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Sub-acute Dietary Exposure to Tyre-Flame Processed Cow Hide (Ponmo) Increases Serum Lead and Copper Levels in Male Wistar Rats Without Significant Changes in Liver Enzymes: A Preliminary Toxicology Study

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Abstract- Background: Cow hide (Ponmo) is a widely consumed animal by product in West Africa. Singeing using scrap tyre flames may introduce toxic heavy metals into the product. However, limited in vivo toxicological data exist on the biological effects of consuming tyre flame processed (TFP) cow hide.
Objective: This preliminary study evaluated sub acute (28 day) dietary exposure to TFP cow hide on serum heavy metal concentrations and liver function markers in male Wistar rats.
Methods: Twenty five male Wistar rats (5–6 weeks, 100–144 g) were randomly assigned to five groups (n=5 per group). Groups 1–3 received 10%, 20%, and 30% TFP cow hide in diet; Group 4 received 20% razor shaved processed (RSP) cow hide (method control); Group 5 received normal rat chow (negative control). After 28 days, serum heavy metals (Pb, Cd, Cu, Zn, As, Ni) were analyzed by atomic absorption spectroscopy, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) by spectrophotometry. Due to the preliminary nature of this study, histopathological examination was not performed, and liver enzyme data alone cannot definitively rule out subclinical hepatotoxicity.
Results: Rats fed 30% TFP cow hide showed the highest serum lead concentration (1.30±0.01 mg/L) compared to the RSP control (0.02±0.01 mg/L; p < 0.05). Copper levels were higher in the 20% TFP (0.22±0.01 mg/L) and 30% TFP (0.12±0.01 mg/L) groups compared to the normal control (0.06±0.02 mg/L; p < 0.05). Nickel was elevated in the 30% TFP group (1.23±0.01 mg/L). Notably, the normal control group showed unexpectedly high arsenic levels (0.72±0.02 mg/L), suggesting possible baseline contamination of the rat chow. No statistically significant differences (p > 0.05) in AST, ALT, or ALP activities were observed between any groups. However, in the absence of histopathological examination, subclinical liver injury cannot be excluded.
Conclusion: In this preliminary sub acute study (28 days, n=5 per group), consumption of TFP cow hide was associated with elevated serum lead and copper concentrations in male Wistar rats. Serum liver enzyme markers showed no significant differences between treatment and control groups. However, histopathological examination is required to definitively assess hepatotoxicity. These findings are hypothesis generating rather than confirmatory. Chronic toxicity studies with larger sample sizes (n ≥ 10), histopathological evaluation, analysis of multiple batches of TFP cow hide, and quantification of heavy metals in the feed itself are necessary before concluding that tyre flamed Ponmo poses a human health hazard.

Keywords: Ponmo; Cow Hide, Heavy Metals, Lead, Sub-Acute Toxicity, Wistar Rats, Tyre Burning, Preliminary Study

I. INTRODUCTION

1.1 Background

Cow hide, locally known as Ponmo in Nigeria, is a popular and affordable animal by product consumed across West Africa (Funke, 2013; Okiei et al., 2009). Its consumption has increased due to rising costs of conventional meat. Two common methods for hair removal exist: boiling followed by razor shaving, and singeing using open flames. The flame method often utilizes scrap tyres, firewood mixed with spent engine oil, or plastics to generate intense heat that efficiently removes hair without cracking the skin (Akwetey et al., 2015).

1.2 Chemical Hazards Associated with Tyre Combustion

Scrap tyres contain numerous hazardous substances. Table 1 summarizes the composition of a typical scrap tyre.

Table 1: Percentage Composition of a Typical Scrap Tyre

Constituent	Percentage (%)	References
Rubber (natural and synthetic)	47	WRAP, 2006
Carbon black	21.5	WRAP, 2006
Metal (steel belts)	16.5	WRAP, 2006
Zinc oxide	1.0–2.0	WRAP, 2006
Sulphur	1.0–1.5	WRAP, 2006
Additives (oils, resins, etc.)	7.5	WRAP, 2006

During combustion, these components release particulate bound heavy metals, including lead, cadmium, zinc, arsenic, and nickel (Rahbar et al., 2015; National Toxicology Program, 2011). Recent studies confirm that tyre combustion releases heavy metals that exceed international soil quality guidelines (Orisakwe et al., 2020; Iwegbue et al., 2021). These metals are deposited onto the surface of cow hide during singeing, potentially contaminating the final food product (Obiri-Danso et al., 2008; Essumang et al., 2007).

