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Assessment of respiratory and reproductive impacts of artisanal refinery activities on male Albino Wistar rats: implications for environmental health

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Abstract

Background Artisanal petroleum refining operations have been known to produce a significant volume of air pollutants. The highest concentration of pollutants is generated during the oven heating or crude boiling phase of the operation. The major pollutant is black carbon or soot. Although these operations are widespread, especially in developing countries, the impact of exposure to emissions from artisanal refinery on both respiratory and reproductive health remains poorly understood.

Objective This study is aimed to examine the effects of controlled subacute exposure to carbon soot emissions generated during the oven heating phase of the refining process, on the respiratory and reproductive systems of male albino Wistar rats.

Methods To simulate the exposure conditions found in artisanal refineries, we developed a replicable fabrication of an artisanal refinery combustion system fitted with an exposure chamber for in vivo studies. 6–8 weeks old adolescent albino Wistar rats were divided into four groups (A, B, C, & D), with group A acting as the general control group and was not exposed to any carbon soot particulate matter. Group B, C, and D were exposed subacutely for four hours each day for 3, 7, 14, 21, and 28 days to varying emission concentrations. Daily exposure measurements were determined using Aeroqual Series 300 Gas Monitor, and average exposure concentration of carbon soot particulate matter ($PM_{2.5}$), for each exposed group were given as: (1.221 ± 0.169 mg/m³, 1.290 ± 0.214 mg/m³ 1.282 ± 0.235 mg/m³). Animals from each group were euthanised on Day 3, 7, 14, 21, and Day 28, respectively. Tissue samples of the lungs and testis were collected for immunohistochemistry and oxidative stress analysis.

Discussion */Conclusion*: Cytoarchitecture of the lungs and testis via histology and immunohistochemistry, showed inflammatory cell infiltration, thickened alveolar walls, diminished alveolar spaces, hyperaemia, and bronchial epithelial hyperplasia in the lungs of Group B, C, and D animals that were exposed to soot. While cytoarchitecture of the testis revealed a distortion of the Leydig cells, vacuolations and mild vacuolations within the spermatid layer, loss of flagella, and some distortion of seminiferous tubule in the lumen.

Notable increase in the mean expression and significant *P*-values determined by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, were observed on Day 14–28 ($P < 0.05$) for tumour-necrosis-factor alpha

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(TNF-α), Day 21–28 ($P < 0.001$) for malondialdehyde (MDA), and Day 21 ($P < 0.001$) for superoxide dismutase (SOD) expression in the lungs of each of the experimental Group (B, C, D) when compared to the control Group A.

Our study provides valuable insights into the health risks associated with exposure to carbon soot particulate matter, thus underscoring the urgent need for necessary control measures to curb air pollution as a result of artisanal refinery activities.

Keywords Artisanal refinery, Air pollution, Carbon soot particulate matter, Respiratory health, Reproductive health, Albino Wistar rats, TNF-α, MDA, SOD, Cytoarchitecture, Inflammatory markers, Oxidative stress

Background

Artisanal petroleum refining activity, characterised by the crude extraction and refining of Petroleum using rudimentary techniques, is a prevalent practice in the Niger Delta region of Nigeria (Bebeteidoh et al. 2020; Elisha and Golden 2022; Nwozor et al. 2020). This is due to the lack of domestic refining capacity to meet the local needs resulting in shortages of the refined products (Umukoro 2018). This unregulated form of petroleum processing poses significant environmental and health concerns for both the artisanal workers and surrounding communities (Obenade and Amangabara 2014; Ojirika et al. 2019). The emissions from artisanal refineries contain a wide range of hazardous substances, including volatile organic compounds (such as benzene, toluene, ethylbenzene, and xylene), particulate matter ($PM_{2.5}$), carbon monoxide (CO), carbon dioxide (CO_2), hydrogen sulphide (H_2S), sulphur dioxide (SO_2), and heavy metals, which can have detrimental effects on human health (Kalagbor et al. 2019; Prioleau 2003; Ragothaman and Anderson 2017; World Bank Group 2016; Yakubu 2017a).

Studies conducted by Akeredolu and Sonibare (2015) and Onakpohor et al. (2020), showed that over 20,000 artisanal refineries have been setup by private investors in the Niger Delta, who take advantage of the cheap labour and availability of raw materials in the area. Most of the artisanal refiners are young men (ages 16–30), seeking to make a living as their former occupations (mostly fishing, farming, and hunting), have been destroyed due to environmental pollution from oil exploration activities by International Oil Companies (IOCs) (Anejionu et al. 2015; Umar et al. 2021). Whereas females also play a role in the artisanal refining activities, their roles are more pronounced in the downstream sector, which includes sales of the finished products (Igben 2021; Goodnews and Wordu 2019). Although, some females have been known to own small scale artisanal refineries (Obenade and Amangabara 2014; Stakeholder Democracy Network 2020).

Operational characteristics of artisanal refinery

The origins of petroleum artisanal refinery can be traced back to the Nigerian Civil war 1967–1970, revived in the early 2000s by the Niger Delta militant's advocacy, a movement largely made up of males (Katsouris and Sayne 2013; Mezie-Okoye 2022).

The process of petroleum artisanal refining is quite simplistic but labour intensive. It requires the use of locally acquired materials comprising of an area of land or camp with access to the river, crude oil obtained illegally by a hole created on a crude oil flow line and transported by a fishing boat popularly called "Cotonou boat" or, hose lines connected directly from the flow lines to the camp. From here, it is emptied and stored in plastic drums where it is aerated for few hours to allow for gas content reduction via evaporation (Akeredolu and Sonibare 2015; Onokpohor et al. 2020). The aerated crude is then emptied into a metal drum set atop an oven, typically a hole in the earth filled with dry wood and kept lit continually by adding raw crude to the burning wood. By varying the oven temperature, the boiling crude then undergoes fractional distillation. The vapours realized from the boiling crude are conveyed from the oven to the collector end by several galvanized pipes connected from the boiling end, cooled through a condenser (A half cut metal drum), intermittently filled with water obtained from nearby water body. The various products which include (kerosene, gasoline, automated gas oil) are then collected at the receiving end for sales/distribution (Glory et al. 2023).

Environmental hazards associated with artisanal refinery activity

The demerits of the process described above far outweigh its benefits. Environmental hazards associated with artisanal refinery activity have been the subject of numerous investigations (Chris and Oghenetekevwe 2023; Chukwunweike et al. 2022; Glory et al. 2023; Sam et al. 2023; Owhor et al. 2023; Kasamatsu et al. 2023). The refining processes employed in these operations often result in the release of toxic emissions and the generation of hazardous waste (Bebeteidoh et al. 2020).

Various studies have highlighted the inefficiency of the artisanal refining process, citing that nearly 80% of the heavy end of the crude cannot be refined and are just discharged into the environment polluting it (Adeloye and Ekade 2021; Ephraim-Emmanuel et al. 2022; Tanee and Yabrade 2016). Contaminants, such as volatile organic compounds, particulate matter, heavy metals, and sulphur compounds, are frequently emitted into the air, soil, and water bodies, leading to ecological damage (Omisakin 2022). Carbon soot comprises the bulk of the emissions, which has been identified as a major contributor to environmental air pollution in the Niger Delta. The carbon soot emitted, is generated as a result of the incomplete combustion of fossil fuel (dry wood and raw crude) from the oven or open-air furnace at the crude boiling unit of the artisanal refinery (Taylor and Francis 2012; Tissari et al. 2009). Due to the lack of regulatory control and adherence to environmental and safety standards, artisanal refinery activity poses significant risks to both the environment and human health including that of the artisanal workers who are constantly exposed to these emissions without any effective control measures to protect them from its adverse effects (Ikezam et al. 2021).

Water pollution is another significant environmental concern associated with artisanal refinery activity. The wastewater generated during the refining process, known as "refinery effluents", contains elevated levels of contaminants, including heavy metals, hydrocarbons, and other toxic substances. Improper disposal or inadequate treatment of these effluents can lead to the contamination of nearby water bodies, such as rivers, streams, and groundwater sources. This contamination not only poses risks to aquatic ecosystems but also jeopardises the availability of clean drinking water for local communities (Nwankwoala et al. 2017). The environmental hazards associated with artisanal refinery activity highlight the urgent need for effective mitigation strategies and regulatory measures (Nemenushcha et al. 2020). Adequate management of air emissions, proper handling and treatment of wastewater, and responsible waste disposal practices are essential to minimise the negative impacts on the environment (Canesto and Téllez 2020). Implementing sustainable practices, promoting cleaner technologies, and raising awareness among operators and communities can help mitigate the environmental hazards and foster a more environmentally conscious approach to oil extraction and refining (Sanchez and Egea 2018). Understanding and addressing these environmental hazards are crucial steps towards achieving sustainable development and protecting both the ecosystems and the health of communities affected by artisanal refinery activity (Sojinu and Ejeromedoghene 2019).

Health risks posed by exposure to artisanal refinery emissions

The vulnerability of the respiratory system to the detrimental effects of air pollutants is well documented in the literature (Kurt et al. 2016). For instance, exposure to air pollution has been shown to increase the risk of hospital admission for asthma in children (Zhao et al. 2021). Short-term exposure to ambient fine particulate pollution has also been found to exacerbate ventilator-associated pneumonia in paediatric intensive care patients undergoing cardiovascular surgeries (Cui et al. 2023). Similarly, air pollution has been associated with an increase in outpatient visits for children with asthma (Zhang et al. 2021). Moreover, fine particulate matters ($PM_{2.5}$) have been implicated in cardiovascular and respiratory health effects (Wan Mahiyuddin et al. 2023).

One of the primary health risks associated with artisanal refinery emissions is respiratory-related illnesses (Okafor 2022). Inhalation of pollutants such as particulate matter (PM), volatile organic compounds (VOCs), sulphur dioxide (SO_2), nitrogen oxides (NO_x), and other combustion by-products can lead to respiratory symptoms, including coughing, wheezing, shortness of breath, and exacerbation of pre-existing respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD). Prolonged exposure to these pollutants may result in chronic respiratory diseases, reduced lung function, oxidative stress, impaired lung function, and increased susceptibility to respiratory infections (Gomes and Florida-James 2014; National Institute of Environmental Health Sciences 2023; Onakpohor et al. 2020).

A recent study conducted by Kanee et al. (2021) in Port Harcourt, a famous oil rich in the Niger Delta city known for its artisanal refinery activities, investigated the PAH (polycyclic aromatic hydrocarbon) levels in Wistar rats exposed to ambient air of the Port Harcourt metropolis. Twenty Wistar rats were imported from a nonpolluted city and exposed to both indoor and outdoor air. The mean concentrations of PAH in indoor and outdoor animals were higher than those of baseline animals, except for Benzo(a)pyrene, which was found in baseline animals but absent in other animal groups. Additionally, dibenz(a,h)anthracene, indeno(1,2,3-c,d)pyrene, pyrene, 2-methyl, and other carcinogenic PAHs were all significantly higher ($P < 0.05$) in outdoor groups. The study concluded that vulnerable groups in Port Harcourt are at the greatest risk of air pollution.

