DOI: https://dx.doi.org/10.4314/jaaps.v12i2.6

J. Afr. Ass. Physiol. Sci.12 (2): 48-60. December 2024

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

https://www.ajol.info/index.php/jaaps

Research Article

Histological Changes in the Lungs and some Haematological and Biochemical Changes in Wistar Rats Following Exposure to Fly Ash Dust

Mabel B. Ehi-Omosun and Maryam F. Akande

Departments of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

Keywords:

Fly ash particles; histoarchitecture; lungs; pneumonitis; measurable biological chemicals; manganese; urea; creatinine

* Address for Correspondence: Email: mabel.ehi-omosun@uniben.edu

Received: 17 November 2024
Revised: 28 December 2024
Accepted: 30 December 2024

ABSTRACT

Background: Fly ash dust is a harmful air pollutant that poses a major environmental and health risk. Previous research has linked fly ash dust to respiratory tract disease but its effects on the lung are not well understood. We aimed at investigating body weight, lung histoarchitecture, and haematological and biochemical changes in Wistar rats exposed to fly ash dust.

Methods: Twenty-four Wistar rats (12 males, 12 females), weighing 250g-280g, were randomly assigned into 4 groups of 6 animals each. Group A rats were placed in a fly ash dust-free chamber while Group B - D rats were exposed to various concentrations of fly ash dust dispersed from 5g, 10g and 20g of fly ash, respectively. The weights of the animals were recorded weekly and body weight gain computed. At the end of 28th day of exposure, the rats were weighed and euthanized under chloroform anaesthesia. Blood samples were collected through cardiac puncture into plain specimen bottles for biochemical analysis and into EDTA anticoagulant bottles for haematological analysis. The lungs were harvested and processed for histological examination. The obtained data were analyzed using the one-way Analysis of Variance, with level of significance set at $P<0.05$.

Results: Exposure to fly ash dust caused significantly reduced weight gain and haematological alterations in rats including decreased lymphocytes, haemoglobin, and red blood cells. Biochemical analysis revealed increased manganese, urea, and creatinine levels, indicative of manganese poisoning and renal impairment. Additionally, low serum bicarbonate (HCO_3^-) levels suggested acidosis. Histopathological examination confirmed normal lung architecture in the control group. There were observable histological variations in the lung architecture of the exposed rats (Groups B-D) which include bronchiolar ulceration, activated lymphoid follicles, and patchy alveolar collapse (evidence of pneumonitis). Given the similar biological responses, these effects of fly ash dust on rats can be reasonably extrapolated to humans, highlighting the importance of protective measures for individual occupationally exposed to fly ash dust.

Conclusion: Fly ash dust caused body weight loss, histopathological changes in lung tissue, haematological and biochemical derangements which are capable of compromising lung, haematological, biochemical and renal functions, potentially resulting in fatal outcomes.

All articles published in this journal are licensed under the **Creative Commons Attribution 4.0 International** (CC BY 4.0) license.

© Copyright 2024 *African Association of Physiological Sciences* -ISSN: 2315-9987. All rights reserved

M.B. Ehi-Omosun's ORCID ID: https://orcid.org/0009-0000-5612-8466.

1. Introduction

Fly ash dust, a byproduct of coal combustion, is an atmospheric pollutant (Igunka-Nnoka *et al.*, 2021). It is generated during the entire value chain, from coal combustion in power plants to transportation, storage, usage, concrete cutting and workers' activities like bag emptying (Eriakhaba *et al.*, 2020; Adeleke *et al.*, 2019). Fly ash dust possesses distinct physicochemical properties shaping its behaviour, applications, and environmental impact (Veeresham *et al.*, 2019; Sikira, *et al.*, 2021). Physically, fly ash dust exhibits a grayish to dark gray colour and comprises particles ranging from 0.01 to 100 µm in size, with the majority being less than 10 µm (Oriabure *et al.*, 2023). This fine particle size contributes to its potential health hazards and environmental dispersion. The density of fly ash dust falls between 2.0 g/cm^3 and 3.0 g/cm^3, significantly influencing its setting behaviour and transportation properties (Robbani *et al.*,2016; Otong and Ikelegbe, 2023). The alkaline pH (8-12) of fly ash dust affects its reactivity and interaction with other substances (Tiwari, 2016; Oriabure *et al.*, 2023; Islam *et al.*, 2020). Understanding these physicochemical properties is crucial for environmental management, industrial applications, and health risk assessments. Fly ash dust's unique physicochemical properties dictate its behaviour, uses, and potential risks. Recognizing these characteristics of fly ash dust informs strategies for mitigating environmental impacts and optimizing beneficial applications.

Many studies (Williams *et al.*, 2017; Oseahumen *et al.*, 2017; Saidi *et al.*, 2021*;* Patwardhan *et al.*, 2018**)** have previously attempted to evaluate the effects of fly ash dust exposure in humans on the basis of spirometry or radiology or both. However, due to lack of suitable model for controlled laboratory exposures, investigations on the effects of fly ash dust in Wistar rats and extent of the associated toxicity have been limited (Bello *et al.*, 2019; Iden, 2016).

