592	0.488	0.453	0.436	0.384	-0.582	-0.391
	p-value	0.072	0.153	0.188	0.208	0.273	0.078	0.264
Final body weight	Pearson Correlation	-0.817*	-0.875*	-0.830*	-0.900*	-0.6	0.789*	0.772*
	p-value	0.004	0.001	0.003	0.000	0.067	0.007	0.009

** Significant correlation with final body weight*

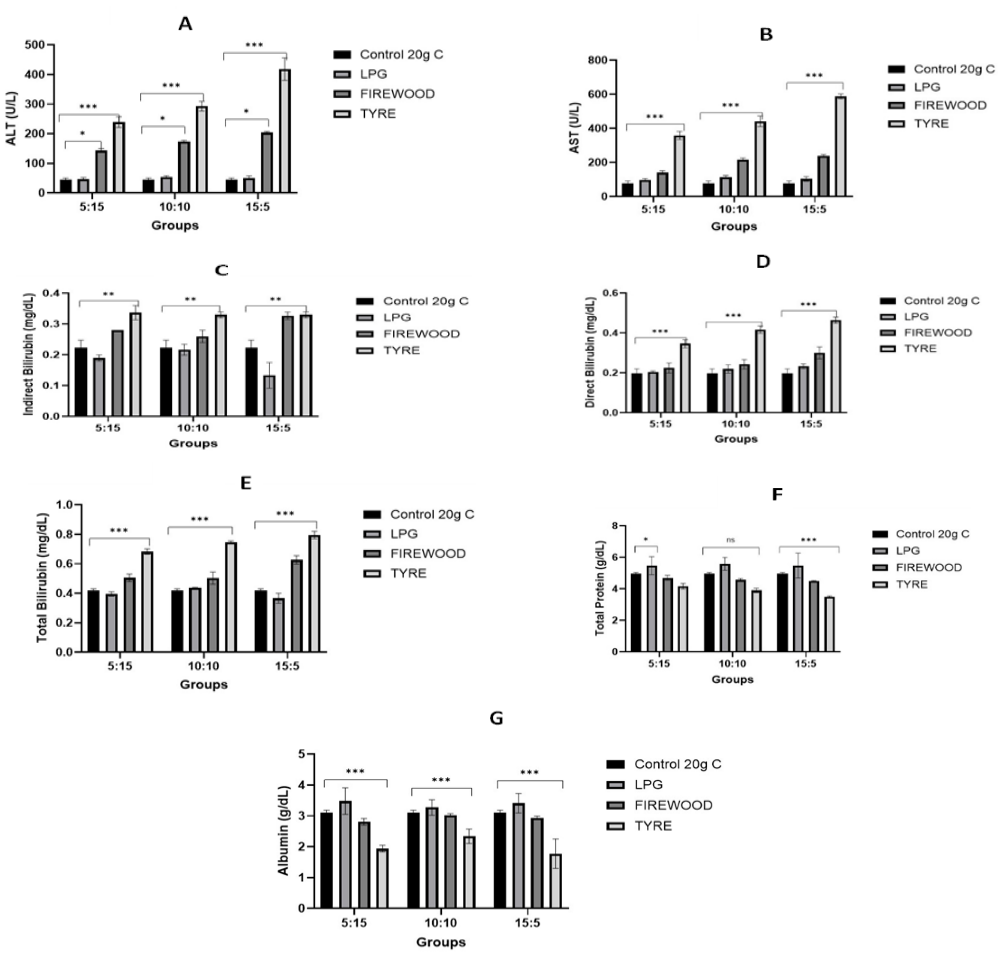


Figure 4. Liver Function Status of the Experimental Rats Exposed to Singed Cow Meat (A = ALT, B = AST, C = Total bilirubin, D = Direct bilirubin, E = Indirect bilirubin, F = Total protein, G = Albumin). ** Significantly different from control at $p < 0.05$; ** significantly different from control at $p < 0.01$; *** significantly different from control at $p < 0.001$*

0.009). The correlation with indirect bilirubin was negative but not statistically significant (r = -0.600, *p* = 0.067).