Additionally, artisanal refinery emissions can have systemic health effects beyond the respiratory system. Exposure to toxic substances present in the emissions, such as heavy metals (e.g. lead, mercury, cadmium), polycyclic aromatic hydrocarbons (PAHs), and benzene, can lead to adverse health outcomes. These substances are known

carcinogens, neurotoxicants, and endocrine disruptors, potentially increasing the risk of cancer, neurological disorders, and hormonal imbalances (Abdel-Shafy and Mansour 2016; Briffa et al. 2020; Ephraim-Emmanuel and Ordinioha 2021; Howard et al. 2021; Tchounwou et al. 2012).

The reproductive system is also vulnerable to the health risks posed by artisanal refinery emissions. Studies have shown that exposure to certain pollutants, such as PAHs and heavy metals, can lead to reproductive disorders, including reduced fertility, impaired sperm quality, and hormonal disruptions. These effects may have long-lasting implications on both male and female reproductive health, impacting fertility rates, and reproductive success (Carré et al. 2017; Ramirez et al. 2017).

In a study seeking to establish a link between environmental black soot (one of the main constituents of artisanal refinery emissions), and derangement in the hypothalamus and testis of rats, exposed male rats to black soot for 4, 8, and 12 weeks, respectively. The hypothalamus and testis of the rats were processed for biochemical analysis. The results showed that black soot exposure for 4, 8, and 12 weeks significantly increased oxidative stress markers both in the testis and in the hypothalamus of rats. In addition, a decrease in the alkaline phosphatase, acid phosphatase as well as lactate dehydrogenase activities in the testis were also recorded. Furthermore, the result demonstrated an upregulation of the protein expression of caspase 3, an indication of increased apoptosis, which led to the disruption of the histological architecture of the hypothalamus and testis (Onyeso et al. 2020).

Another study conducted by Odinga et al. (2016) on the effect of effluent from the Port Harcourt Refining Company on the hepatic and reproductive function of Wistar albino rats. The effluent was administered to the rats for a period of 14 days. The rat's hormonal and hepatic function test showed significant increase in LP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), and testosterone levels, indicating impaired reproductive hormonal function and hepatotoxicity. The histological examination of the hepatic cells indicated that the effluent induced proximal degeneration of the integrity of the hepatic cells, inflammation, hepatocyte degeneration, partial architectural distortion, and haemorrhage.

The above studies show the health risks associated with ambient air and water pollution from various sources. However, studies establishing a direct link between exposure to artisanal refinery activities and its respiratory and reproductive effects are limited. This study seeks to investigate the potential respiratory and reproductive health impacts of artisanal refinery activities, with specific focus on the effects of exposure to carbon soot particulate matter.

Lastly, this study aims to aid in the understanding the risks associated with artisanal refinery activities in order to assist in the developing of a multi-faceted approach, which includes regulatory measures, improved occupational health and safety standards, implementing emission control technologies, providing personal protective equipment for workers, promoting proper waste management practices, raising community awareness and alternative economic empowerment, in order to protect the health and well-being of both workers and communities residing near artisanal refineries (Yakubu 2017b).

Methods

Study design

The animal experimental model study design was used for the study, (Johnson and Besselsen 2002). Only male rats were utilised for this study because an overwhelming percentage of the artisanal refinery workers are young men (ages 16–30) (Anejionu et al. 2015; Umar et al. 2021).

Soot particle generation

Carbon soot was generated by the design and fabrication of a combustion system, identical to those used in the artisanal refinery, based on similar studies conducted by (Chan et al. 2013; Lee et al. 2010; Onakpohor et al. 2020). The combustion system comprised of a combustor chamber consisting of a drum measuring 15×15cm in diameter, fabricated entirely of metal sheets. The drum was then fitted with an opening to allow for air supply and placed at the bottom of a metal stand measuring approximately 1.5m in height. Dry wood (charcoal), obtained from the Choba market (VWQ2 + F74, 500,102, Oduoha-Emuoha, Rivers), was then placed inside the combustor chamber. Raw crude identified as light crude, by determination of its density using a pycnometer, was procured from Shell BP. The combustion rate of the crude was ascertained using the material balance principle. We weighed 50 g of the crude and placed it in a tin can, allowing it to burn for intervals of 5, 10, and 15 min. We then measured the remaining crude post-combustion.

Table 1 Showing the combustion rate of the crude at various time intervals

Weight	5 min	10 min	15 min
Mass of initial crude (g)	50.07	51	50.18
Mass of left-over crude (g)	36.85	30.01	21.61
Combusted crude (g)	13.22	20.99	28.57
Combustion rate (g/min)	2.64	2.099	1.904

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A graph of combusted crude against time

$y = 1.535x + 5.5767$
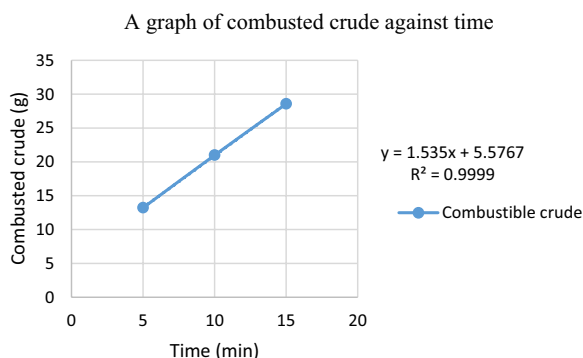$R^2 = 0.9999$

— Combustible crude

Fig. 1 A graph of combusted crude against time

Table 1 presents the combustion rates at these specific time intervals, while Fig. 1 illustrates the relationship between the amount of crude combusted and time. From the figure, it can be deduced that the average crude combustion rate is 2.2143 g/min.

Figure 2 depicts an isometric model of an artisanal refinery combustion chamber, locally fabricated. The central element, a cylindrical tank constructed from metal sheets, measured 20 cm in height and 15 cm in diameter. It was specifically designed to hold the raw crude and was strategically placed on a metal stand, positioned above the combustion chamber. The flow of crude was meticulously regulated by a valve, which was connected to a 2.5 cm diameter metal pipe. This pipe linked the tank to the combustion chamber. To counteract and minimize the high temperatures associated with emissions, an exhaust pipe of 5 cm diameter was enveloped within a recycled water-cooling system. This exhaust system further branched out into three distinct metal pipes, with diameters of 1.27 cm, 1.91 cm, and 2.54 cm, respectively. Each

of these pipes was equipped with control valves, ensuring the delivery of varying emission concentrations to three separate exposure cages. The design was adept at facilitating a study of diverse emission impacts under varying conditions.

Animal housing and care

Forty male Wistar rats (all 6–8 weeks old, weighing between 170 and 210 g) were imported from a non-air-polluted (free of petroleum-based activity) location, the University of Nigeria, Nsukka, to Anatomy animal house, College of Medicine, Rivers State University, Port Harcourt. The rats were allowed to acclimatize for two weeks, under maintained laboratory conditions, including a controlled temperature (25 ± 2.0 °C), a 12-h light/12-h dark cycle, and ad libitum access to standard rat feed (Eastern Premier Mills Limited, Calabar) and water. The animals were identified by special markings on the ears, and wire meshed cages with sawdust bedding were used for housing. Cross-ventilation in the animal house ensured a clean environment.

Simulation activity of artisanal refinery crude boiling unit for controlled exposure

In this study, animals were divided into four groups (A, B, C, and D), with ten animals contained in each group. Group A acted as the general control group and was not exposed to any carbon soot particulate matter. Group B, C, and D were placed in three metal cages with wire mesh openings to allow for ventilation. The exposure cages, measuring 30×27 cm each, were placed on top of the combustion equipment stand, above the crude oil tank. The temperature of each cage was continuously monitored by a Thomas Enviro-Safe® Liquid-In-Glass
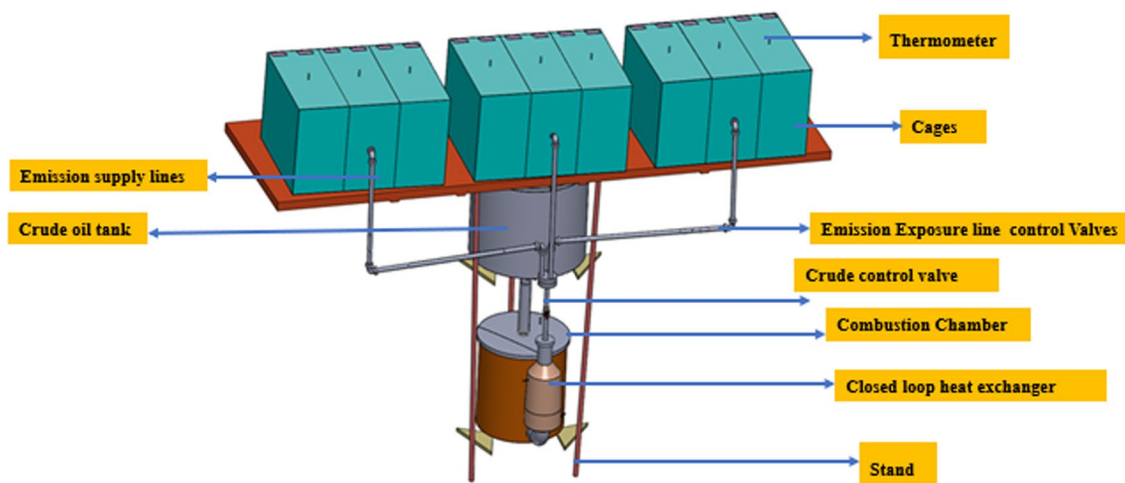

Fig. 2 Isometric model of locally fabricated artisanal refinery combustion chamber

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thermometer with a range of (0–100 °C), suspended from the roof of each cage by a drilled hole. Rats were monitored for signs of distress, and appropriate measures were taken to ensure their well-being. Veterinary support was provided, and strict animal care protocols were implemented to minimise harm and guarantee ethical treatment.

The rats were exposed for four hours each day, exposure was controlled by the varying of the pipe diameter attached to each cage, and manually controlling the flow by opening and closing of the valves attached to each the afore mentioned pipes. During this time, measurement on the quantity of the various emissions (O_3, H_2S, CO_2, SO_2, CH_4, CO, VOC, and $PM_{2.5}$) in mg/m^3, were taken using Aeroqual Series 300 Gas Monitor. Based on the daily exposure gas monitor readings, Group B was exposed to an average concentration of 1.221 ± 0.169 mg/m^3 of carbon soot particulate matter ($PM_{2.5}$). Group C, the third group, encountered an average concentration of 1.290 ± 0.214 mg/m^3 of the same particulate matter. The last group, Group D, was exposed to an average concentration of 1.282 ± 0.235 mg/m^3 of the carbon soot particulate matter ($PM_{2.5}$). The exposure of these groups to the carbon soot was methodically carried out over a span of 28 days. To assess the impact of sub-acute carbon soot exposure, two animals from each group were euthanised on Day 3, Day 7, Day 14, Day 21, and Day 28, for subsequent analysis.

