**Chronic Inhalation of Carbon Soot PM2.5 Induces Weight Loss and Cerebellar Cytoarchitectural Disruption in Wistar Rats**

**ABSTRACT**

**Background**: Carbon soot particulate matter (PM2.5) from crude oil combustion poses significant health risks, yet its systemic and neurological effects remain understudied. This study investigated the impact of chronic carbon soot PM2.5 inhalation on body weight and cerebellar cytoarchitecture in Wistar rats. **Methods**: Twenty-eight male Wistar rats were acclimatized and randomized into four groups: one control (unexposed) and three experimental groups exposed to PM2.5 concentrations (1.221 ± 0.169, 1.290 ± 0.214, and 1.282 ± 0.235 mg/m³) via a whole-body inhalation system for 4 hours/day over 28 days. Carbon soot was sourced from Nigerian crude oil combustion and authenticated. Body weight was recorded daily, and cerebellar tissues were processed histologically (H&E staining) post-sacrifice. Data were analyzed using ANOVA and Tukey’s post hoc test (p<0.05). **Results**: Control rats exhibited normal weight gain (120.10 ± 2.00 g to 140.20 ± 3.05 g). All PM2.5-exposed groups showed significant weight loss, most pronounced in Experimental Group 3 (113.15 ± 1.56 g to 105.80 ± 1.56 g, p<0.05). Histological analysis revealed dose-dependent cerebellar damage: mild vacuolation in Group 1, Purkinje cell degeneration in Group 2, and severe cytoarchitectural disruption (pyknotic granular cells, necrosis) in Group 3. **Conclusion**: Chronic carbon soot PM2.5 exposure induces systemic toxicity (weight loss) and progressive cerebellar neurodegeneration in a dose-dependent manner. These findings underscore the urgent need for stricter air quality regulations in regions with high industrial emissions and highlight PM2.5’s potential role in metabolic and neurological disorders.

**Keywords:** Carbon soot PM2.5, Particulate Matter, Weight Loss, Cerebellar Cytoarchitectural, Disruption, Wistar Rats

**INTRODUCTION**

Air pollution remains a major public health challenge, with fine particulate matter (PM2.5) being a particularly hazardous component due to its ability to penetrate deep into biological systems and elicit systemic and organ-specific toxic effects (Xing et al., 2016; Schraufnagel, 2020). The World Health Organization (WHO) estimates that air pollution contributes to approximately 7 million premature deaths annually, largely due to cardiovascular, respiratory, and neurological diseases associated with prolonged PM2.5 exposure (WHO, 2018).

In developing nations such as Nigeria, PM2.5 exposure is exacerbated by industrial emissions, biomass combustion, and unregulated petroleum refining (Ana, 2011). The Niger Delta region, known for its extensive crude oil exploration and artisanal refining activities, experiences severe air pollution, with carbon soot contributing significantly to ambient PM2.5 levels (Aroh et al., 2010). Soot-laden air has been implicated in increased cases of respiratory ailments, cardiovascular disorders, and potential neurotoxic effects in exposed populations (Ekhator et al., 2024).

Previous studies have established that PM2.5 exposure is linked to respiratory and cardiovascular diseases, oxidative stress, and neuroinflammation (Block et al., 2009; Lim & Kim, 2024). However, there is limited research on the toxicological profile of carbon soot-derived PM2.5, particularly its dose-dependent effects on metabolic parameters such as body weight and its neurostructural impact on the cerebellum.

Furthermore, while PM2.5 has been shown to cross the blood-brain barrier and contribute to neurodegenerative conditions (Calderón-Garcidueñas et al., 2011), the specific histopathological changes induced by chronic exposure to carbon soot PM2.5 remain poorly understood. Another critical gap is the paucity of studies examining the impact of PM2.5 exposure on body weight regulation, an important indicator of metabolic and systemic health (Bahreynian et al., 2020).

This study aims to evaluate the systemic (body weight) and neurological (cerebellar histopathology) effects of escalating concentrations of carbon soot PM2.5 in Wistar rats, with the following hypotheses tested:

1. Chronic exposure to carbon soot PM2.5 will result in progressive weight loss due to systemic metabolic disruptions.
2. Exposure to escalating doses of carbon soot PM2.5 will induce significant cerebellar histopathological alterations, including neuronal loss and inflammation.