Conversely, liver weight displayed moderate positive correlations with ALT (r = 0.592, *p* = 0.072), AST (r = 0.488, *p* = 0.153), total bilirubin (r = 0.453, *p* = 0.188), direct bilirubin (r = 0.436, *p* = 0.208), and indirect bilirubin (r = 0.384, *p* = 0.273). However, none of these correlations reached statistical significance. Liver weight also showed a non-significant negative correlation with total protein (r = -0.582, *p* = 0.078) and albumin (r = -0.391, *p* = 0.264).

The levels of oxidative stress biomarkers, including malondialdehyde (MDA), total antioxidant capacity (TAC), 4-hydroxynonenal (4-HNE), and glutathione (GSH), were evaluated in brain, liver, and kidneys of rats administered with different proportions of cow meat singed using LPG, firewood, and scrap tyres (Figures: 5, 6, and 7).

MDA levels, an indicator of lipid peroxidation, were significantly increased in all the organs corresponding with higher consumption of firewood- and tyre-singed cow meat compared to the control group. Rats administered 15 g of tyre-singed cow meat had the highest MDA levels in the brain (11.79 ± 1.41 µM), liver (11.79 ± 1.42 µM), and kidney (11.83 ± 1.32 µM), which were significantly (P= 0.0060, 0.0059, 0.0060,) elevated respectively compared to the corresponding organs in control rats (1.73 ± 0.17 µM, 1.72 ± 0.16 µM, and 1.73 ± 0.16 µM) respectively. Conversely, MDA levels in rats administered LPG-singed cow meat showed minimal and non-significant variation compared to the control.

The levels of 4-HNE, a prominent oxidative stress biomarker, increased significantly with firewood- and tyre-singed cow meat consumption. Rats administered 15 g of tire-singed meat exhibited the highest 4-HNE levels in brain (485.01 ± 39.09 nM), liver (485.02 ± 39.11 nM), and kidney (484.73 ± 38.65 nM), which were substantially higher (p = 0.0033, 0.0033, 0.0001), respectively than the respective organs in the control rats (54.89 ± 3.64 nM, 54.86 ± 3.68 nM, and 54.93 ± 3.60 nM. Notably, LPG-singed cow meat consumption had a comparatively smaller effect on 4-HNE levels.

TAC levels were markedly reduced in all organs of rats administered firewood- and tyre-singed cow meat. The lowest TAC levels were observed in the group, consuming 15 g of tyre-singed cow meat, with recorded values of 0.56 ± 0.12 mmol Trolox equivalents/L, 0.56 ± 0.12 mmol Trolox equivalents/L, and 0.54 ± 0.09 mmol Trolox equivalents/L in brain, liver, and kidney respectively. These reductions were statistically significant (p = 0.0010, 0.0010, 0.0009), respectively, compared to the control group with corresponding values of 3.64 ± 0.28 mmol Trolox equivalents/L, 3.66 ± 0.27 mmol Trolox equivalents/L, and 3.65 ± 0.28 mmol Trolox equivalents/L, in respective brain, liver and kidney.

GSH level, an indicative of antioxidant defense, was significantly depleted in rats that consumed firewood- and tyre-singed cow meat. The lowest GSH concentration was observed in the 15 g tyre-singed meat administered group, with values of 0.19 ± 0.01 mM (brain), 0.19 ± 0.01 mM (liver), and 0.21 ± 0.04 mM (kidney), representing a drastic decline (p = 0.0001) for all organ tissue compared to the corresponding organs of the control rats (1.44 ± 0.00 mM, 1.45 ± 0.01 mM, and 1.44 ± 0.00 mM). LPG-singed cow meat consumption had negligible effects on GSH level in the various organs of the treated rats.