Several authors have reported that exposure to fly ash dust may result in some metabolic disorders, cardiovascular diseases and severe inflammation (Gheethi *et al.*, 2022; George, *et al.*, 2018). Not much has been reported on fly ash dust exposure on the tissue morphology and parameters of haematological and biochemical functions. It will be of public interest to know the effects of fly ash dust exposure on tissue morphology, haematological and biochemical parameters, and body weight. The haematological parameters commonly examined to assess toxic stress induced by fly ash dust include white blood cells, lymphocytes, monocytes, neutrophils, eosinophil, basophils, platelets, reticulocytes, haemoglobin, hematocrit, red blood cells, and other red cell indices (Ullah *et al.*, 2016). Furthermore, biochemical indices, including bicarbonate, urea, and creatinine as well as the basic biochemical components of fly ash dust (aluminium, silicon, calcium, manganese, iron, and zinc), are evaluated due to their potential environmental health concerns (Vandel *et al.*, 2022).

Exposure to fly ash dust may cause several health complications and pathogenesis because fly ash dust contains many types of chemical elements. These include aluminium, silicon, calcium, manganese, iron and zinc which at high levels of exposure can trigger biochemical changes in the body leading to some derangements in biochemical parameters and may lead to pathogenesis of various biochemical diseases including respiratory disorders, manganism, and renal diseases (Gungor and Usunobun, 2017; Fletcher and Peto, 2017; Jarikre *et al.*, 2019). The most reported occupational hazards for fly ash workers are allergy, pneumonitis, and other respiratory illnesses (Finkelman *et al.*, 2022; Ibe, *et al.*, 2021; Mark *et al.*, 2016).

Excessive accumulation of manganese in the blood or tissues causes a neurodegenerative disease called manganism, characterized by tremors, insomnia, anorexia, irritability, general malaise and hearing impairment (Mark *et al.*, 2016; Drake *et al.*, 2015). Source of manganese poisoning could be occupational, environmental or iatrogenic. Bicarbonate (HCO_3^-) is a byproduct of body's metabolism (Mohamed *et al.*, 2016. The blood brings bicarbonate to the lungs, and then it is exhaled as carbon dioxide (Ehiabhi *et al.*, 2018; Elgailani and Alsakka *et al.*, 2016. Low HCO_3^- level indicates poor health in the form of acidosis. Urea and creatinine are waste products

of the body's metabolism (Chooli *et al.,* 2015; Cheesbrough, 2015). Typically, they dissolve in the blood and are then filtered and excreted out through urine by the kidney (Cheong *et al.,* 2018). Urea also known as carbamide ($CO(NH_2)_2$) is an organic compound, formed by the metabolism of proteins (Betkoval *et al.,* 2019; Ahmed *et al.,* 2016)). Creatinine is a waste product that forms when creatinine phosphate, which is found in the muscle, breaks down (Bankole *et al.,* 2021; Johnson *et al.,* 2018). High level of urea and creatinine in the blood indicate uremia as the kidney will be unable to filter these byproducts (Gheethi *et al*., 2022; Heyder *et al.,* 2022***)***. Prompt treatment is essential, as unresolved manganism, acidosis, and uremia can culminate in general malaise, kidney failure, and severe respiratory and cardiac dysfunction, posing a significant risk to life (Ghanapathy *et al.,* 2020; Moore and Dalley, 2015).

Signs and symptoms of fly ash dust related kidney and respiratory failure include fatigue, wheezing, dyspnoea, cyanosis, dizziness, uremia, multiple-organ failure and death if left untreated (Fermandesi *et al.,* 2020; Harrison, 2015; Bergmeyer *et al.,* 2017). Hence, the present study investigates the effects of fly ash dust on the tissue morphology, haematological and biochemical parameters and body weight of Wistar rats.

1.1 Objectives of the Study:
The objectives were to;

1. investigate the effects of fly ash dust on the weight of Wistar rats
2. evaluate changes in the haematological parameters of Wistar rats exposed to fly ash dust
3. evaluate changes in some biochemical indices of Wistar rats exposed to fly ash dust
4. determine histological changes in the lungs of Wistar rats exposed to fly ash dust.

2. Materials and Methods

2.1 Experimental Animals: Twenty-four (24) adult Wistar rats (both sexes) of 250 g and 280 g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria and were utilized for this experiment. Animals were kept in groups of six per cage, with wood shavings as bedding, under controlled environmental conditions: 12-hous light-dark cycle, 50-60% humidity, and temperature ranging from 25-28^{0}C. The housing conditions were designed to minimize stress and ensure the comfort and well-being of the animals. The animals were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed manufactured by Bendel Flour Mill, Ewu, and clean water *ad libitum*. The supplier, Bendel Flour Mill, situated in Ewu, Edo State, Nigeria, delivered Standard Animal Feed in pellet Form. The relevant details are: batch number: BMI/EWCB/009 and expiration date July 2024. We ensured that the feed's nutritional value remained uncompromised by using it within the recommended time frame.

2.2 Ethical Consideration: Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (The approval number obtained is CMS/REC/2012/338). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