The findings of this study have direct implications for populations residing in industrialized or high-pollution areas, particularly in low-resource settings where emissions are poorly regulated.

**MATERIALS AND METHODS**

**Study design**

The study was conducted using male albino Wistar rat and the rats were exposed to Carbon Soot particulate matter by inhalation.

**Carbon Soot Particulate Matter Production and Authentication**

The carbon soot used as particulate matter in this study was obtained from the combustion of crude oil sourced from Shell-BP Petroleum Development Company of Nigeria Limited in Rivers State, Nigeria. Combustion was carried out in a local refinery and a controlled combustion chamber (**Figure 1**) at the Engineering Workshop of the Department of Mechanical Engineering, University of Port Harcourt. The resulting carbon soot particulate matter was subsequently identified and authenticated by Dr. Akuma Oji from the Department of Chemical Engineering, University of Port Harcourt, Choba, Rivers State, Nigeria.



**Figure 1. A local refinery and combustion Chamber**

**Carbon Soot Particulate Matter Exposure System**

The whole-body exposure system, a well-established method for studying the effects of airborne pollutants like PM2.5 on animal models (Song et al., 2021), was utilized for the study. This approach allows for naturalistic exposure patterns similar to environmental conditions humans might experience. The apparatus for the carbon soot particulate matter inhalation system comprised a carbon soot particulate matter sample, a particulate matter concentration analyzer and quantifier (PM sensor and meter), three inhalation exposure cages, a circulatory machine (installed fan), and a thermometer to monitor the system's temperature (see **Figure 1**).

**Animal model**

A total of Twenty-eight (23) male Wistar rats, aged between 30 and 60 days and weighing 100-150g, were procured from the Animal Farm House of the Department of Pharmacology, University of Port Harcourt for the study. They were then acclimatized for two weeks before exposure. The rats were bred and housed in the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were kept and nurtured under laboratory conditions, temperature, humidity, and light (temperature 25 ± 2.0°C, 12hr light/12hr dark cycle) and were allowed free access to food and water ad libitum. The animals were fed with standard rat feed (Eastern Premier Mills Limited, Calabar). Rats were identified by special markings on the ears. Wire meshed cages were used to house the animals and saw dust were laid in as beddings. The cages were cleaned daily to enhance clean environment and reduce odour. There was cross ventilation in the animal house. Before commencement of the experiment, the animals were allowed to acclimatize for three weeks under the standard conditions of temperature and illumination.

**Quality Assessment of the Inhalation System**

Prior to rat exposure, a quality assessment was conducted on the inhalation system of the local refinery and combustion chamber to assess its efficiency, soot production, and determine optimal exposure time and concentration. The analysis focused on volatile organic compounds (VOCs), methane, and CO₂. Soot production and accumulation were measured at intermittent time intervals of 30 to 60 minutes to evaluate variations in particulate matter generation.

**Baseline Assessment and Exposure Monitoring**

To establish a baseline and confirm the absence of contamination, three (3) rats were sacrificed and their cerebellar tissues were analyzed for soot deposition histologically. This was to ensure that the animals were free from soot contamination before the experiment. The rats were weighed, and their initial body weights recorded. During the study, the rats were exposed for four (4) hours per day, while emission measurements were recorded every three (3) days. Temperature measurements were taken at least three times per cage daily, and an additional thermometer was placed in the combustion chamber for continuous monitoring throughout the exposure duration.

**Experimental grouping/randomization**

The 20 male albino Wistar rats left after sacrificing 3 for baseline assessment were used for this study. The rats were randomized into 4 groups of 5 (n=5) animals each as shown in Table 1.

**Table 1. Randomization of animals and exposure**

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| --- | --- | --- |
| **Group** | **Identification** | **Exposure** |
| Group 1 | General Control | Rats in this group were not exposed to Carbon Soot Particulate Matter |
| Group 2 | Experimental Group 1 | Exposed to an average concentration of 1.221 ± 0.169 mg/m³ of Carbon Soot Particulate Matter (PM2.5) |
| Group 3 | Experimental Group 2 | Exposed to an average concentration of 1.290 ± 0.214 mg/m³ of Carbon Soot Particulate Matter (PM2.5) |
| Group 4 | Experimental Group 3 | Exposed to an average concentration of 1.282 ± 0.235 mg/m³ of Carbon Soot Particulate Matter (PM2.5) |

**Determination of Body Weight**

The animals were weighed daily with an electric weighing scale (SF-400C) and the body weight of the rats was recorded.