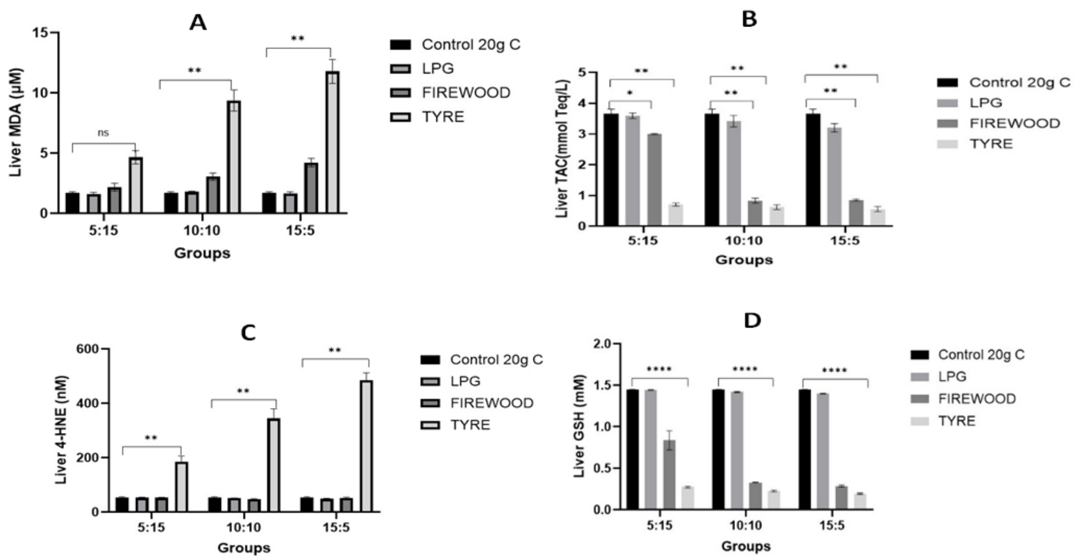


Figure 5. Oxidative Stress Status of the Liver of Experimental Rats Exposed to Singed Cow Meat (A = MDA, B = TAC, C = GSH, D = 4-HNE) ** Significantly different from control at p < 0.05; ** significantly different from control at p < 0.01; *** significantly different from control at p < 0.001*

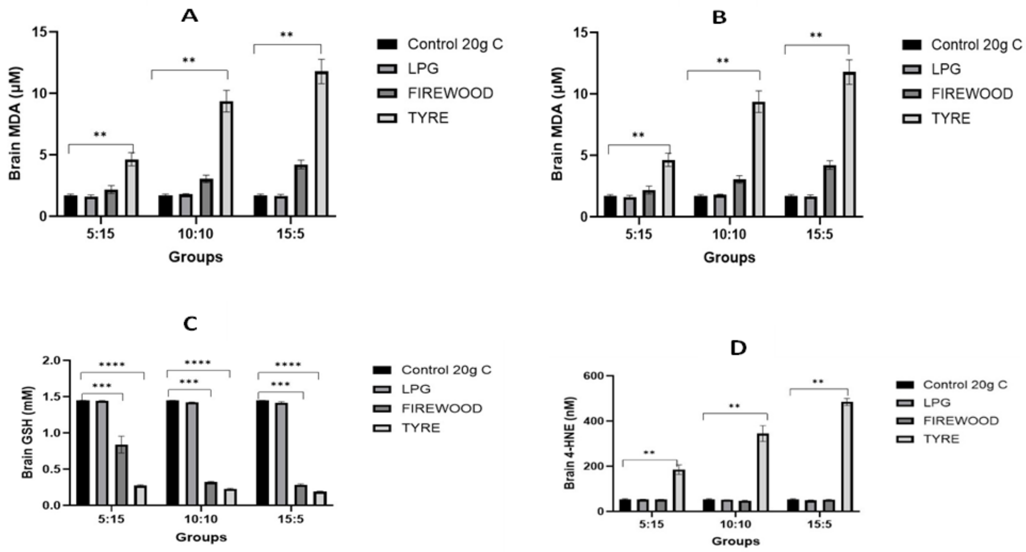


Figure 6. Oxidative Stress Status of the Brain of Experimental Rats Exposed to Singed Cow Meat (A = MDA, B = TAC, C = GSH, D = 4-HNE) ** Significantly different from control at p < 0.05; ** significantly different from control at p < 0.01; *** significantly different from control at p < 0.001*