2.3 Experimental design: This study employed 24 animals, randomly assigned to four groups of six rats each. To mitigate external influences, we employed stringent controls in our study design. Rats were housed in standardized conditions, with six per cage, identical cage, size, bedding materials, and ventilation. A uniform diet was ensured by providing the same standardized feed from Bendel Flour Mill, and with unrestricted access to clean water. Additionally, a controlled 12-hour light/dark cycle was maintained, with standardized lighting intensity and wavelength, to further reduce environmental variability. These controls ensured that any differences observed between groups were due to the experimental treatment and not to environmental factors. We employed a randomized block design to divide

the 24 rats into four groups of six rats each. To minimize bias, we employed a rigorous methodology, utilizing a computer-generated randomization program to assign rats to groups. We ensured that the groups were well-matched for critical variables, including age, sex, and body weight. Additionally, we implemented a blind procedure for group assessment and treatment administration, and maintained researcher blinding throughout data collection and analysis, to prevent any potential bias. By taking these measures, we aimed to ensure the validity of our study results. Group A rats which served as control, were placed in a fly ash dust-free dust distributor glass chamber (DDGC). Group B rats were exposed to fly ash dust dispersed from 5 g of fly ash daily for 30 days. Group C rats were exposed to fly ash dust dispersed from 10 g of fly ash daily for 30 days. Group D rats were exposed to fly ash dust dispersed from 20 g of fly ash daily for 30 days. The dosages of 5 g, 10 g, and 20 g of fly ash used in this experiment were determined based on a thorough review of existing literature on toxicological studies involving fly ash exposure in animal models (Tiwari, 2016; Patwardhan *et al.*,2018; Veeresham *et al.,* 2019). We also considered factors such as animal weight, exposure duration, and route of administration. The dosages were administered via inhalation for 1 hour per day, for a total of 30 consecutive days, utilizing dust distributor glass chamber (DDGC). The weights of the animals in each group were taken and recorded weekly and the difference between them and previous weights were noted.

2.4 Method of Termination and Sample Collection:

At the end of 30th day of exposure, the animals were weighed and subsequently euthanized under chloroform anaesthesia. Blood samples were obtained via cardiac puncture and divided into two groups: EDTA anticoagulant bottles for haematological testing and plain specimen bottles for biochemical analysis. The lung of each rat was excised and immediately fixed in 10% formal saline for 24 hours to prevent tissue degradation and autolysis before the histological procedures. The tissue sections were cut in the sagittal plane with a thickness of 5µ (5 micrometer), using a microtome and processed according to the method of Drury and Wallington, (1980). Consistency was ensured by using a precision microtome with a digital micrometer, allowing for precise control over section thickness. The trimmed tissues underwent manual histological processing for microscopy involving fixation in 10% neutral buffered formalin (P^H 7.4) for 24-48 hours at room temperature, followed by embedding in paraffin wax using a Leica TP1020 tissue processor at 58 ^{0}C, 100 mbar, and 12 hours. Subsequently, tissues were stained with Hematoxylin and Eosin (H&E) using a Thermo Scientific Gemini tissue stainer at 37 ^{0}C for 30 minutes. This H&E staining enabled visualization of tissue morphology and architecture, allowing for the evaluation of histological changes and their correlation with experimental findings. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x40.

White blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, red blood cells, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelets, and reticulocytes were analysed using an auto-analyzer (2006 model, manufactured by Hoddlier and Stoughton Group of Company, London with recognized biochemical kit (2010 model, Diagnostic Merck, London).

Measurement of Biochemical Indices: Calcium was assayed for by using a convenient and accurate method for determination of total serum calcium concentration in blood samples called Flame Atomic Emission Spectrophotometry (FAES) (Cheesbrough, 2015). Serum level of silicon was measured by Direct Current Plasma Emission Spectrometer (DCPES) in blood samples (Mark *et al.,* 2016). The concentration of Aluminium in blood was measured using the analytical techniques known as Electrothermal Atomic Absorption Spectrometry (Cheesbrough, 2015). Flameless Atomic Absorption Spectroscopy (FAAS) was the method used for the quantitative analysis of manganese in blood sample (Harrison,, 2015). Double bean ultraviolet - visible spectrophotometric (UVVS) method was used for the measurement of iron in blood sample

(Cheesbrough, 2015). There are different analytical techniques that have been employed to measure zinc in biological samples. However, in this present study, Flame Atomic Absorption Spectrometry (FAAS) was the method of choice used for the measurement of zinc in blood sample (Mark *et al*., 2016).

Electrolyte analysis was conducted on a Roche Diagnostics Electrolyte Analyzer, manufactured by Roche Diagnostic Groups of Company, Germany. We employed multiple test methods to measure electrolytes, urea, and creatinine. Specifically, serum electrolytes were analyzed using ion-selective potentiometry, while blood urea was measured using the hypobromite kowarsky method. Creatinine levels were determined using the calorimetric method of Folins and Benis (Cheesbrough, 2015).

2.5 Statistical analysis: Statistical analysis was carried out with Statistical Software Package, Microsoft Excel, (2010) and Statistical Package for Social Sciences (S.P.S.S.) version 20. Results were presented as Mean (X) ± Standard error of mean (SEM). The one-way Analysis of Variance (ANOVA) was used to determine the significance of the difference in means at 95% confidence interval. $P \leq 0.05$ was considered significant.

3. Results

Body Weight Findings: Changes in body weights of the animals in all the experimental groups are presented in Table 1. The results revealed a significant increase ($p<0.05$) in body weight across all experimental groups (A-D). However, weight gain was significantly lower in exposed groups (B-D) compared to the control group (A), suggesting fly ash's inhibitory effect on weight gain.

Haematological Test Findings: Findings of the study for haematological analysis in all the experimental groups are presented in Tables 2. The table shows that fly ash dust decreased some haematological parameters such as lymphocytes and basophils, haemoglobin and haematocrit, red blood cells and other red cell indices with associated reticulocytosis in the exposed rats across all the groups. The haematological abnormalities observed in the animals may precipitate anaemia and heightened susceptibility to infections potentially compromising haematological homeostasis and increasing the risk of mortality.