Immunohistochemistry of lung and testis tissues

Tissues of the lungs and testis were prepared for sectioning by fixing them for two weeks in formal saline to prevent autolysis and bacterial decomposition. The tissues were then dehydrated in alcohol and cleared for two hours in pure xylene before being embedded in molten wax. The tissues were sectioned at 5µm, arranged on various slides, and placed in a hot oven prior to H and E staining. Slides were cover slipped and mounted using DPX mountant and allowed to dry. Photomicrographs was taken using Zeiss Axioscope microscope.

Determination of the cytokine activity tumour-necrosis-factor alpha (TNF–α) of the lungs

The TNF-α assay Kit was used to determine the cytokine activity. The right lungs tissue was used for this purpose. Plasma cytokines were measured using rat Multi-Analyte ELIS-Array kit (Qiagen), which contains tumour-necrosis-factor alpha (TNF-α) and was regulated on activation. The normal T cell expressed and secreted (RANTES Qiagen).

Photomicrography

The lung tissue sections were observed under a digital brightfield microscope (OMAX 40-2000X 3MP Digital Compound Microscope, USA) and photomicrographs were taken with 400 × magnification.

Experimental protocols for biochemical parameters (estimation of oxidative markers) in the lung tissue

Fresh lung specimens were homogenized in Tris–HCl buffer (5 mmol/L containing 2 mmol/L Eth-ylene-Diamine-Tetra-Acetic acid (EDTA), pH 7.4) to give a 10% (w/v) lung homogenate. The homogenates were then centrifuged at 1000 rpm for 10 min at 4 °C, and the supernatants were immediately used for the determination of oxidant-antioxidant status. Malondialdehyde (MDA) and superoxide dismutase (SOD) as an index of the extent of lipid peroxidation were determined following the manufacturer's instruction provided by Bio diagnostic Company (biodiagnostic_eka@lycos.com and info@bio-diagnostic.com). The parameters that were used to determine oxidative stress in this study are malondialdihyde (MDA) and superoxide dismutase (SOD) activity assay.

Analysis of haematological parameters

Blood was collected from rats on the day of sacrifice in sterilized vials containing 4.0% potassium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for estimation of haematological parameters and a smear was prepared from freshly collected blood. The mean packed cell volume (PCV), haemoglobin (Hb) estimation, mean red blood cell count (RBC), white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), neutrophil, lymphocyte, eosinophil, and mean monocyte percentage were estimated as per method describe by (Schalm et al. 1975).

Ethical considerations

Approval for this research was obtained from the Research Ethics Committee of the University of Port Harcourt. The study adhered to ethical guidelines and animal welfare regulations throughout the experimental procedures. Measures were taken to minimise pain, distress, and discomfort to the animals.

Statistical analysis

The data were analysed using IBM SPSS Statistics (Version 25). Data obtained from the various assessments were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using two-way analysis of variance (ANOVA) for weight, exposure

concentration TNF, SOD AND MOD. A one-way analysis of variance (ANOVA) was conducted for haematological analysis, followed by post hoc Tukey tests for multiple comparisons. A *P*-value less than 0.05 was considered statistically significant.

Results

Result of the initial and final mean body weight of rats

The comprehensive analysis of the initial and final body weights of the rat subjects from the General Control group and Groups B, C, and D is in Table 2. The initial mean body weights with their corresponding standard error means (SEM) for the general control group were documented as follows: 124.67g ± 3.11g, 146.87g ± 1.75g, and 172.05g ± 2.81g, while those for the exposed Group (A, B, C and D): 180.60g ± 7.51g, 205.4g ± 12.4g, 219.8g ± 13.3g, and 251.4g ± 12.3g.

Table 2 shows mean body weights and their standard errors (SE) of rats between the groups A, B, C, and D, and within each of the group A, B, C, and D, both before and after exposure to soot. The weights are recorded from the initial body weight, followed by Day 1 exposure, then at weekly intervals up to Week 4.

Means with a = significant increase across the row ($P < 0.5$), means with b = significant decrease across the row ($P < 0.5$), means with c = significant increase along the column ($P < 0.5$), and means with d = significant decrease along the column ($P < 0.5$).

The data suggest significant weight changes across all groups with a significance level ($P < 0.05$.). The weights are presented in grams (g), and all values are expressed as Mean ± SEM.

([b] $P < 0.05$.) in Fig. 3, indicating significant difference in the results obtained at various times of exposure and across the different groups.

Table 2 Comparative analysis of mean body weights of rats prior and post soot exposure

Weight (g)	Group A	Group B	Group C	Group D
Initial body weight before exposure (g) (Mean ± SE)	124.6 ± 3.11[bd]	146.87 ± 1.75[a]	172.05 ± 2.81[ac]	213.89 ± 6.64[a]
Final body weight after Day 1 Exposure (g) (Mean ± SE)	133.06 ± 3.66[bc]	147.09 ± 1.36[a]	173.16 ± 3.15[ac]	213.78 ± 6.54[a]
Final body weight after Week 1 exposure (g) (Mean ± SE)	156.14 ± 3.00[ac]	163.47 ± 2.30[ac]	184.53 ± 3.76[ac]	16.07 ± 8.08[bd]
Final body weight after Week 2 exposure (g) (Mean ± SE)	175.45 ± 3.43[bc]	186.00 ± 3.22[ac]	209.17 ± 4.42[ac]	242.75 ± 9.75[ac]
Final body weight after Week 3 exposure (g) (Mean ± SE)	178.60 ± 8.51[bc]	205.2 ± 10.5[ac]	214.0 ± 10.6[ac]	246.4 ± 14.6[ac]
Final body weight after Week 4 exposure (g) (Mean ± SE)	180.60 ± 7.51[bc]	205.4 ± 12.4[a]	219.8 ± 13.3[ac]	251.4 ± 12.3[ac]
Weight change (g) (Mean ± SE)	55.93[a]	58.53[a]	47.75[b]	37.51[b]
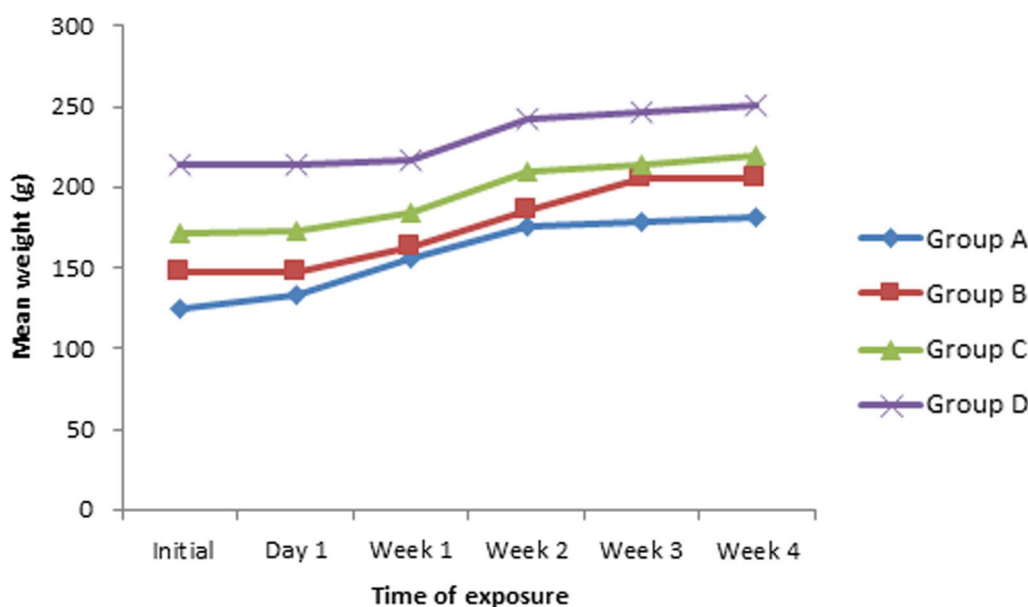
Fig. 3 Graph of mean body weight of each group (**A**, **B**, **C**, **D**) against exposure time

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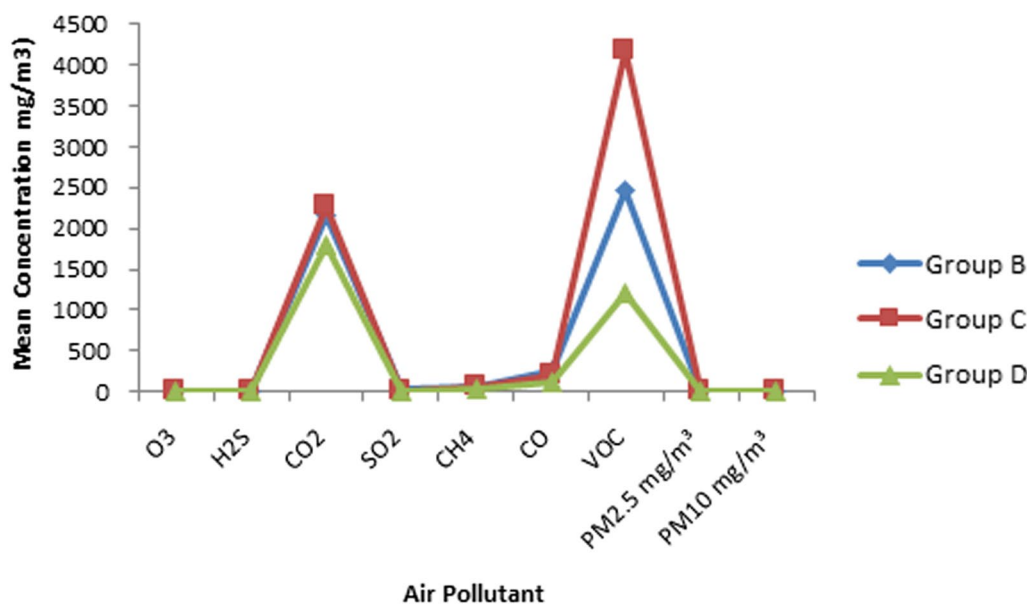
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Fig. 4 Comparative Analysis of Mean Concentrations of Air Pollutants Exposed to Rats

Mean concentration of air pollutant that rats were exposed to

Figure 4 represents the mean concentrations of various air pollutants—O_3, H_2S, CO_2, SO_2, CH_4, CO, VOC, $PM_{2.5}$, and PM_{10}—that were exposed to the rats in Groups B, C, and D. Each data point is accompanied by its respective standard error mean (SE), highlighting the precision of the measurements. The concentrations of CO_2, VOC O_3, H_2S, SO_2, CH_4, CO, $PM_{2.5}$, and PM_{10} are expressed in milligrams per cubic metre (mg/m^3). [a] $P > 0.05$ across the different groups indicating no significant difference in the results. [b]$P < 0.05$ across the various pollutants, indicating significant difference in the effect of the different air pollutants.