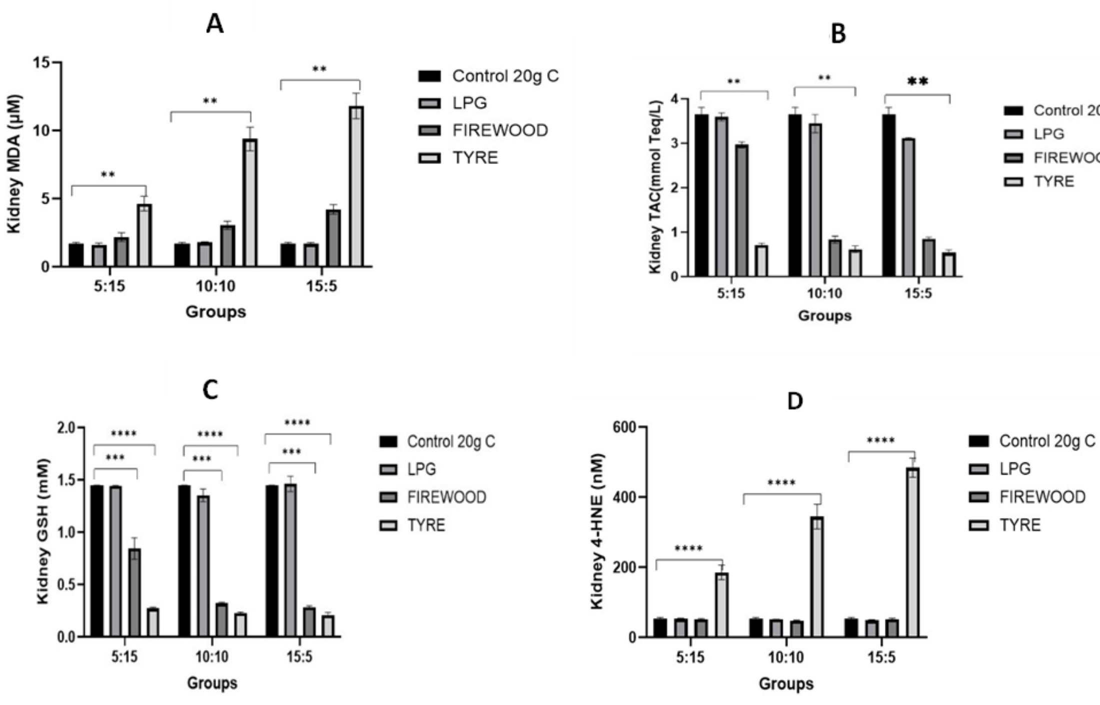


Figure 7. Oxidative Stress status of the Kidney of Experimental Rats Exposed to Singed Cow Meat (A = MDA, B = TAC, C = GSH, D = 4-HNE) ** Significantly different from control at p < 0.05; ** significantly different from control at p < 0.01; *** significantly different from control at p < 0.001*

3.4 Multivariate Regression Analysis of Oxidative Stressors and Hepatorenal Function Markers in Rats Administered Cow Meat Singed with Diverse Fuel Sources

The multivariate regression analysis revealed significant correlations between oxidative stress markers (4-HNE and GSH) and liver and kidney function parameters for rats administered cow meat singed with different fuel sources.

For the liver function tests, ALT showed a strong positive correlation with 4-HNE (r = 0.551, p < 0.001) and a significant negative correlation with GSH (r = -0.557, p < 0.001), indicating that oxidative stress increases ALT levels while antioxidant activity reduces them. Similarly, AST exhibited a stronger positive correlation with 4-HNE (r = 0.705, p < 0.001) and a moderate inverse correlation with GSH (r = -0.397, p < 0.001), suggesting a strong relationship between oxidative damage and AST elevation.

Total bilirubin was positively correlated with 4-HNE (r = 0.510, p = 0.003) and inversely associated with GSH (r = -0.576, p = 0.001). Direct bilirubin also followed a similar trend, with 4-HNE showing a strong positive correlation (r = 0.751, p < 0.001) and GSH demonstrating a negative correlation (r = -0.329, p = 0.006). However, indirect bilirubin had a non-significant correlation with 4-HNE (r = 0.09, p = 0.689) but was significantly negatively correlated with GSH (r = -0.834, p = 0.006).