Biochemical Test Findings: Results of the biochemical tests of the animals in all the experimental groups are presented in Tables 3 and 4. Comparative analysis of serum levels of calcium, silicon, aluminium, manganese, iron zinc in the control and exposed groups (B, C, and D) revealed a statistically significant elevation in manganese levels alone (Figure 3). Significant ($p<0.05$) elevations in urea, and creatinine levels were observed in groups B-D compared to the control group (A), whereas bicarbonate levels exhibited a significant ($p<0.05$) decline in groups B-D relative to the control group (Figure 4).

Histological Findings: Histological findings in all the experimental groups are presented in Figure 1. The photomicrograph of the control group (group A), shows normal features of the lung such as alveoli, terminal bronchiole, interstitial space and bronchial artery (figure 1A). In the rats exposed to fly ash dust dispersed from 5 g of fly ash (group B), there were mild bronchiolar ulceration, activated lymphoid follicles and patchy alveolar collapse (figure 1B). The group exposed to fly ash dust dispersed from 10g of fly ash (Group C), presented severe bronchiolar ulceration, activated lymphoid follicles and patchy alveolar collapse (figure 1C). In the rats exposed to fly ash dust dispersed from 20 g of fly ash (group D), there were severe bronchiolar ulceration, activated lymphoid follicles and patchy alveolar collapse (figure 1D).

Table 1: Weekly Body Weight Gain: Comparative Analysis across Groups (g)

Exposure Period	Group A	Group B	Group C	Group D	P Value
Ist week	5.60 ± 0.68g	0.60 ± 0.19g *	0.42 ± 0.16g *	0.38 ±0.16g *	0.000
2nd week	6.70 ± 0.93g	0.30 ± 0.05g *	0.30 ± 0.09g *	0.20± 0.14g *	0.000
3rdweek	7.40 ± 1.24g	0.06 ± 0.17g *	0.20 ± 0.05g *	0.16± 0.07g *	0.000
4thweek	7.74 + 0.60g	0.18 ± 0.09g *	0.36 ± 0.10g *	0.04± 0.08g *	0.000

n=6; g=gram; Values are Mean ± S.E.M

Table 2: Initial and Final Body Weights of Rats across All Experimental Groups

Groups	Initial body weight (g)	Final body weight (g)	Weight Difference	P-value
Group A (Control)	5.60 ± 0.68g	7.74 ± 0.60g	2.94 ± 3.34g	0.000
Group B (5g of fly ash Exposure)	0.60 ± 0.19g *	0.68 ± 0.09g *	0.80 ± 0.14g	0.000
Group C (10g of fly ash Exposure)	0.42 ± 0.16g *	0.46 ± 0.10g *	0.68 ± 2.20g	0.000
Group D (20g of fly ash Exposure)	0.38 ± 0.16g *	0.44 ± 0.18g *	0.58 ± 0.10g	0.000

n=6; Values are Mean ± S.E.M. The P-value speaks to the difference between the initial and final weights.

Table 3: Comparison of Haematological Parameters In all the Experimental Groups

Haematological Parameters	Groups				P- Values
	A (Control)	B (5 g of cement)	C (10 g of cement)	D (20 g of cement)	
WBC (10^3/uL)	11.06 ± 3.00	10.48 ± 2.34	9.66 ± 0.88	8.22 ± 1.65	0.799
LYM (%)	79.78 ± 1.07	75.04 ± 3.52	70.02 ± 5.58	59.20 ± 3.04*	0.007
MON (%)	26.84 ± 6.70	21.50 ± 1.38	20.40 ± 1.74	18.94 ± 2.09	0.474
NEU (%)	8.64 ± 0.99	7.92 ± 1.02	6.20 ± 1.29	5.34 ± 0.96	0.155
EOS (%)	1.94 ± 1.37	1.38 ± 0.54	1.02 ± 0.46	0.70 ± 0.27	0.715
BAS (%)	9.56 ± 1.15	6.12 ± 1.82	4.66 ± 0.79	3.92 ± 0.58*	0.018
RBC (10^6/ul)	10.14 ± 0.73	9.12 ± 0.22	8.98 ± 0.30	7.02 ± 0.21*	0.001
HGB (g/dl)	13.04 ± 0.73	12.12 ± 0.33	11.10 ± 0.35*	10.76 ± 0.23*	0.010
HCT (%)	43.30 ± 1.66	42.56 ± 2.06	38.38 ± 1.35	35.52 ± 1.34*	0.012
MCV (Fl)	46.60 ± 0.38	45.32 ± 0.61	44.34 ± 0.40*	42.84 ± 0.47*	0.000
MCH (pg)	17.16 ± 0.21	16.90 ± 0.24	16.30 ± 0.17	15.10 ± 0.25*	0.000
MCHC (%)	35.78 ± 0.36	35.06 ± 0.32	34.98 ± 0.30	34.02 ± 0.02*	0.005
PLT (10^3/uL)	826.00 ± 18.87	802.60 ± 59.09	798.40 ± 5.83	741.20 ± 52.47	0.674
RET (%)	0.12 ± 0.02	0.54 ± 0.11*	0.98 ± 0.40*	1.60 ± 0.72*	0.000

Values are Mean ± S.E.M *P<0.05 vs. control

Key: White blood cells (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (NEU), Eosinophils (EOS), Basophils (BAS), Red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Platelets (PLT), Reticulocytes (RET).