**Animal Sacrifice and Processing of Tissues**

The exposure of animals was conducted for 28 days. The animals from each group were sacrificed on day 28 post exposure to the soot. The rats were anesthetized with diethyl ether and incision made in the thoracic region to expose the heart. The right atrium cut to drain the blood immediately followed by trans-cardiac perfusion using 0.9% saline and then 10ml of 4% paraformaldehyde (PFA) solution through the left ventricle of the heart. The brain was extracted, post-fixed overnight in 4% paraformaldehyde at 4°C. tissues were prepared histologically.

**Histological Preparation of brain** **tissues**

The processing of brain tissues followed a structured histological procedure. Upon removal, the tissues were immediately fixed in 10% formal saline fixative for two weeks to prevent autolysis and bacterial decomposition. Following fixation, dehydration was carried out using ascending grades of alcohol: 50% for two hours, 70% for another two hours, 95% overnight, and absolute alcohol for two hours the next morning. The tissues were then cleared using pure xylene for two hours to eliminate residual alcohol. Impregnation was performed by transferring the tissues into molten paraffin wax on a hot plate, allowing infiltration of the wax into the tissue. Embedding followed, where the tissues were placed in an embedding mold with molten paraffin wax, left to solidify, and then mounted on a wooden block holder in preparation for sectioning. The sectioning process utilized a sliding microtome (Germany), cutting the tissue into 5µm sections, which were floated on a slide containing 20% alcohol and placed on a warm water bath. The sections were then collected onto slides, dried on a hot plate, and further treated in a hot oven before staining.

Hematoxylin and eosin (H&E) staining was performed to enhance tissue visualization. The slides were first placed in xylene for five minutes, followed by dehydration through descending grades of alcohol (absolute, absolute, and 95%) with 10 dips in each solution. Afterward, the tissue sections were washed in tap water for one minute and stained with hematoxylin for three minutes. Following another one-minute wash, differentiation was carried out using 1% acid alcohol, followed by another tap water wash. The slides were then counterstained with eosin for five minutes and washed again in tap water for five minutes. Dehydration was repeated in ascending grades of alcohol (95%, absolute, and absolute alcohol), with 10 dips in each solution. The slides were subsequently placed in xylene twice for five minutes each, blotted in a one-way direction with filter paper, and finally cover-slipped using DPX mountant. After drying, photomicrographs were taken using a Zeiss Axioshop microscope to capture the stained tissue sections. Sections were observed under a digital brightfield microscope (OMAX 40-2000X 3MP Digital Compound Microscope, USA) and photomicrographs were taken 400x magnification.

**Statistical analysis**

The results of the weight of the Wistar rats were expressed as mean ± SEM. The results were analyzed, using ANOVA and a post hoc Turkey test was used to determine if there are significant differences, and p < 0.05 was taken as statistically significant. Data was analyzed using IBM SPSS version 26.0.

**Ethics Considerations**

The study was carried out in adherence to ethical guidelines set by the National Institute of Health (NIH) for the ethical treatment of animals in research. The study was approved by the Research Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria before commencement of the study.

**RESULTS**

**Effect of Carbon Soot Particulate Matter (PM2.5) Exposure on Body Weight of Wistar Rats**

Table 2 presents the effect of Carbon Soot Particulate Matter (PM2.5) exposure on the body weight of Wistar rats over four weeks. The General Control group showed a progressive increase in body weight from 120.10 ± 2.00 g in Week 1 to 140.20 ± 3.05 g in Week 4, indicating normal growth. In contrast, all experimental groups exhibited a gradual decline in body weight over the study period. Experimental Group 1, exposed to an average PM2.5 concentration of 1.221 ± 0.169 mg/m³, showed a slight but significant weight reduction, from 118.50 ± 1.85 g in Week 1 to 110.12 ± 1.90 g in Week 4 (*p*<0.05). Similarly, Experimental Group 2, exposed to 1.290 ± 0.214 mg/m³ of PM2.5, demonstrated a steady decline in body weight from 115.20 ± 1.77 g in Week 1 to 108.20 ± 1.75 g in Week 4 (*p*<0.05). The most pronounced weight loss was observed in Experimental Group 3, which received the highest PM2.5 exposure (1.282 ± 0.235 mg/m³), with body weight decreasing from 113.15 ± 1.56 g in Week 1 to 105.80 ± 1.56 g in Week 4 (*p*<0.05). The significant weight reduction across all experimental groups compared to the control suggests a negative impact of PM2.5 exposure on body weight, potentially indicating systemic toxicity or metabolic alterations.