Total protein and albumin exhibited inverse correlations with oxidative stress, as 4-HNE negatively correlated with total protein (r = -0.446, p = 0.007) and albumin (r = -0.569, p = 0.022), while GSH was positively associated with total protein (r = 0.631, p = 0.001) and marginally with albumin (r = 0.448, p = 0.054).

For kidney function, BUN was significantly associated with oxidative stress, showing a positive correlation with 4-HNE (r = 0.492, p = 0.012) and a negative correlation with GSH (r = -0.573, p = 0.006). Creatinine displayed a strong positive correlation with 4-HNE (r = 0.874, p = 0.015), while its association with GSH was not significant (r = 0.113, p = 0.691). Uric acid was positively correlated with 4-HNE (r = 0.698, p = 0.002), suggesting that an increase in oxidative stress contributes to elevated uric acid levels.

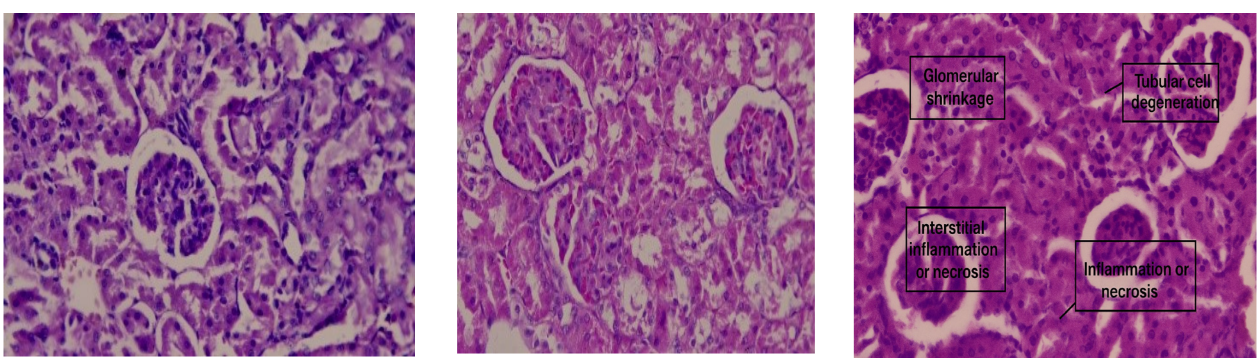
The multiple correlation coefficient (Multiple R) and R-square values further confirmed the strength of the associations, with values ranging from 0.812 to 0.995 for Multiple R and 0.66 to 0.99 for R-square. These findings suggest a robust relationship between oxidative stress and liver and kidney dysfunction, emphasizing the detrimental role of lipid peroxidation in organ function impairment.

3.5 Histopathology

Histopathological examination of the kidney, liver, and brain was performed on rats in the experimental groups to evaluate potential pathological changes associated with singed meat consumption.

Kidney Histology

The control group (Group A) exhibited normal renal architecture, characterized by well-defined glomeruli and tubules with no evidence of necrosis, cellular damage, or inflammation. The tissues for the group administered the highest LPG-singed meat (15 g) exhibited the same features as the control group. The kidney sections from the 15 g firewood-singed meat treated group (Group B) showed mild histological alterations, including slight congestion in the glomeruli and early signs of tubular cell degeneration, indicative of cellular stress. However, these changes were not severe (Figure 8).