Histology

Cytoarchitecture of the lungs

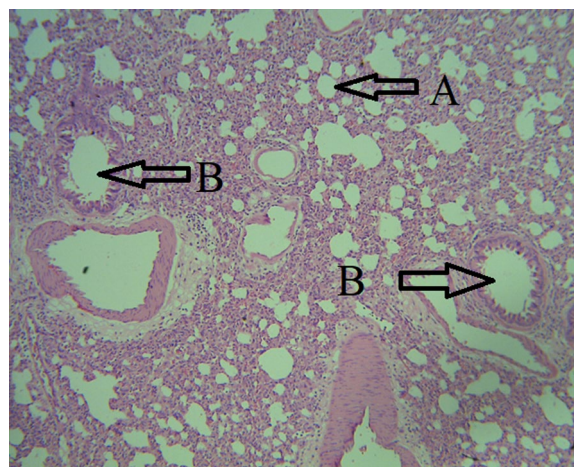Figure 5 represents a normal cytoarchitecture of the lungs with no inflammations in the (A)-Alveoli or (B)-Bronchiole.

Cytoarchitecture of the lungs for Group A to Group D for Day 3 after exposure to Soot. H and E stain   Figure 6 shows the cytoarchitecture of the H and E-stained lungs for Day 3 after exposure to Soot. There are four groups labelled as 1 (Group A/general control), 2 (Group B), 3(Group C), and 4 (Group D). The result showed the normal pulmonary architecture consisting of alveoli (A) (alveolar ducts, alveolar sacs) and the bronchiole (B) for Group A. On the alveoli (A) of Group B, C, and Group D

Fig. 5 Representative photomicrograph of the Normal cytoarchitecture of the lungs H and E stain Magnification: × 100 (A-Alveoli; B-Bronchiole)

animals that were exposed to soot, mild emphysematous air spaces were observed in the alveoli of the lungs of each group.

Cytoarchitecture of the lungs for Group A to Group D for Day 28 after exposure to Soot. H and E stain   The cytoarchitecture of the H and E-stained lungs for Day 28 after exposure to Soot is shown in Fig. 7. There are four groups labelled as 1, 2, 3, and 4. Group 1 represents (General control), Group 2 represents (Group B), Group 3 (Group C), and Group 4 (Group D). The result
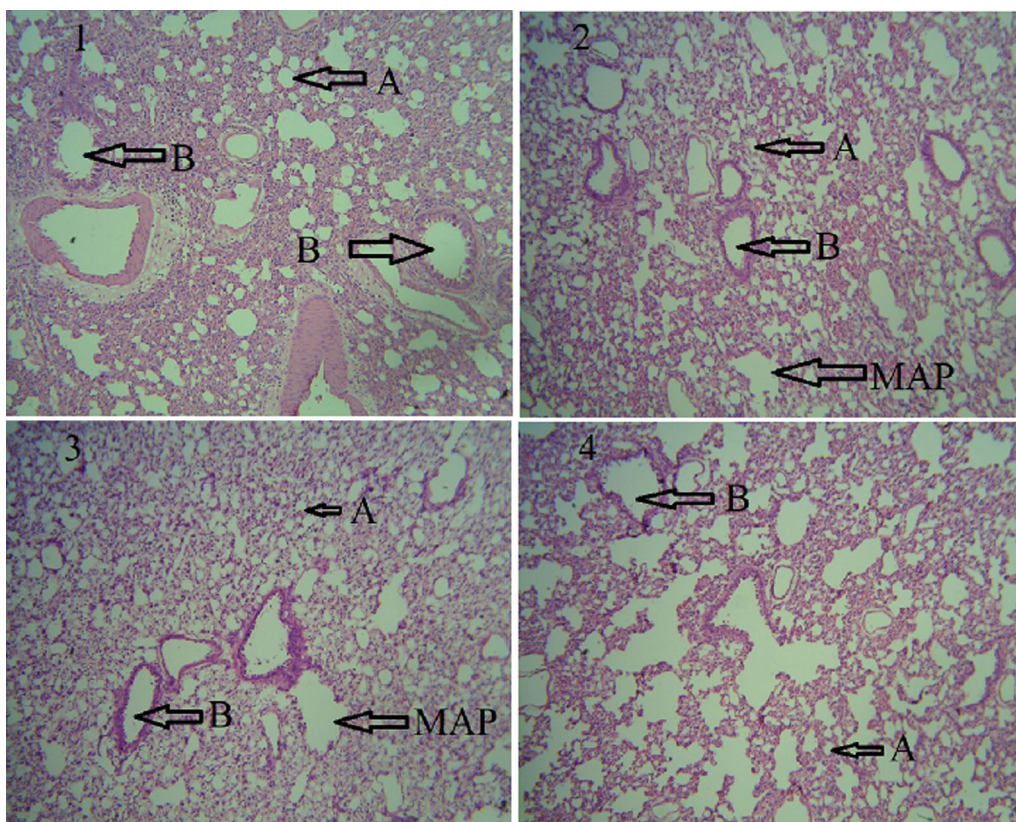
Fig. 6 Representative photomicrograph of the cytoarchitecture of the lungs. Group 1 (General control), Group 2 (Group B), Group 3 (Group C), Group 4 (Group D) for Day 3 post Soot exposure. H and E stain, Magnification: × 100. (A-Alveoli; B-Bronchiole, MAP-mild emphysematous air spaces)

showed the normal histology of the lungs in the alveoli (A) alveolar ducts and the bronchiole (B) for Group A. For the lungs of Group B, C and Group D animals that were exposed to soot, inflammatory cell infiltration, thickened alveolar walls, diminished alveolar spaces, hyperaemia, and bronchial epithelial hyperplasia were observed.

Prussian blue reaction product (deep blue precipitate) Cytoarchitecture of the lungs for Group A, B, C, and Group D for Day 7 after exposure to Soot. H and E stain   Figures 8, 9, 10, 11 shows Day 7 lungs tissue cytoarchitecture after staining with the Prussian blue reaction product (deep blue precipitate), after counterstaining with neutral red, for Group A (general control), Group B, C and Group D. The Arrows on the bronchoalveolar lavage (BAL) in group A, indicate no Prussian blue reaction product (deep blue precipitate), demonstrating the absence of ferric iron in the lungs. For the lungs of Group B, C and Group D animals that were exposed to soot, the presence of Prussian blue reaction product (deep blue precipitate) on the on the bronchoalveolar lavage (BAL) demonstrates the presence of ferric iron in the lungs.

Cytoarchitecture of the testis
Figure 12 represents a normal cytoarchitecture of the testis with no distortion of the seminiferous tubules, Leydig cells, sperm cells, spermatid layers, and flagella in the lumen.

Cytoarchitecture of the Testis for Group A, B, C, and Group D for Day 3 after exposure to Soot. H and E stain   Figure 13 shows the cytoarchitecture of the H and E-stained Testis for Day 3 after exposure to soot. There are four groups labelled as 1, 2, 3, and 4. Group 1 represents (Group A/General control), Group 2 represents (Group B) Group 3 (Group C) and Group 4 (Group D). The result showed normal cellular architecture of seminiferous tubules, Leydig cells, Sertoli cells, sperm cells, spermatid layers and flagella in the lumen in all stages for Group A. In Group B, C and Group D rats that were exposed to soot, few mildly distorted Leydig cells, mild vacuolations within the spermatid layer and partial loss of flagella in the lumen.

Cytoarchitecture of the Testis for Group A, B, C and Group D for Day 28 after exposure to Soot. H and E stain   The cytoarchitecture of the H and E-stained Testis for Day
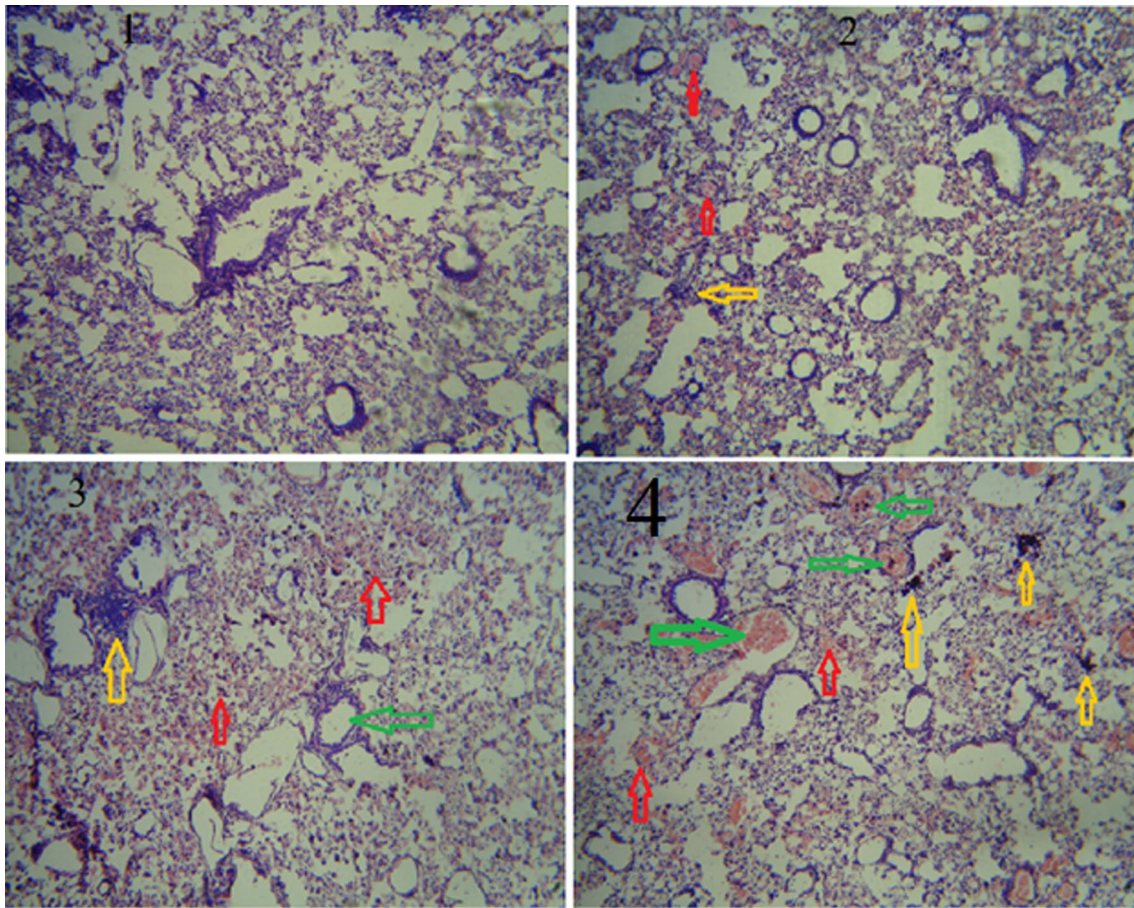
Fig. 7 Representative photomicrograph of the cytoarchitecture of the lungs. Group 1 (General control), Group 2 (Group B) Group 3 (Group C), Group 4 (Group D) for Day 28 post Soot exposure. H and E stain, Magnification: ×100. (The red arrows indicate sites of hyperaemia, the yellow arrow indicates site of inflammatory cell infiltration, and the green arrow indicates site of bronchial stenosis and mucus secretion)
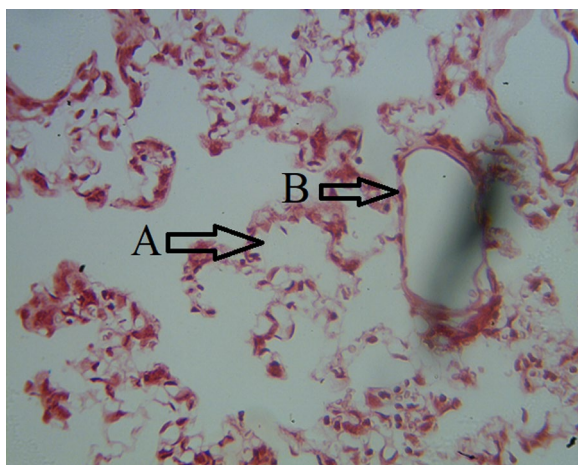