Table 4: Serum Trace Element Concentrations in Fly Ash-Exposed and Unexposed Rats

Trace element	Group A	Group B	Group C	Group D	P Values
Ca (mg/dl)	166.73 ± 38.48	213.46 ± 24.87	223.68 ± 14.36	243.68 ± 24.00	0.258
Si (mg/dl)	26.80 ± 4.02	29.02 ± 3.93	30.46 ± 3.03	34.34 ± 3.24	0.521
Al (mg/dl)	242.56 ± 55.54	245.14 ± 17.91	245.28 ± 8.92	266.76 ± 15.06	0.936
Mn (mg/dl)	0.34 ± 0.01	1.02 ± 0.17*	1.07 ± 0.02*	1.70 ± 0.07*	0.000
Fe (mg/dl)	25.80 ± 8.38	26.76 ± 4.31	27.92 ± 3.37	28.64 ± 3.99	0.983
Zn (mg/dl)	1.08 ± 0.32	1.64 ± 0.23	1.72 ± 0.20	2.20 ± 0.57	0.225

Values are Mean ± S.E.M *P<0.05 compared with control

Table 5: Serum Bicarbonate (HCO_3^-), Urea, and creatinine Profiles in Experimental Groups

	Group A	Group B	Group C	Group D	P Value
Bicarbonate (Mmol/L)	20.11 ± 3.23	12.56 ± 1.31*	10.87 ± 3.41*	8.13 ± 12.44*	P<0. 001
Urea (mg/dl)	24.11 ± 3.23	84.11 ± 3.23*	90.87 ± 3.41*	94.10 ± 0.41*	P <0. 001
Creatinine (mg/dL)	0.60 ± 12.34	1.24 ± 0.28*	1.80 ± 12.24*	2.20 ± 2.26*	P <0. 001

Values are Mean ± S.E.M *P<0.05 compared with control

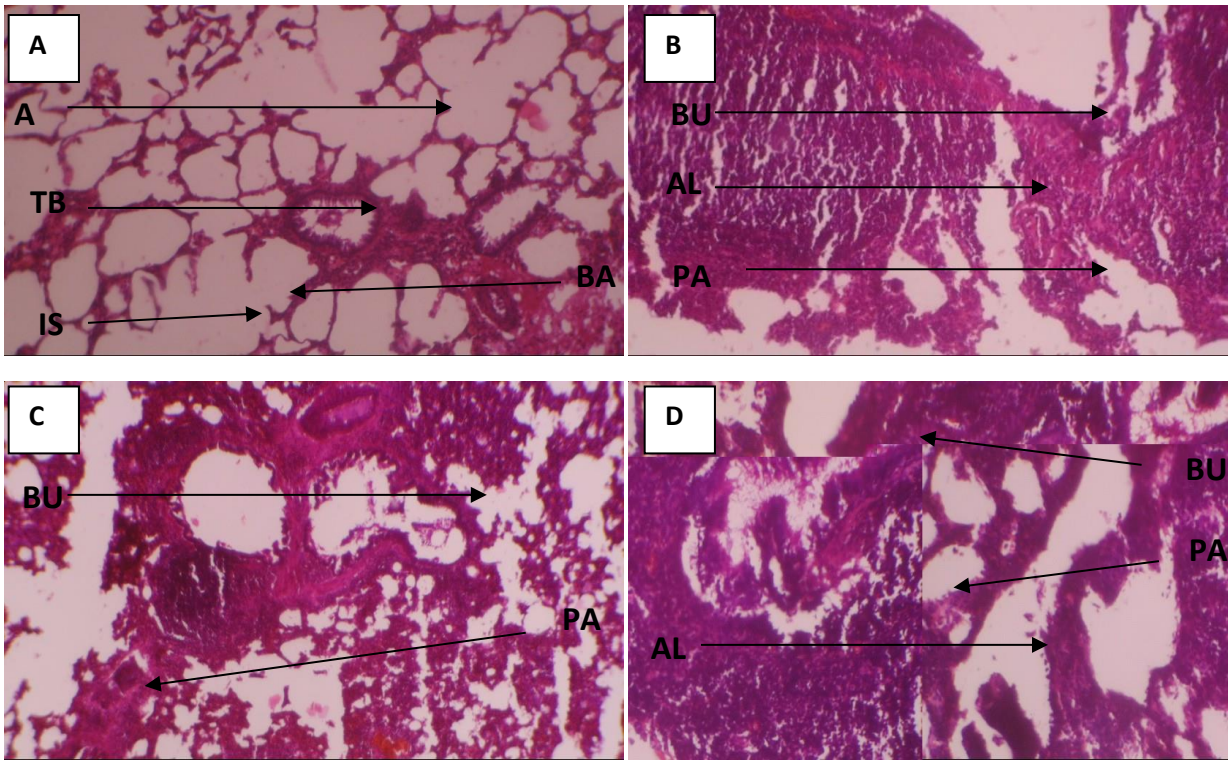

FIGURE 1: Representative photomicrographs of H&E sections of the lungs of the experimental animals at the end of the study

'A' shows the Group A (Control group) showing normal alveoli **'A'**, terminal bronchiole **'TB'**, interstitial space **'IS'**, and bronchial artery **'BA'**. 'B' shows Group B exposed to cement dust dispersed from 5 g of cement daily for 28 days showing bronchiolar ulceration **'BU'**, activated lymphoid follices **'AL'**, and patchy alveolar collapse **'PA'**. 'C' shows Group C exposed to cement dust dispersed from 10 g of cement daily for 28 days showing severe bronchiolar ulceration **'BU'**, and patchy alveolar collapse **'PA'.** 'D' shows Group D exposed to cement dust dispersed from 20 g of cement daily for 28 days showing severe bronchiolar ulceration **'BU'**, patchy alveolar collapse **'PA'** and activated lymphoid follicles **'AL'**.