1.3 Health Effects of Heavy Metals

Heavy metals are non-biodegradable and bioaccumulate in human tissues, leading to chronic toxicity affecting the kidneys, liver, nervous system, and hematopoietic system (Tchounwou et al., 2025). Table 2 summarizes the major health effects of heavy metals relevant to this study.

Table 2: Health Effects of Selected Heavy Metals

Heavy Metal	Major Target Organs	Health Effects	Reference
Lead (Pb)	Nervous system, kidneys, hematopoietic system	Neurodevelopmental deficits, hypertension, nephropathy, anemia	Obeng-Gyasi et al., 2021
Copper (Cu)	Liver, kidneys, eyes	Liver damage, Wilson disease (chronic), gastrointestinal distress	ATSDR, 2022
Nickel (Ni)	Lungs, nasal cavity, skin	Carcinogenic (Group 1), contact dermatitis, respiratory toxicity	IARC, 2023
Cadmium (Cd)	Kidneys, bones, lungs	Renal dysfunction, bone demineralization, carcinogenic	Godt et al., 2022
Arsenic (As)	Skin, lungs, bladder, liver	Carcinogenic (Group 1), hyperkeratosis, peripheral neuropathy	IARC, 2023

Lead exposure, even at low levels, is associated with neurodevelopmental deficits in children and cardiovascular disease in adults. A recent systematic review confirmed dietary lead exposure as a significant contributor to non-communicable diseases globally (Okeke et al., 2024). Subclinical hepatotoxicity following low level heavy metal exposure has also been documented, where histological damage may occur without significant transaminase elevation (Eze et al., 2024; Orisakwe et al., 2022).

1.4 Knowledge Gap and Rationale

Despite widespread consumption of tyre flamed Ponmo, limited in vivo toxicological research exists. Most studies focus on chemical analysis of the product rather than biological effects (Okiei et al., 2009; Iwegbue et al., 2021). Recent investigations highlight knowledge gaps regarding chronic low level heavy metal exposure from informally processed meat products in sub Saharan Africa (Adetunji et al., 2023; Okonkwo et al., 2025).

This preliminary study aimed to evaluate sub acute toxicity of TFP cow hide in Wistar rats by measuring serum heavy metal concentrations and liver function parameters. Importantly, we acknowledge that liver function enzymes alone are insufficient to rule out hepatotoxicity, and our findings should be interpreted as hypothesis generating.

II. MATERIALS AND METHODS

2.1 Collection and Preparation of Cow Hide Samples

Fresh cow hide was purchased from a slaughter point at Ogbor Hill, Aba South Local Government Area, Abia State, Nigeria. The hide was divided into two portions:

Tyre flame processed (TFP): The first portion was processed using flames from burning scrap tyres until hair was completely singed, followed by thorough washing and drying.

Razor shaved processed (RSP): The second portion was boiled in water for 45 minutes, after which hair was shaved off using a razor blade.

Both products were chopped, dried, and milled into powder. Importantly, heavy metal content of the processed powders was not analyzed in this preliminary study, representing a limitation.

2.2 Animal Housing, Grouping, and Ethical Compliance

Animals: Twenty-five male Wistar rats (Rattus norvegicus), aged 5–6 weeks and weighing 100–144 g, were obtained from the Animal House, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Housing conditions: Animals were housed in stainless steel cages with plastic bottoms under humid tropical conditions with a 12 hour light/dark cycle, with access to clean tap water ad libitum, and acclimatized for one week.

Ethical approval: All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Michael Okpara University of Agriculture, Umudike (Approval No. MOUA/BCH/2024/019). Humane endpoints were observed throughout.

Euthanasia: At the end of the 28 day treatment period, rats were euthanized by cervical dislocation without anesthetic agents (to avoid interference with assays, as approved by the IACUC).

2.3 Experimental Design and Grouping

Rats were randomly assigned to five groups (n=5 per group):

Group	Treatment	Diet Composition
Group 1	10% TFP	10% TFP cow hide + 90% normal rat chow
Group 2	20% TFP	20% TFP cow hide + 80% normal rat chow
Group 3	30% TFP	30% TFP cow hide + 70% normal rat chow
Group 4	20% RSP (Control 1)	20% RSP cow hide + 80% normal rat chow
Group 5	Normal Control (Control 2)	100% normal rat chow

Eighty grams of feed was provided per cage daily for 28 days.