Group A: Control (Normal rat diet)

Group B: Consumption of 15 g firewood-singed meat plus 5 g standard rat diet

Group C: Consumption of 15 g tyre-singed singed meat plus 5 g standard rat diet

Figure 8. Kidney Photomicrographs of Rats fed with Different fuel source singed cow meat. Stained with H&E X400

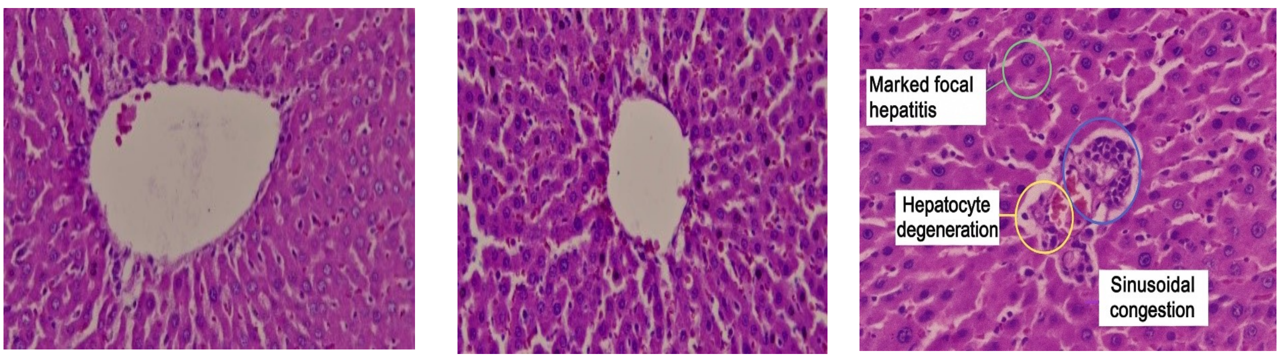
Liver Histology

The photomicrograph of liver tissues in the control group (conventional-diet) showed normal hepatic architecture, with intact central veins, well-organized hepatic cords, and minimal pathological alterations, similar to those treated with LPG-singed cow meat. Liver photomicrographs of the 15 g firewood-singed cow meat- administered group (Group B) revealed moderate structural changes, including sinusoidal congestion, slight inflammatory infiltration, and hepatocyte degeneration, indicating early hepatotoxic effects. However, liver photomicrograph of 15 g tyre-singed cow meat-treated group (Group C) exhibited severe histopathological alterations with pronounced dilation of the central vein, marked inflammatory infiltration, and significant hepatocyte degeneration. These changes suggest substantial hepatic stress and injury, with a higher degree of liver toxicity in Group C compared to Group B (Figure 9).

Brain Histology

Microscopic examination of brain sections revealed varying degrees of neurotoxicity across the experimental groups. The control group (Group A) displayed normal neuronal morphology, characterized by intact neuronal bodies, well-defined neuropil, and the absence of necrosis or inflammatory infiltration. In contrast, the 15 g firewood-singed, cow meat-treated group (Group B) exhibited mild to moderate neuronal degeneration, cellular necrosis, and some disorganization in neuronal arrangements. Additionally, vacuolation within the neuropil was observed, indicating early signs of neurotoxicity.

In the 15 g tyre-singed cow meat-treated group (Group C), severe necrotic changes were evident, with extensive vacuolation in the neuropil and prominent neuronal shrinkage. There were also significant perivascular oedema, astrocyte proliferation, and infiltration of inflammatory cells around blood vessels, suggesting vascular impairment, neuronal neuroinflammation, and direct neuronal toxicity (Figure 10).

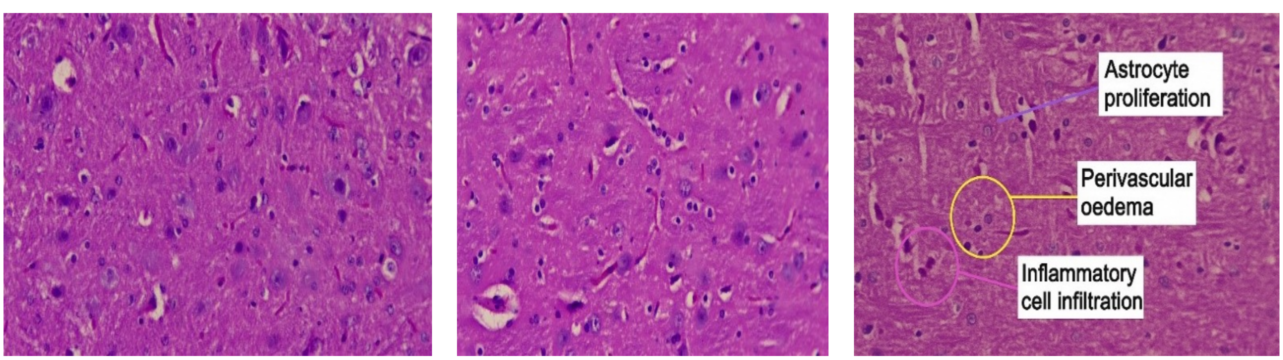


Group A: Control (Normal rat diet)