Fig. 8 Representative photomicrograph of the cytoarchitecture of the lungs. Group A (General control), for Day 7 post Soot exposure. Tissue stained negative to Prussian blue stain, Magnification: ×100. (A-Alveoli, B-Bronchial wall)
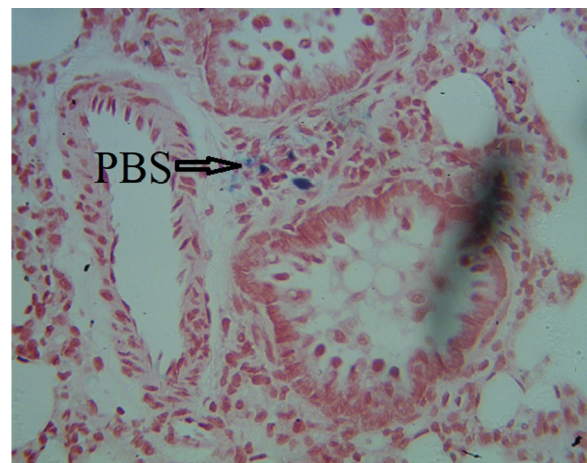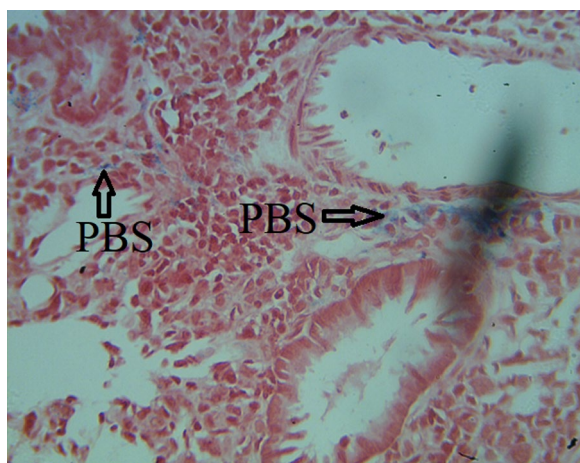

Fig. 9 Representative photomicrograph of the cytoarchitecture of the lungs. Group B for Day 7 post Soot exposure. Tissue stained positive to Prussian blue stain, Magnification: ×100. (PBS-Prussian Blue Stain reaction)

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Fig. 10 Representative photomicrograph of the cytoarchitecture of the lungs. Group C for Day 7 post Soot exposure. Tissue stained positive to Prussian blue stain, Magnification: ×100. (PBS-Prussian Blue Stain reaction)
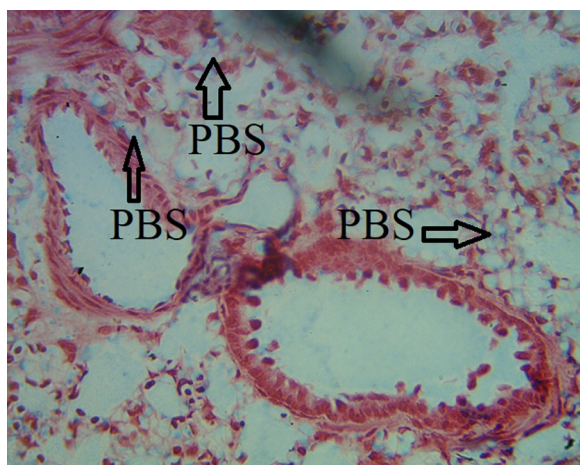

Fig. 12 Representative photomicrograph of the cytoarchitecture of the Testis H and E stain Magnification: ×100 (S-Seminiferous tubules, F-flagella sperm cells in the lumen)

Fig. 11 Representative photomicrograph of the cytoarchitecture of the lungs. Group D for Day 7 post Soot exposure. Tissue stained positive to Prussian blue stain, Magnification: ×100. (PBS-Prussian Blue Stain reaction)

28 after exposure to Soot is shown in Fig. 14. There are four groups labelled as 1, 2, 3 and 4. Group 1 represents (General control), Group 2 represents (Group B), Group 3 (Group C) and Group 4 (Group D). The result showed normal cellular architecture of seminiferous tubules, Leydig cells, Sertoli cells, sperm cells, spermatid layers and flagella in the lumen in all stages for Group A. Significant distortions were recorded in the Leydig cells, deformation in the Seminiferous tubule, vacuolatio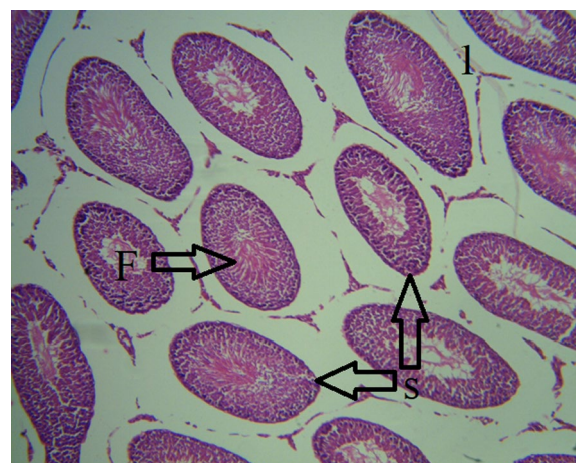ns and mild vacuolations within the spermatid layer, and loss of flagella in the lumen of Group B, C and Group D rats that were exposed to soot.

Expression of tumour-necrosis-factor alpha (TNF-α) in the lungs

Note: Data are presented as mean ± SEM for each group. Comparisons were made with Group A (control). $^{b}P < 0.05$ vs. control. Statistical significance was determined by Two-way Analysis of Variance (ANOVA) for TNFα: (pg/ml).

Figure 15 presents the temporal dynamics of tumour-necrosis-factor alpha (TNF-α) expression in the lung tissues of both control and soot-exposed experimental groups, quantified from Day 3 to Day 28. The expression levels are meticulously recorded as mean values with their respective standard error means (SEMs) for each group.

Statistical comparisons were made against Group A, the control group, which was not exposed to soot. The data revealed remarkable variations in TNF-α expression between the control and experimental groups. Notably, from Day 14 onwards, the experimental groups (Group B, C, and D) demonstrated a statistically significant elevation in TNF-α expression levels compared to the control group, with *P*-values less than 0.05, indicating a robust response to soot exposure.

Furthermore, an intriguing trend was observed in the experimental groups: A consistent amplification in TNF-α expression was witnessed from Day 3 to Day 28, suggesting a progressive inflammatory response to continued soot exposure. This persistent rise in TNF-α expression underscores the potential chronic
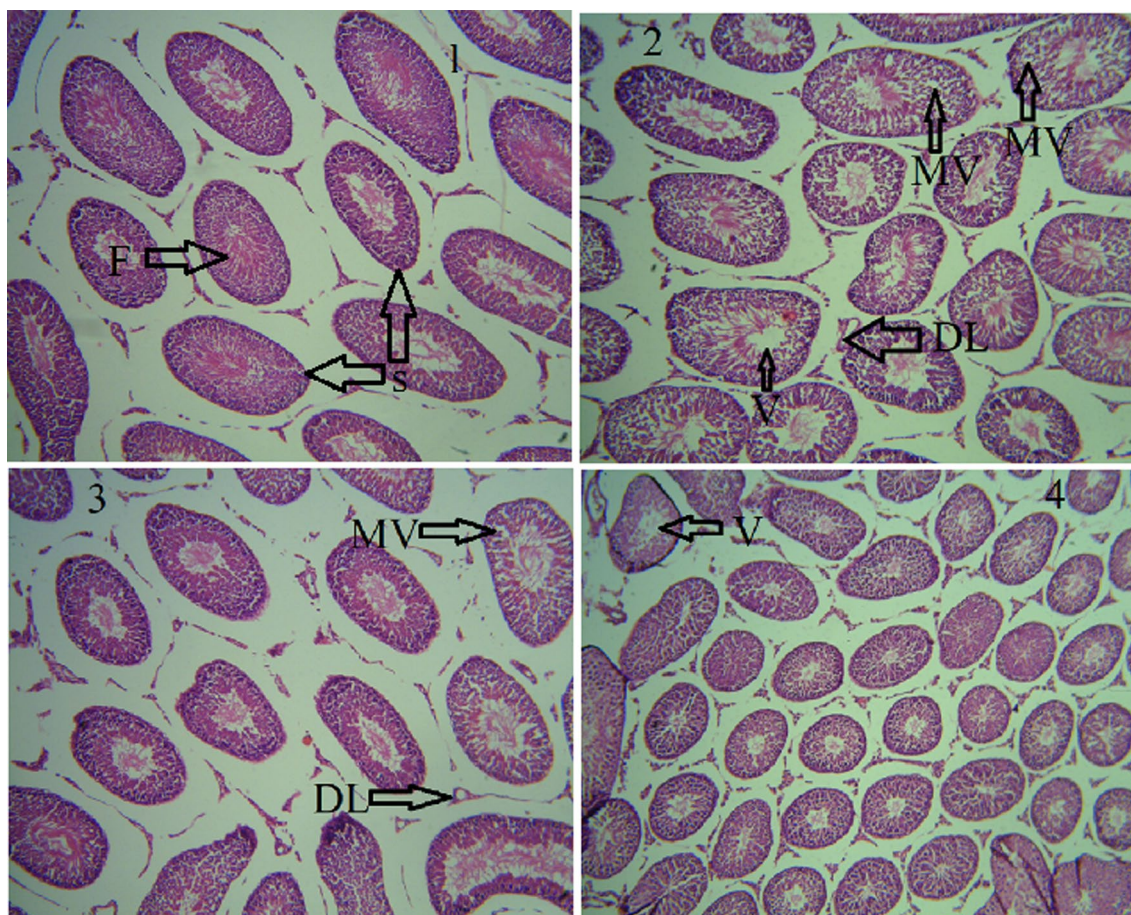
Fig. 13 Representative photomicrograph of the cytoarchitecture of the Testis. Group 1(General control), Group (Group B), Group 3 (Group C), Group 4 (Group D) for Day 3 post-soot exposure. H and E stain, Magnification: ×100. (V-Vacuolation, MV-Mild Vacuolation, DL-Distortion of Leydig cell, F-Flagella of spermatozoa in the lumen of Testis)

inflammatory effect of soot on lung tissue and the pivotal role of TNF-α as a key mediator in this process.