4. Discussion

Fly ash dust inhalation has been linked to various respiratory diseases and other health issues in humans and animals. Despite this, research on its effects has been limited due to the lack of suitable laboratory equipment. This study investigated the impact of fly ash dust on lung histology, body weight, and haematological and biochemical parameters in Wistar rats.

Exposure to fly ash dust led to reduced weight gain, likely due to its toxicological properties. Consistent with our findings, Ehiabhi *et al.*, (2018) suggested that the reduced weight gain observed in rats exposed to fly ash dust is indicative of the toxic or harmful effects of its constituent chemical elements. Inhalation of fly ash dust triggered a systemic inflammatory response (Figure 1B-1D) in exposed animals, leading to pulmonary toxicity, which likely impacted their overall health and contributed to reduced weight gain. The implications of these results are crucial for the development of innovative weight management strategies in humans.

Findings of the study for haematological parameters showed that exposure to fly ash dust causes derangements in haematological parameters such as lymphocytes, basophils, haemoglobin and haematocrit (Table 3). The

exposed groups showed a reduction in lymphocytes and basophils, suggesting compromised immune function and heightened susceptibility to infections. Additionally, the exposed animals exhibited reduced red blood cell parameters, including mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, resulting in microcytic normochromic anemia. This observation aligns with the findings of Chooli *et al.,* (2015). Furthermore, decreased hemoglobin and hematocrit levels were observed in exposed groups, with a corresponding increase in reticulocytes, indicating an effective erythropoietic response. These haematological changes are indicative of various disease states, including microcytic hypochromic anemia, basopenia, lymphocytopenia, and reticulocytosis.

The results of this study demonstrate that fly ash dust exposure causes significant alterations in serum biochemical parameters, including urea, creatinine, bicarbonate, and manganese (Tables 4 and 5). The observed decrease in bicarbonate levels in exposed groups suggests acidosis, while increased levels of manganese, urea, and creatinine indicate manganism and uremia, aligning with previous research by Chooli *et al*. (2015). These biochemical derangements are indicative of a range of diseases, including manganese poisoning, renal failure, and respiratory acidosis, highlighting the potential for mortality due to these conditions.

Histological examination of lung tissues from Groups B, C, and D revealed consistent findings, including bronchiolar ulceration, activated lymphoid follicles, and patchy alveolar collapse (Figures 1B, 1C, and 1D). These lesions may lead to severe respiratory complications, such as chest pain, lung infections, airway obstruction, impaired lung function, and haemoptysis. Moreover, patchy alveolar collapse may cause impaired gas exchange, hypercapnia, pulmonary hypertension, and hypoxemia, potentially resulting in organ failure. Activated lymphoid follicles indicate inflammation, tissue damage, and immune system activation. Our findings are in agreement with Poinen-Rughooputh *et al*.'s (2016) study, which induced pneumonia using silica dust.

These findings suggest that fly ash dust exposure can lead to severe health complications, including, respiratory problems, renal failure, manganese poisoning, pneumonitis, bronchiolitis, and alveolitis. Given the similar biological responses, these effects of fly ash dust on rats can be reasonably extrapolated to humans, highlighting the importance of protective measures for individuals occupationally exposed to fly ash dust.

This study provides valuable insights into the harmful effects of fly ash dust on respiratory and overall health. Its findings can inform strategies for preventing and managing fly ash-related health hazards, particularly in industrial settings. Preventing fly ash dust-related bronchiolo-alveolar toxicity and its complications requires a multi-faceted approach. This includes adhering to proper safety protocols, such as wearing personal protective equipment like face masks, face shields, goggles, hand gloves, boots and coveralls, to minimize exposure. Regular medical check-ups are also crucial, especially for cement factory workers and those in related occupations, to mitigate occupational health risks. Additionally, raising public awareness through regular information campaigns can help prevent health hazards. Finally, adopting modern machines and technologies in cement factories, particularly in developing countries can reduce environmental cement dust emissions, further minimizing risks.

5. Conclusions

This study demonstrates that fly ash dust exposure causes significant harm to respiratory and overall health in Wistar rats, leading to histomorphological changes, hematological and biochemical derangements, and weight loss. These findings underscore the need for protective measures and regular health monitoring for individuals occupationally exposed to fly ash dust, particularly in industrial settings.

6. The Novel Aspects of This Study

This study reveals pivotal findings on the toxic effects of fly ash dust, specifically:

The adverse impact of fly ash dust on body weight and lung histoarchitecture, demonstrating

the potential for severe health consequences; Comprehensive haematological analysis uncovering alterations in lymphocytes, basophils, reticulocytes, haemoglobin, haematocrit, red blood cells, and other red cell indices, indicating disrupted blood cell production and function; Biochemical disruption in manganese, bicarbonate, urea, and creatinine levels, highlighting the potential for fly ash dust-induced metabolic and renal dysfunction

7. Recommendations

To mitigate the risks associated with fly ash, we suggest the following measures: Implementing robust safety protocols, including the provision of personal protective equipment, should be prioritized. Regular medical check-ups for factory workers will also help identify potential health issues early. Additionally, public awareness campaigns can educate the community about the risks and prevention strategies. Lastly, investing in modern machinery and technologies can significantly reduce environmental fly ash dust emissions.