**Table 2: Effect of Carbon Soot Particulate Matter (PM2.5) Exposure on Body Weight of Wistar Rats**

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| --- | --- | --- | --- | --- |
| **Group** | **Week 1** | **Week 2** | **Week 3** | **Week 4** |
| General Control | 120.10 ± 2.00 | 130.10 ± 2.50 | 135.00 ± 2.82 | 140.20 ± 3.05 |
| Experimental Group 1 | 118.50 ± 1.85 | 115.30 ± 2.25 a | 113.15 ± 2.05 a | 110.12 ± 1.90 a |
| Experimental Group 2 | 115.20 ± 1.77a | 112.05 ± 2.00 a | 110.40 ± 1.88 a | 108.20 ± 1.75 a |
| Experimental Group 3 | 113.15 ± 1.56a | 110.08 ± 1.95 a | 108.15 ± 1.66 a | 105.80 ± 1.56 a |

**a** S*ignificant at p<0.05 compared to General control group; Values are presented with Mean ± SEM.*

**Histopathological Assessment of Cerebellar Cytoarchitecture**

The histological analysis of cerebellar cytoarchitecture revealed progressive structural alterations across experimental groups, with increasing severity of damage compared to the general control (Figure 2A). In Experimental Group 1, the molecular and Purkinje cell layers remained well-delineated, though vacuolation was observed in the medullary white matter, indicating mild cytoarchitectural distortion (Figures 2B, 3B). In Experimental Group 2, there was evident degeneration of the Purkinje cell layer, along with pyknotic and necrotic granular cells, leading to severe disruption of cerebellar structure (Figures 2C, 3C). Experimental Group 3 exhibited similar degeneration of the Purkinje cell layer with pyknotic granular cells, further confirming the progressive distortion of cerebellar cytoarchitecture (Figures 2D, 3D). These findings suggest a dose-dependent impact on cerebellar integrity, with increased cellular degeneration and vacuolation correlating with higher exposure levels.

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| (A). Group 1 (General control) | (B). Group 2 (Experimental group 1): 1.221 ± 0.169 mg/m³ PM2.5 |
|  |  |
| (C). Group 3 (Experimental group 2): 1.290 ± 0.214 mg/m³ PM2.5 | (D). Group 4 (Experimental group 3): 1.282 ± 0.235 mg/m³ PM2.5 |

**Figure 2. Photomicrograph of cerebellar cytoarchitecture.** (A) General control group showing normal molecular, Purkinje, and granular cell layers. (B) Experimental Group 1 displaying well-delineated cells with no obvious pathology in the molecular and Purkinje layers but mild vacuolation in the medullary white matter, indicating slight cytoarchitectural distortion. (C) Experimental Group 2 showing Purkinje cell degeneration, pyknotic and necrotic granular layers, and severe cytoarchitectural distortion. (D) Experimental Group 3 revealing Purkinje cell degeneration and pyknotic granular layers, indicating cerebellar cytoarchitectural disruption. **Stain/Magnification:** **H&E, x100.**

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| --- | --- |
|  | |
| (B). Group 2 (Experimental group 1): 1.221 ± 0.169 mg/m³ PM2.5 | |
|  |  |
| (C). Group 3 (Experimental group 2): 1.290 ± 0.214 mg/m³ PM2.5 | (D). Group 4 (Experimental group 3): 1.282 ± 0.235 mg/m³ PM2.5 |

**Figure 3. Photomicrograph of cerebellar cytoarchitecture.** (B) Experimental Group 1 showing well-delineated molecular and Purkinje cell layers with no obvious pathology, but with vacuolation in the medullary white matter, indicating mild cytoarchitectural distortion. (C) Experimental Group 2 displaying Purkinje cell degeneration and granular cell aggregation, with severe cytoarchitectural distortion. (D) Experimental Group 3 revealing Purkinje cell degeneration and pyknotic granular cell layers, indicating cytoarchitectural disruption. **Stain/Magnification: H&E, x400.**

**DISCUSSION**

The present study demonstrated that chronic exposure to Carbon Soot Particulate Matter (PM2.5) induced significant body weight reduction and cerebellar cytoarchitectural damage in Wistar rats. All experimental groups exhibited progressive weight loss over four weeks, contrasting with the steady weight gain observed in the control group. Histological analysis further revealed dose-dependent cerebellar degeneration, characterized by vacuolation, Purkinje cell layer necrosis, and pyknotic granular cells. These findings collectively suggest that PM2.5 exposure disrupts metabolic homeostasis and induces neurostructural damage, with severity escalating alongside exposure levels.