These findings lend vital insights into the pathophysiological mechanisms underlying soot-induced lung inflammation and could serve as a foundation for further exploration into preventive and therapeutic strategies.

Expression of Malondialdehyde (MDA) in lungs
Note: Values are presented as mean ± SEM for each group. Experimental Groups (B, C and D) are compared to Group A (control): [b]$P < 0.05$ vs control. Statistical significance was determined by Two-way Analysis of Variance (ANOVA) for MDA, MDA unit µmol/ml.

As exhibited in Fig. 16, the temporal dynamics of malondialdehyde (MDA) expression, a key marker of oxidative stress, were investigated in the lung tissues of both control and soot-exposed groups. These results are presented as mean values accompanied by their respective standard error of the mean (SEM).

For the control group (Group A), the MDA levels maintained a relatively stable expression pattern across the experimental timeline from Day 3 to Day 28. In contrast, the soot-exposed Groups (B, C, and D) demonstrated a conspicuous elevation in MDA expression, with the most significant differences being observed on Day 21 and Day 28 ([b]$P < 0.001$).

This temporal pattern signifies an escalation of oxidative stress in the lung tissues of the soot-exposed rats, marked by an increasing trajectory of MDA expression from Day 3 through to Day 28. It's noteworthy that the induction of this oxidative stress response appears to be both persistent and progressive, further reinforcing the detrimental impact of soot exposure on lung tissue integrity.

The statistical comparisons were made against Group A (control), with P-values < 0.05, < 0.01, and < 0.001 indicating varying degrees of statistical significance. These P-values were derived from a one-way Analysis of
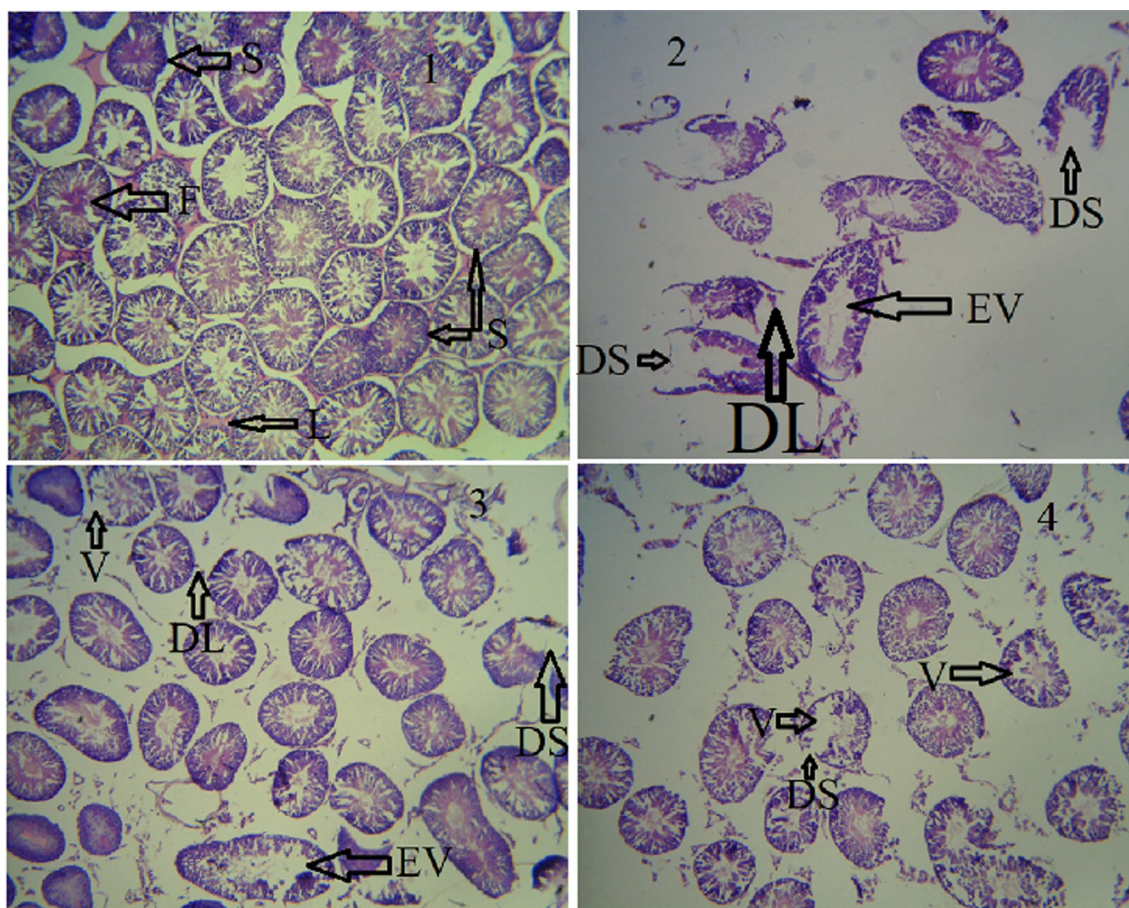
Fig. 14 Representative photomicrograph of the cytoarchitecture of the Testis. Group 1(General control), Group 2 (Group B) Group 3 (Group), Group 4 (Group D) for Day 28 post Soot exposure. H and E stain, Magnification: × 100. (EV-Excessive Vacuolation, V-Vacuolation, DL-Distortion of Leydig cell, F-Flagella of spermatozoa in the lumen of Testis, S-Seminiferous Tubule, DS- Distortion of Seminiferous Tubule)

Variance (ANOVA), supplemented by a Tukey's post hoc test for multiple comparisons.

Expression of Superoxide Dismutase (SOD) in the lungs

Note: in Fig. 17 values are presented as mean ± SEM for each group. Experimental Groups (B, C, and D) are compared to Group A (control). [a]$P > 0.05$ across the different days indicating no significant difference in the results as days go by. [b]$P < 0.05$, across the different groups, indicating significant difference. Statistical significance was determined by Two-way Analysis of Variance (ANOVA). SOD Unit: (u/ml).

Changes in the expression of superoxide dismutase (SOD) in lung tissues, following exposure to soot, are comprehensively depicted in Fig. 17. Data across time points, from Day 3 to Day 28, are presented as mean ± standard error of the mean (SEM) for each group.

A comparative analysis was performed between the experimental groups (Group B, Group C and Group D) and the control group (Group A). The analysis revealed a significant down-regulation of SOD expression in the experimental groups relative to the control group, particularly on the 21st day of exposure ($P < 0.001$).

Furthermore, a temporal trend of decreasing SOD expression was observed across the experimental groups from Day 3 to Day 28. This pattern indicates an inverse correlation between the duration of soot exposure and SOD levels in lung tissue, suggesting a potential detrimental impact of prolonged soot exposure on the antioxidant defence mechanisms of the lungs.

Effects of soot exposure to haematological parameters in Wistar rats

Note: in Fig. 18 the Values are given as mean ± SEM for each group. Indicate significant difference at [b]$p < 0.05$, compared to Group A (Control). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Note: in Fig. 19 Values are given as mean ± SEM for 5 rats in each group. Experimental groups are compared

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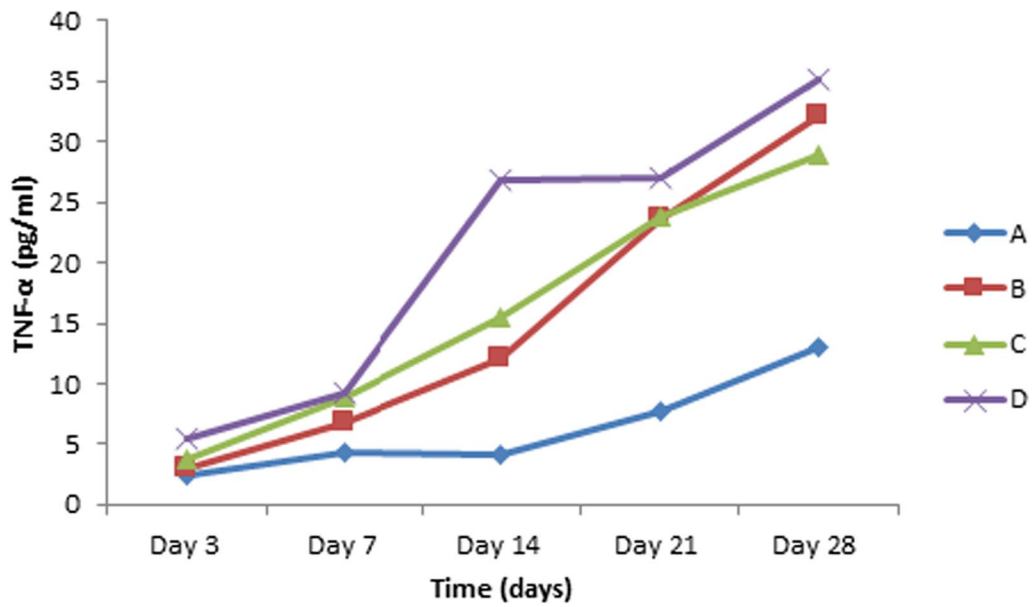
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Fig. 15 Longitudinal changes in the Mean Expression of Tumour-Necrosis-Factor alpha (TNF-α) (pg/ml) in the lungs from Day 3 to Day 28 post-exposure
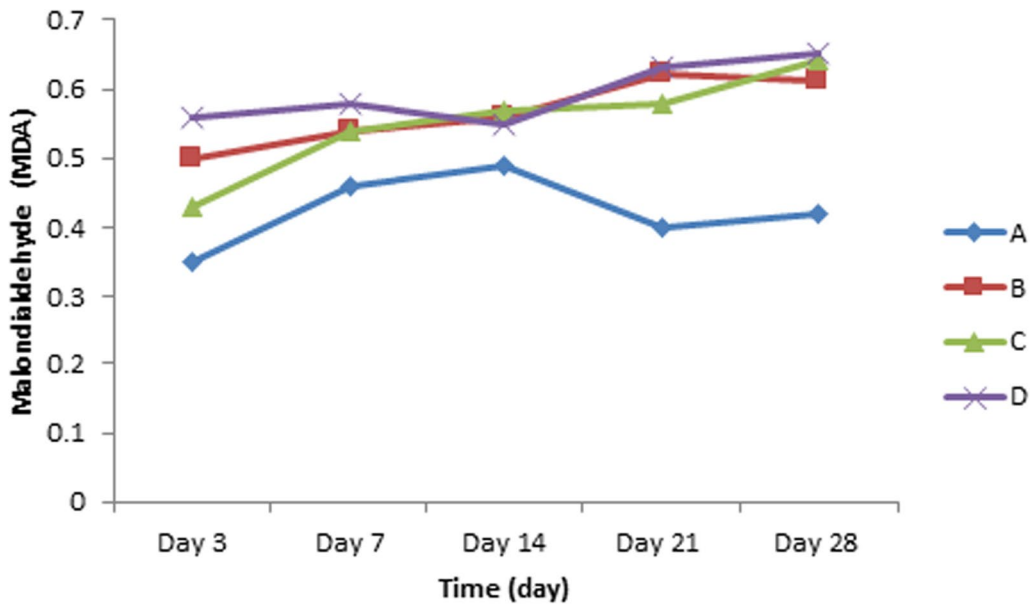