Group B: Consumption of 15 g firewood-singed meat plus 5 g standard rat diet

Group C: Consumption of 15 g tyre-singed singed meat plus 5 g standard rat diet

Figure 9. Liver Photomicrographs of Rats fed with Different fuel source singed cow meat. Stained with H&E X400



Group A: Control (Normal rat diet)

Group B: Consumption of 15 g firewood-singed meat plus 5 g standard rat diet

Group C: Consumption of 15 g tyre-singed singed meat plus 5 g standard rat diet

Figure 10. Brain Photomicrographs of Rat fed with Different fuel source singed cow meat. Stained with H&E X400

4 Discussion

This study evaluated the physiological and toxicological effects of consuming cow meat singed with different fuel sources. The findings reveal that the type of singeing fuel substantially influenced growth performance, biochemical responses, and tissue integrity in rats. Among the treatment groups, tyre-singed meat produced the most severe adverse effects, while LPG-singed meat caused the least.

Rats administered tyre-singed meat exhibited markedly reduced body weight gain and poor feed conversion efficiency, indicating impaired nutrient utilization. These findings are consistent with those observed by Gideon *et al.* (2024) and Sunu (2014), who reported growth retardation and gastrointestinal irritation in animals exposed to tyre-singed meat, attributed to contamination with heavy metals and PAHs. The combustion of tyres releases toxic residues—particularly Pb, Cd, and PAHs—that disrupt energy metabolism and enzyme activity, leading to reduced feed efficiency and stunted growth (Jedrychowski *et al.*, 2015; Wan Mahari *et al.*, 2025). Conversely, rats administered LPG- and firewood-singed meat exhibited better growth and feed efficiency, confirming that cleaner fuels produce fewer toxicants and thus lower physiological stress (Woko *et al.*, 2020).

Variations in relative organ weights further support the differential toxic impact of the fuels. Increased liver and kidney weights in rats administered tyre-singed meat reflect compensatory hypertrophy and metabolic stress due to toxicant exposure (Lazic *et al.*, 2020; Wang *et al.*, 2019). The highest values in the 15 g tyre- administered group suggest hepatocellular and renal overload, consistent with studies linking heavy metals and PAHs to hepatic and renal injury (Rahman *et al.*, 2022; Abdel-Hamid *et al.*, 2023). In contrast, the LPG group exhibited minimal organ weight changes, implying reduced toxic burden.

Biochemical markers corroborate these patterns. Elevated BUN, creatinine, and uric acid levels in tyre-singed meat groups signify nephrotoxicity and impaired kidney clearance, similar to the renal stress reported by Lee and Kim (2024) and Obasi *et al.* (2023). These biomarkers increased in a dose-dependent pattern, suggesting cumulative toxicant exposure. Similarly, significant elevations in ALT and AST levels indicate hepatocellular damage, most pronounced in the tyre group. These findings corroborate previous reports associating PAHs and heavy metals to hepatic inflammation and enzyme leakage (Nsonwu-Anyanwu *et al.*, 2021; Liao *et al.*, 2024). The LPG group maintained enzymatic profiles proximal to physiological norms, confirming its relative safety profile.

Hepatic protein metabolism was significantly altered across the treatment groups. The observed reductions in total protein and albumin levels in rats administered tyre- and firewood-singed meat indicate an impairment of the liver's biosynthetic capacity (Adegbola *et al.*, 2024; Paranjape & Garcia-Pereira, 2024). The decline in serum albumin levels reflect a compromised nutritional and hepatic status, while the positive correlation between body weight and protein indices highlights the influence of nutritional adequacy on maintained liver function. These biochemical disturbances are consistent with oxidative and inflammatory damage induced by PAHs and heavy metals (Goutam *et al.*, 2022; Kuppusamy *et al.*, 2020).