2.4 Sample Collection

At the end of treatment, rats were euthanized, and blood was collected by cardiac puncture into plain tubes. Blood was allowed to clot, then centrifuged to obtain serum. Serum samples were stored at −20°C until analysis.

2.5 Heavy Metal Analysis

Sample digestion: Serum samples (2 mL) were digested with $HCl:HNO_3$ (1:3) in a water bath for 30 minutes.

Analysis: Heavy metal concentrations (lead, cadmium, copper, zinc, arsenic, nickel) were determined using an atomic absorption spectrometer (AAS Buck Scientific VG210).

Quality control: Calibration standards were analyzed every 20 samples. All samples were analyzed in duplicate.

2.6 Liver Function Tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured using Randox commercial kits (Randox Laboratories, UK) following:

ALT/AST: Reitman and Frankel (1957) method
ALP: King and Kind (1957) method

Absorbances were read using a spectrophotometer (Spectronic 20, Labtech, USA).

2.7 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). One way analysis of variance (ANOVA) followed by Student's t test was performed using SPSS version 21. Statistical significance was set at $p < 0.05$. No adjustments were made for multiple comparisons (e.g., Bonferroni correction) due to the preliminary nature of the study.

III. RESULTS

3.1 Heavy Metal Concentrations in Rat Serum

Table 3 presents the heavy metal concentrations in serum of rats fed different dietary inclusions of TFP cow hide.

Table 3: Heavy Metal Concentrations in Rat Serum (mg/L)

Group	Lead (Pb)	Cadmium (Cd)	Copper (Cu)	Zinc (Zn)	Arsenic (As)	Nickel (Ni)
10% TFP	0.73±0.01^{c}	0.04±0.01^{b}	0.04±0.01^{c}	0.07±0.01^{a}	0.10±0.01^{e}	0.57±0.01^{c}
20% TFP	1.27±0.01^{b}	0.05±0.01^{b}	0.22±0.01^{a}	0.03±0.01^{b}	0.06±0.01^{d}	0.50±0.01^{d}
30% TFP	1.30±0.01^{a}	0.06±0.01^{b}	0.12±0.01^{b}	0.03±0.01^{b}	0.14±0.01^{c}	1.23±0.01^{a}
20% RSP	0.02±0.01^{e}	0.11±0.01^{a}	0.03±0.01^{c}	0.03±0.01^{b}	0.30±0.01^{b}	0.19±0.01^{e}
Normal Control	0.45±0.02^{d}	0.06±0.02^{b}	0.06±0.02^{c}	0.06±0.02^{a}	0.72±0.02^{a}	0.64±0.02^{b}

*Values are mean ± SEM. Different superscript letters within a column indicate significant difference ($p < 0.05$). TFP = Tyre-flame processed; RSP = Razor-shaved processed. *

3.2 Liver Function Markers

Table 4 shows the effect of TFP cow hide on serum AST, ALT, and ALP activities.

Table 4: Liver Function Markers in Rat Serum

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
10% TFP	115.40±4.15^{ab}	87.60±2.68^{ab}	10.40±0.51^{a}
20% TFP	120.60±5.91^{ab}	80.20±1.46^{bc}	11.40±1.08^{a}
30% TFP	117.80±3.01^{ab}	79.00±3.41^{c}	10.20±0.49^{a}
20% RSP	130.20±1.80^{a}	92.20±3.44^{a}	12.60±1.12^{a}
Normal Control	122.80±5.12^{ab}	88.60±3.50^{ab}	12.40±0.60^{a}

Values are mean ± SEM. Different superscript letters within a column indicate significant difference ($p < 0.05$).

IV. DISCUSSION

4.1 Interpretation of Findings in Context of Study Limitations

This preliminary study evaluated heavy metal bioaccumulation and liver function following sub acute exposure to TFP cow hide. The results demonstrate that TFP cow hide consumption is associated with elevated serum lead, copper, and nickel concentrations. However, the following limitations must be considered when interpreting these findings.

4.2 Lead Bioaccumulation

Lead concentrations in TFP fed rats (0.73–1.30 mg/L) exceeded the permissible limit for meat products (0.1 mg/kg) established by the Codex Alimentarius Commission (2023) and USDA (2006). This finding is consistent with previous reports of heavy metal contamination in TFP cow hide (Iwegbue et al., 2021; Okonkwo et al., 2025).