Fig. 16 Temporal Expression of Malondialdehyde (MDA) in lung Tissue Post Soot Exposure

with group A (control). No significant difference at a 95% confidence). [b]$P < 0.05$ statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Note: Values in Fig. 20 are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ([b]$P < 0.05$) statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Note: in Fig. 21 values are given as mean ± SEM. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ([a]$P < 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.
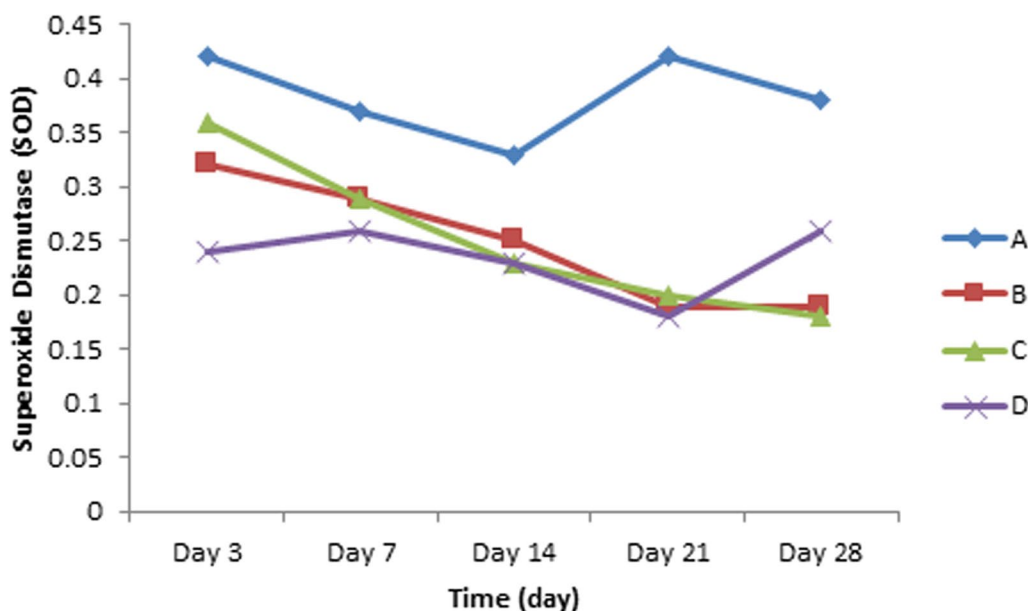
Fig. 17 Graph Expression of Superoxide Dismutase (SOD) in lung Tissue Post Soot Exposure
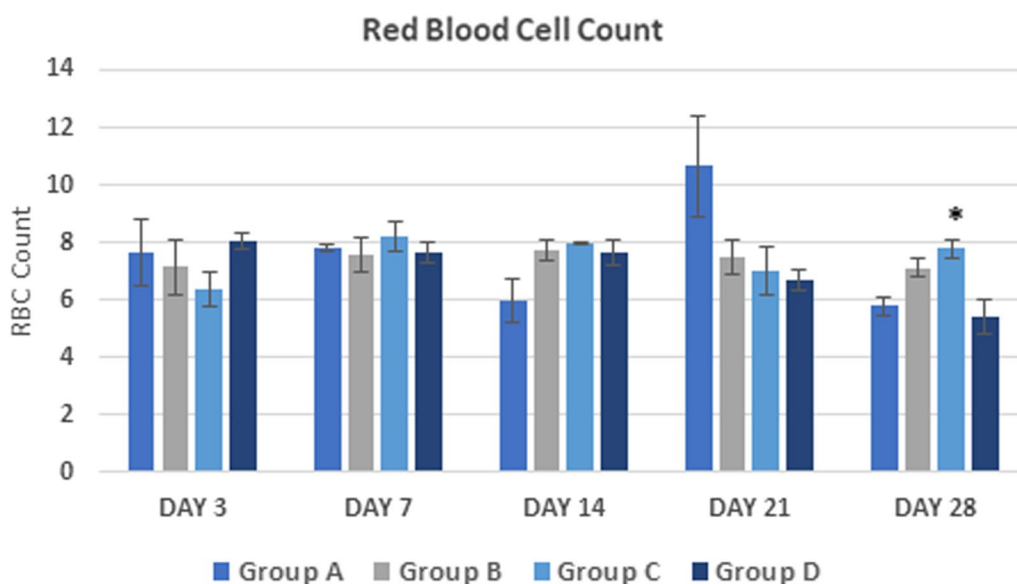
Fig. 18 Red Blood Cell count following soot exposure

Note: In Fig. 22 values are given as mean ± SEM for each group. Experimental Groups are compared with Group A (control). $^{b}P < 0.05$, $^{b}P < 0.01$ vs. control. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Values are given as mean ± SEM for each group in Fig. 23. Experimental Groups are compared with Group A (control). $^{b}P < 0.05$, $^{b}P < 0.01$ vs. control. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test.

In Fig. 24, values are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ($^{a}P > 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.
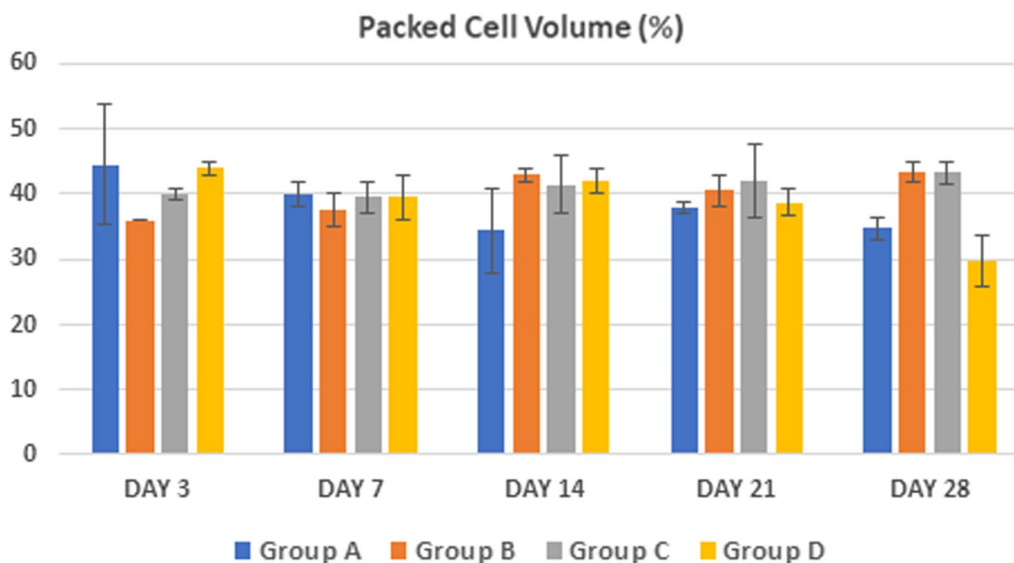
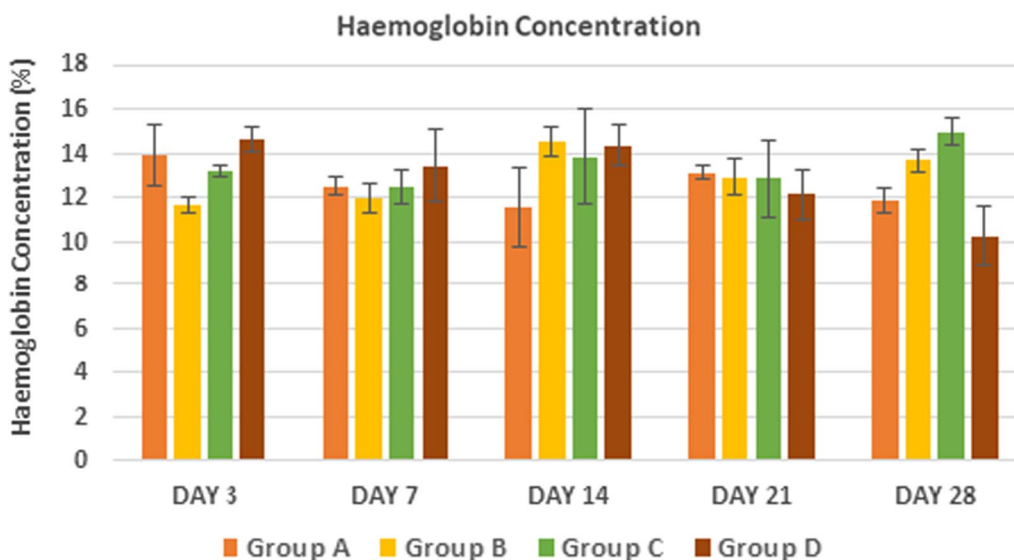**Fig. 19** Packed Cell Volume following soot exposure

Fig. 20 Haemoglobin concentration following soot exposure

Note: Values are given as mean ± SEM for 5 rats in each group in Fig. 25. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ($P > 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

In Fig. 26, the values are displayed as mean ± SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ([b]$P > 0.05$). P: statistical

level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test.