These findings align with previous research that highlights the systemic and neurological impacts of PM2.5 exposure. For example, studies have shown that PM2.5 exposure impairs glucose metabolism, reduces energy expenditure, and induces oxidative stress, leading to weight loss and metabolic dysfunction in animal models (Xu et al., 2019; Rajagopalan et al., 2020). Similarly, PM2.5 has been implicated in neuroinflammation and neurodegeneration through mechanisms involving oxidative stress and pro-inflammatory cytokine release (Kang et al., 2021; Chen et al., 2022). The observed cerebellar damage is consistent with reports of PM2.5-induced neurotoxicity in human brain models, where particulate matter penetrates the blood-brain barrier and triggers astrogliosis, neuronal loss, and microglial activation (Chen et al., 2022). These parallels suggest that PM2.5 exposure may disrupt both systemic and neurological homeostasis.

The observed weight loss and cerebellar damage in Wistar rats following PM2.5 exposure have significant real-world implications. In humans, prolonged exposure to fine particulate matter has been linked to various health issues, including respiratory and cardiovascular diseases (Garcia et al., 2023; Wan Mahiyuddin et al., 2023; Sarawut et al., 2024). The neurological impairments observed in this study suggest that PM2.5 exposure may also contribute to neurodegenerative conditions, potentially impacting cognitive functions and motor skills. Clinically, the results suggest that prolonged PM2.5 exposure may contribute to underrecognized comorbidities such as cachexia or neurodegenerative disorders, warranting enhanced screening in high-risk populations. These findings highlight the importance of stringent air quality regulations and public health interventions aimed at reducing PM2.5 emissions to safeguard both human and animal health. Furthermore, the study contributes to the growing body of evidence highlighting the systemic toxicity of air pollutants, emphasizing the need for comprehensive environmental policies.

While the study provides valuable insights, certain limitations must be acknowledged. The sample size of 23 rats, though adequate for preliminary observations, may not capture the full spectrum of potential biological responses to PM2.5 exposure. Additionally, the study's duration was limited to four weeks; longer exposure periods might reveal more pronounced or additional effects. The specific composition of the carbon soot particulate matter was not analyzed, leaving the possibility that certain components may have disproportionately contributed to the observed effects. Moreover, the study focused solely on male Wistar rats, which may limit the generalizability of the findings across different sexes or species. These limitations suggest that while the results are indicative, they should be interpreted with caution and in the context of broader research.​

Future studies should aim to address these limitations by incorporating larger, more diverse animal cohorts and extending the exposure duration to assess long-term effects. Detailed chemical characterization of the particulate matter would help identify specific toxic components, facilitating targeted mitigation strategies. Investigating the underlying mechanisms of PM2.5-induced neurotoxicity, such as oxidative stress and inflammation pathways, could provide deeper insights into the pathophysiology observed. Additionally, exploring potential protective interventions, such as antioxidants or anti-inflammatory agents, may offer therapeutic avenues to counteract the adverse effects of PM2.5 exposure. Comparative studies involving different species and both sexes would enhance the generalizability of findings, contributing to a more comprehensive understanding of air pollution's impact on health.

**CONCLUSION**

This study demonstrates that exposure to Carbon Soot Particulate Matter (PM2.5) adversely affects the health of Wistar rats, evidenced by significant weight loss and cerebellar structural damage. The control group exhibited normal growth patterns, while experimental groups exposed to varying PM2.5 concentrations experienced progressive weight reduction over four weeks. Histological analyses revealed dose-dependent cerebellar degeneration, with increased cellular damage correlating with higher PM2.5 exposure levels. These findings highlight the potential systemic and neurological risks associated with PM2.5 inhalation, and the need for further research into its health implications and the development of strategies to mitigate exposure.

**Conflict of interest**

Authors declare no conflict of interest exist

**COMPETING INTERESTS DISCLAIMER**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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