8. References

Adeleke, D.E., Mojhammed, V. and Mohammad, H.S. (2019): 'Evaluation of particulate matter'. *Journal of Air Pollution and Health*; 4(4): 221-226.

Ahmed, W., Nisa, S., Mohammed, N. and Hussain, R. (2016): 'Assessment of particulate matter (PM_{10} and $PM_{2.5}$) and associated health problems in different areas of fly ash industries' warehouse, Hattar, Haripur'. *Journal of Science and Technology*; 37(2): 7-15.

Bankole, C.O.J., Ukwok, C.E., Anakor, A.C.F., Ademisi, C.C. and Nwobodo, E. (2021): 'Coal combustion-induced pulmonary pneumonia in Wistar rats'. *Journal of Environmental Science, Toxicology and Food Technology;* 9(1): 25-38.

Bello, N.M., Pinter, E., Elsaid, F.G., Elsawy, H.A. (2019). 'Effects of fly ash dust on some biochemical and molecular parameters in male rats. *Toxicology of Reports.6*:210-219. doi:10.1016/j.toxrep.2019.02.006

Bergmeyer, H.J., Bernt, Z.H. and Lyoussi, B (2017): 'Acute and chronic toxicological studies of fly ash dust in experimental Wistar rats'. *Journal of Ethnopharmacology*; 9(3): 43-50.

Betkovai, A.A., Susor, Y., and Zhang, X. (2019): 'Air pollution exposure and lung function in highly exposed subjects in Beijing, China'. *Journal of Particle and Fibre Toxicology*; 11(1): 51-60.

Buzek, J. and Chastel, O. (2010): 'Directive 2010/63/EU of the European parliament and of the Council. Protection of animals used for scientific purposes (text with EEA relevance)'. *Official Journal of the European Union;* 34(1): 276.

Cheesbrough, M.J. (2015): *District laboratory practice in tropical countries*. Second edition. Cambridge University press publishers, United Kingdom, London.

Cheong, K.U., Ukpanukpona, R.U., Otu, D.O. and Ajanaku, V.A. (2018): 'Effects of cement dust on Haematological parameters in Mice'. *World Journal of Pharmaceutical Research*; 7(9): 180-187.

Chooli, K.P., Ibrahim, D., Yeng, C., Subramaniam, S. and Sreenivasan, S. (2015): 'Subchronic toxicity study of cement dust in Rats'. *International Journal of Biomedical Research*; 18(3): 50-64.

Drake, R.L., Vogl, W., Mitchell, A.W. and Gray, H. (2015): *Gray's anatomy for students.* Second edition.Churchill living press publishers, Philadelphia, United States of America.

Drury, R.A. and Wallington, E.A. (1980) '*Carleton's Histological technique*. Fifth edition, Oxford University Press, New York.

Ehiabhi, O.O., Desalu, E.O., Okoegwule, K.O., Adewole, T.O. and Erhabor, G.E. (2018): 'Respiratory symptoms and lung function patterns in workers exposed to fly ash dust and coal dust in Mining industry'. *Journal of Occupational Health*; 27(3): 145-160.

Elgailani, I.E.H. and Alsakka, M.M. (2016): 'Determination of some chemical elements in different hemoglobin

samples from different patients by UV-Visible Spectrophotometer'. *Journal of Advances in Analytical Chemistry*; 6(2): 48-56.

Eriakhaba, D., Elgorashi, E. E., Ntloedibe, D.T., Van Staden, J., and Ethnomedicine, A. (2020). 'Fly ash occupational and environmental health issues among Anegbettar fly ash industry workers'. *South African Journal of Biochemistry;* 73(3): 378–383.

Fernandesi, A., Krusinski, A. and Milanowski, J. (2020): 'Effects of air pollution on human respiratory system'. *Journalof Agricultural and Environmental Medicine*; 15(1): 278-293.

Finkelman, A., Gupta, H.O, Urban, T., Ahrens, W. and Becher, H. (2022): 'Exposure to cement dust-related occupational groups and laryngeal cancer risk: Result of a population based case-control study'. *International Journal of Cancer*; 108(6): 907-914.

Fletcher, C. and Peto, R. (2017). 'The natural history of chronic airflow obstruction'. *The British Medical Journal*; 1(6): 1645-1648.

Ganapathy, A., Tandon, R., Baxia, M. and Kaler, S. (2020): 'Cadaveric study of lung anatomy: A surgical overview'. *Journal of Medical Respiratory Innovation*; 3(1): 149-153.

George, B.M., Nayak, S.B. and Marpalli, S. (2018): 'Morphological variations of the lungs: A study conducted on Indian cadavers'. *Journal of Anatomy and Cell Biology*; 47(4): 253-260.

Gheethi, A., Pillai, S., More, U. and Sontakke, A. (2022): 'Measurement of some biochemical and haematological parameters in clinical samples'. *Journal of Cement and concrete research and Clinical Biochemistry*; 30(2): 162-178.

Gungor, U.I. and Usunobun, M.I. (2017): 'Current developments in the physiology and management of bronchitis'. *Nigerian Journal of Physiological Science*; 6(20): 19-29.

Harrison, R.J.(2015): *Textbook of Medicine with relevant physiology andanatomy*. Second edition. Cambridge University press publishers, Hodder and Stoughton, London.