Comparison with permissible limits:

Standard	Permissible Limit (mg/kg)	This Study (30% TFP)	Exceeds Limit?
Codex Alimentarius (2023)	0.1 (lead in meat)	1.30 mg/L	Yes – by 13-fold
USDA (2006)	0.1 (lead)	1.30 mg/L	Yes – by 13-fold
WHO (2022)	0.01 (lead in drinking water)	1.30 mg/L	Yes – by 130-fold

4.3 Copper Findings – Non-Monotonic Dose-Response

An unexpected finding was the non-monotonic dose response for copper: the 20% TFP group had higher serum copper (0.22 mg/L) than the 30% TFP group (0.12 mg/L). Possible explanations include:

4.4 Liver Enzymes – Normal but Not Conclusive

No significant differences in AST, ALT, or ALP were observed between TFP groups and controls. However, as noted in the introduction, normal liver enzymes do NOT rule out hepatotoxicity.

Evidence from literature:

Study	Finding	Implication
Eze et al. (2024)	Subclinical hepatotoxicity can occur without transaminase elevation	Liver enzymes are insensitive to mild injury
Orisakwe et al. (2022)	Histological damage precedes enzyme elevation	Histopathology is more sensitive
Nwachukwu et al. (2024)	Human consumers of Ponmo had normal liver enzymes but elevated blood lead	Supports need for histopathology in animal studies

4.5 Comparison with Previous Studies

Table 5: Comparison with Previous Studies on Heavy Metals in TFP Cow Hide

Study	Sample Type	Key Findings	Comparison with This Study
Okiei et al. (2009)	TFP cow hide (product)	Detected Pb, Cd, Zn in product	This study confirms bioaccumulation in serum (novel)
Obiri-Danso et al. (2008)	TFP hides in Ghana	Elevated heavy metals in product	Consistent with our findings
Iwegbue et al. (2021)	Nigerian TFP cow hide	Pb, Cd, Ni exceeded	Consistent with our findings

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Study	Sample Type	Key Findings	Comparison with This Study
		permissible limits	
Okonkwo et al. (2025)	Risk assessment of Ponmo consumption	Estimated daily intake of heavy metals	This study adds in vivo data

Novel contribution of this study: To the best of our knowledge, this is the first study to report serum heavy metal concentrations following controlled dietary exposure to TFP cow hide in an animal model. However, the preliminary nature of this study limits its conclusiveness.

4.6 Public Health Implications

While this study demonstrates that TFP cow hide consumption is associated with elevated serum lead and copper in rats, direct extrapolation to human health risk is not possible from this preliminary study. Human risk assessment would require: chronic exposure studies in animal models, human biomonitoring studies (blood lead levels in regular Ponmo consumers), quantitative risk assessment (dose–response modeling) as well as analysis of multiple batches of TFP cow hide from different sources

Recent human studies, such as Nwachukwu et al. (2024), have reported associations between Ponmo consumption and blood lead levels in Nigerian adults, supporting the potential relevance of these findings to human health. However, causality has not been established.

V. CONCLUSION

5.1 Summary of Findings

In this preliminary sub-acute study, dietary exposure to tyre flame processed cow hide was associated with:

Finding	Certainty	Comment
Elevated serum lead (up to 1.30 mg/L)	Moderate	Dose-dependent; exceeds permissible limits
Elevated serum copper (non-monotonic)	Low	Inconsistent dose-response; requires confirmation
Elevated serum nickel (up to 1.23 mg/L)	Moderate	Dose-dependent
No significant changes in AST, ALT, ALP	Moderate	But histopathology is required to rule out hepatotoxicity
Unexpected high arsenic in normal control	High	Suggests baseline feed contamination

5.2 Main Conclusion

Consumption of tyre flame processed cow hide was associated with elevated serum lead and copper concentrations in male Wistar rats. Serum liver enzyme markers showed no significant differences between treatment and control groups. These findings are hypothesis generating rather than confirmatory.

5.3 Final Statement on Human Health Implications

Chronic toxicity studies with larger sample sizes ($n \geq 10$), histopathological evaluation, analysis of multiple batches of TFP cow hide, and quantification of heavy metals in feed are necessary before concluding that tyre flamed Ponmo poses a human health hazard.

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Author Contributions:
- NN: Conceptualization, investigation, data curation, writing – original draft
- II: Supervision, methodology, validation, writing – review & editing
- CE: Formal analysis, visualization, writing – review & editing

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