The oxidative stress data provide mechanistic insight into these systemic effects. The marked elevation of MDA and 4-HNE levels, alongside reduced total antioxidant capacity (TAC) and glutathione (GSH), indicates that exposure to tyre- and firewood-singed meat triggered lipid peroxidation and antioxidant depletion. These findings mirror previous reports that PAHs and metals promote reactive oxygen species (ROS) formation, leading to membrane and DNA damage (Zhang *et al.*, 2023; Thirumoorthy *et al.*, 2022). The strong correlations between oxidative stress markers and liver and kidney function indices confirm that oxidative damage is a central mechanism of toxicity. In contrast, the LPG group maintained stable antioxidant status, underscoring the lower oxidative load from cleaner fuel combustion (Carlsten *et al.*, 2020).

Histopathological examination further validated the biochemical results. While the control and LPG groups exhibited preserved tissue architecture, the administration of tyre-singed meat induced extensive hepatic necrosis, renal tubular degeneration, and significant neuronal damage. Firewood-singed meat produced moderate histological lesions, suggesting partial exposure to toxicants. The severe tissue alterations observed in tyre- administered rats are characteristic of PAH- and heavy metal toxicity (Nsonwu-Anyanwu *et al.*, 2021; Tartaglione *et al.*, 2023).

Overall, the study demonstrates that combustion fuel type is a critical determinant of the safety of singed meat. Tyre-singed meat poses the greatest risk due to its concentrated load of PAHs and heavy metals, which impair metabolism, exacerbate oxidative stress, and induce multi-organ damage. While firewood-singed meat presents moderate toxicity, LPG-singed meat offers the safest alternative. Unlike previous studies that focused primarily on the quantification of contaminants, this study provides integrative biochemical and histological evidence associating toxicant exposure to systemic dysfunction. The findings emphasize a critical public health imperative: discouraging the utilization of scrap tyres in meat processing and advocating for cleaner combustion technologies, such as LPG, to minimize toxic exposure and protect consumer health.

5 Conclusions

This study demonstrates that the consumption of tyre-singed cow meat induces significant systemic toxicity, characterized by dose-dependent deleterious effects on the liver, kidneys, and brain. The observed histopathological and biochemical alterations confirm that the combustion of scrap tyres generates a potent mixture of contaminants—likely dominated by heavy metals and polycyclic aromatic hydrocarbons (PAHs)—that exceed the physiological coping mechanisms of the model organism. In contrast, LPG-singed meat exhibited minimal adverse effects, underscoring its viability as a significantly safer alternative for meat processing.

These toxicological responses highlight a critical public health risk and underscore the urgent need for stringent regulatory interventions to prohibit the use of tyres in the food supply chain. Such policy measures should be grounded in toxicological evidence on the composition of harmful residues in singed meat. Therefore, future studies should focus on precise quantification of specific toxicants to establish Maximum Residue Limits (MRLs) and define No Observed Effect Levels (NOELs) for safe human exposure. Comprehensive chemical characterization across various combustion fuels will further validate these biological findings and guide policy toward safer meat-handling practices and protect consumer health.

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Authors' Contribution: PMA: Field sampling & Benchwork, Data Analysis, Writing of the Original Draft, Review, Revision & Editing. **ANE and CKF**: Supervision, Review, Revision & Editing. **OCE and TCU:** Writing of the Original Draft, Review, Revision & Editing. **TBOI:** Data Analysis, Writing of the Original Draft, Review, Revision, & Editing. **OEO:** Conceptualization, Supervision, Data Analysis, Visualization, Writing of the Original Draft, Review, Revision & Editing. **All authors:** Validation and Approval.

Ethics approval: Ethical approval for this study was obtained from the Research Ethical Committee at the University of Port Harcourt, Nigeria (UPH/CEREMAD/REC/MM73/014).

Conflicts of Interest: The authors declare that there are no financial or non-financial conflicts of interest related to the content of this manuscript.

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