Heyder, J., Gebhart, J., Rudolf, G., Schiller, C.F. and Stahlhofen, W. (2022): 'Deposition of particles in the human respiratory tract in the size range 0.005–15 μm'. *Journal of Aerosol Science*; (17)5: 811-825.

Ibe, C.S., Onyeanusi, B.I., Salami, S.O. and Nzalak, J.O. (2021): 'Microscopic anatomy of the lower respiratory system of the giant pouched rat (*Cricetomysgambianus*'. *International Journal of Morphology*; 29(1): 27-33.

Iden, E. (2016): 'Occupational Health and Safety Measures (Nigeria): A Copy of My Appeal Letter to ILO. Industry'.*Journal of Atmospheric Environment*; 47(5): 384–388.

Igunka-Nnoka, D.O.A., Hong, Y.C., and Farnsworth, N.S. (2021): 'Fly Ash exposure and lung cancer risk'. *Journal of Environmental Scienec and Health;* 25(2): 1033–1050.

Islam, M.S., Paul, G., Ong, H.X., Young, P.M., Gue, Y.T. and Saha, S.C. (2020): 'A review of respiratory anatomical development, air flow characterization and particle deposition'. *International Journal of Environmental Research and Public Health*; 17(3): 380-387.

Jarikre, H.M. and Rahman, R.O.A. (2019): 'The origin, composition, toxicological profile and early age hydration mechanisms of fly ash'. *International Journal of Environmental Science and Technology;* 11(5): 129-133.

Johnson, B.I. Knudson F.I. and Ikhifa, K.O. (2018): 'Mortality and morbidity among fly ash exposed workers'. *International Journal of Occupatioal and Environmental Medicine*; 19(2): 77-86.

Mark, H.B., Robert, S.P., Thomas, V.T., Justin, K.M. and Michael, B.O. (2016): *The Merck manual of diagnosis and therapy.* Eighteenth edition.Merck Research laboratory publishers, USA.

Mohamed, R.M.S.R., Nizam, N.M.S., Al-Gheethi, A.A., Lajis, A. and Kassim A.H.M. (2016):'Particulate matter levels in ambient air adjacent to industrial area'.

American Journal of Industrial Medicine; 12(1): 42-56.

Moore, K.L. and Dalley A.E. (2015): *Textbook of Clinically Oriented Anatomy*. Sixth edition. Wolters Kluwer Press Publishers, Philadelphia, London.

Oriabure, R.K., Patel, R., Patel, M., Gupta, S.R., &Laloo, D. (2023). 'Fly ash dust exposure and respiratory health in adult Wistar rats. *Journal of Ethnopharmacology;* 112(1): 163-176.

Oseahumen, D., Lal, S., and Rizvi, S. I. (2017). 'Health effects of coal dust in experimental adult Wistar rats'. *Indian Journal of Occupational Environmental Medicine;* 58(6): 2010-2023.

Otong, D.A and Ikelegbe, O.O. (2023): 'Ambient air quality and human health in fly ash producing industries in rural communities of Edo State in Nigerian Meteorological Society (NMetS)'.*Journal of Cementand Concrete Research*; 3(4): 157–169.

Patwardhan, M.N., Gohil, J.S. and Rao, V.I. (2018): 'Impact of fly ash dust on respiratory system of the adult Wistar rat. *Journal of Environmental Science and Technology;* 20(8): 21-35.

Poinen-Rughooputh, S., Rughooputh, M.S., Guo, Y., Rong, Y. and Chen, W. (2016): 'Occupational exposure to silica dust and risk of lung cancer: An updated meta-analysis of epidemiological studies'. *British Medical Council Journal of Public Health*; 16(5): 11-37.

Robbani, M., Karalliedde, L., Buckley, N.E., Dohila, J.S. and Mitra, V.I. (2016). 'Fly ash exposure in the workplace'. *The Lancet Global HealtH;* 4(11): 746-747.

Saidi, Z., Yifred, B. and Tadesse, T. (2021): 'Chronic respiratory symptoms associated with fly ash workers in Dejen community, Amhara Regional State. '*Journal of Ethiopia Multidisciplinary Respiratory Medicine*; 11(1): 43-46.

Sikira, O.B.K., El-Banna, A.K. and Ebaid, A.E. (2021): 'Morphological study of rabbit lung, bronchial tree and pulmonary vessels using corrosion cast technique'. *Al-azharAssuit Medical Journal*; 13(3): 41-46.

Tiwari, J.G. (2016): 'The effects of inhaled fly ash dust on the lung and other target organs'. *Journal of Occupational Hygiene*; 2(3): 10-22.

Ullah, A.K., Singh, E., Sudha, S. and Abubakah, J.O (2022): 'Haematological and cytogenetic studies in workers occupationally exposed to fly ash dust'. *International Journal of Human Genetics*; 2(3): 59-68.

Vandel, H.O. Alumiasunya, S.O. and Obalola, D.A. (2016): 'Occupational exposure to fly ash dust. *Journal of Occupational and Environmental Medicine*; 2(3): 57-69.

Veeresham, D.O., Ejindu, C.K., Oaikhena, H.A. and Okoli, H.A. (2019): 'Health effects of fly ash exposure in fly ash related occupations'. *Journal of Medicine and Biomedical Research*; 6(2): 4-12.

Williams, O.A., Dalandi, F.E.S., Elkamel, A.O., Ahmadi, L.E. and Iyoha, M. (2017): 'Fly ash, coal combustion and environmental pollution'. *Journal of Atmospheric Pollution Research*; 5(3): 769-778.