Note: Values are given as mean ± SEM for 5 rats in each group in Fig. 27. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ($P > 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Note: Values in Fig. 28 are given as mean ± SEM for 5 rats in each group. Experimental groups are compared
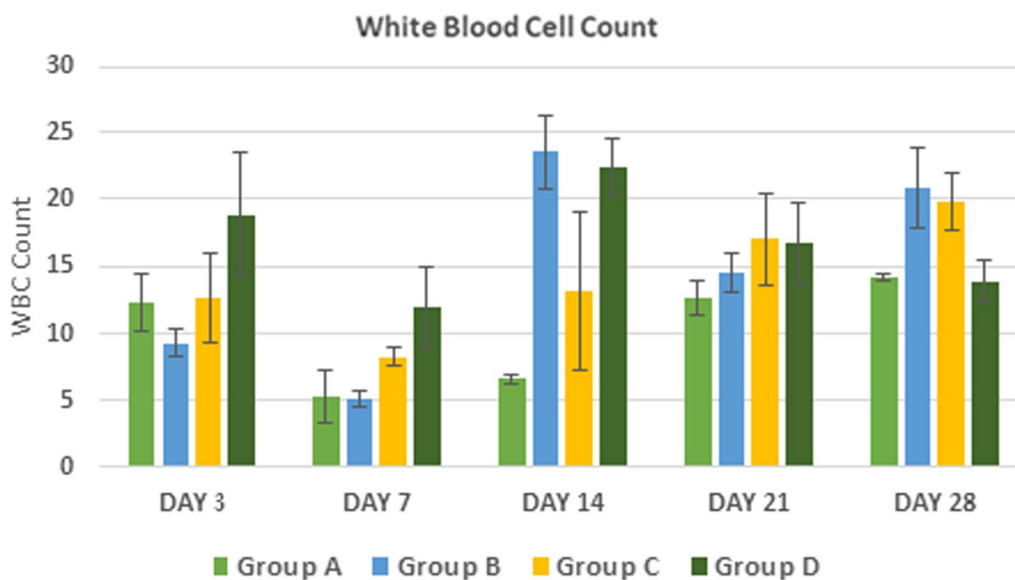
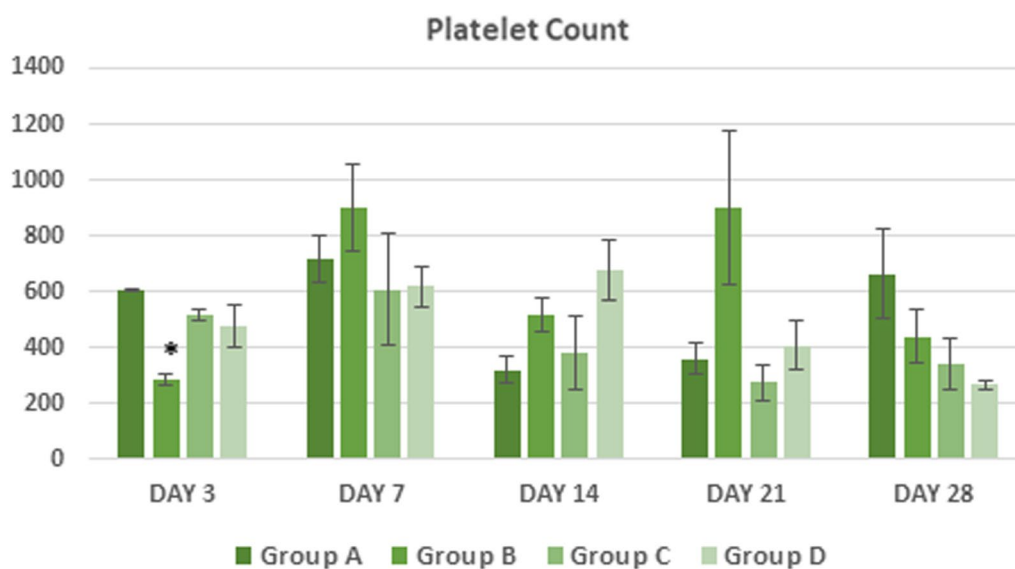**Fig. 21** White Blood Cell Count following soot exposure

Fig. 22 The platelet count following soot exposure

with Group A (control). No significant difference at a 95% confidence interval ($^bP > 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Note: in Fig. 29 the values are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with Group A (control). No significant difference at a 95% confidence interval ($^bP > 0.05$). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

The comprehensive analysis of haematological parameters following exposure to soot in Wistar rats is presented in Fig. 18 through 19. These parameters include packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), along with percentages of neutrophils, lymphocytes, eosinophils and monocytes.

In the early phase of exposure (Day 3 to Day 21), the haematological parameters across all experimental
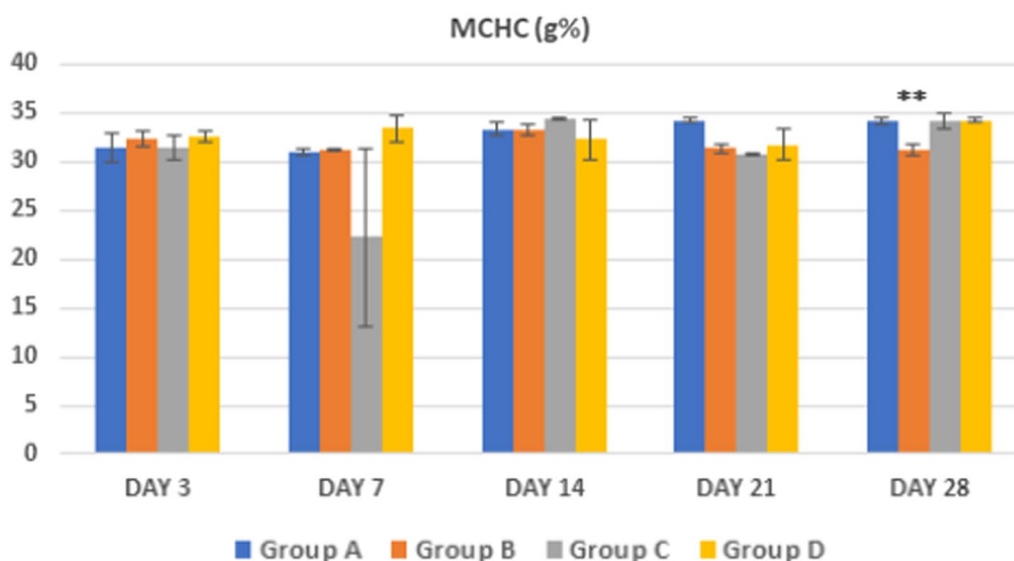
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Fig. 23 Mean corpuscular haemoglobin concentration following soot exposure
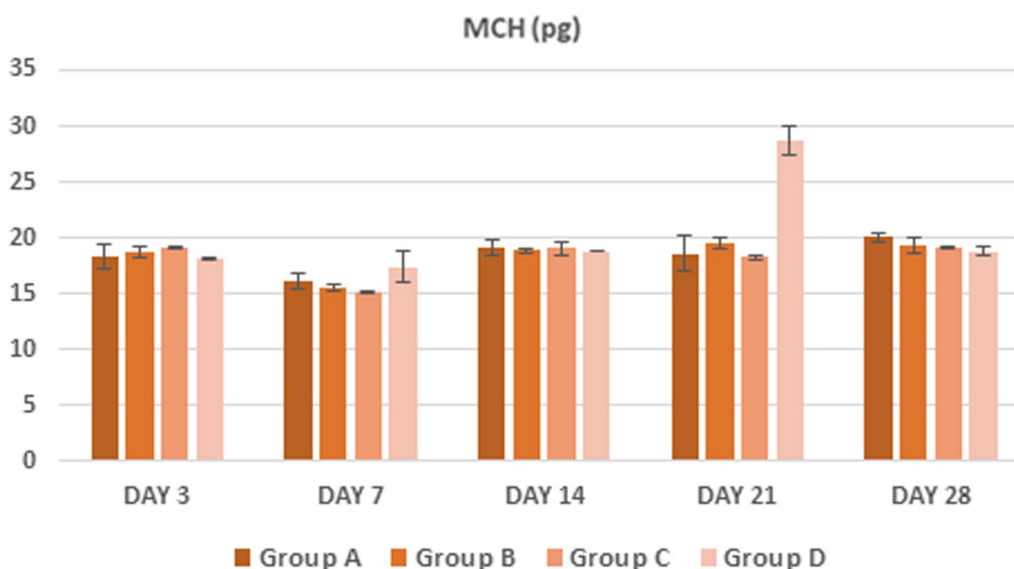
Fig. 24 Mean corpuscular haemoglobin following soot exposure

groups didn't differ significantly from the control group, with the exception of a notable decrease in Platelet count in Group B on Day 3 ($P < 0.05$).

However, on Day 28, a few marked deviations were observed. Group B exhibited a significant decrease in mean corpuscular haemoglobin concentration (MCHC) as compared to the control group ($P < 0.05$). Moreover, an elevated red blood cell count (RBC) was detected in this group, significantly surpassing that of Group A, the general control ($P < 0.05$).

The intragroup comparison over time revealed compelling trends. In Group D, the packed cell volume (PCV) demonstrated a significant reduction from Day 3 to Day 28 ($P < 0.05$). This downward trend in PCV was echoed in other experimental groups as well, with these groups consistently exhibiting lower PCV values compared to Group A from Day 3 to Day 28.

Haemoglobin (Hb) percentage also showed a gradual decrease in the experimental groups over the study period. Specifically, Group D displayed a statistically
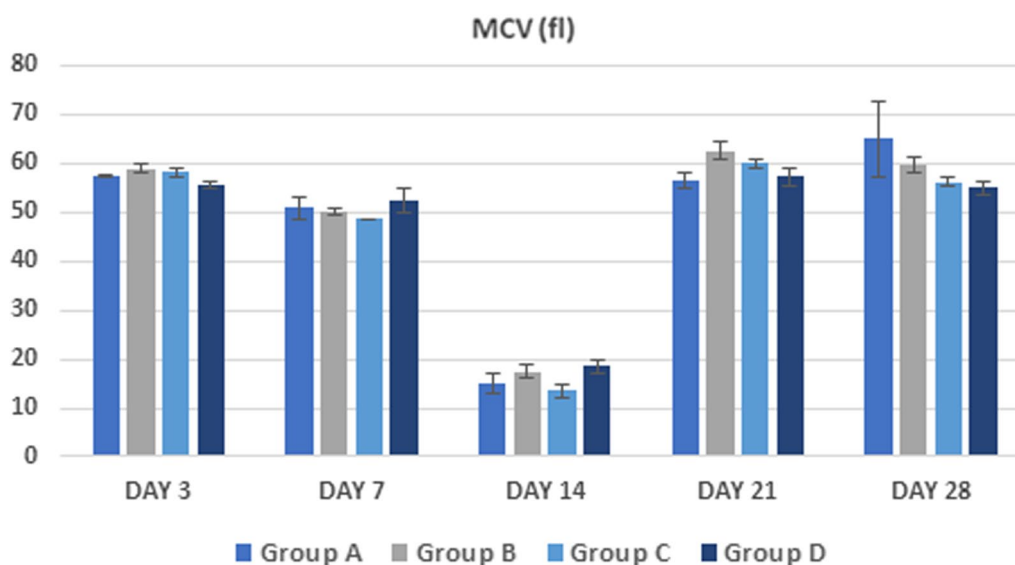
Fig. 25 The mean corpuscular volume following soot exposure
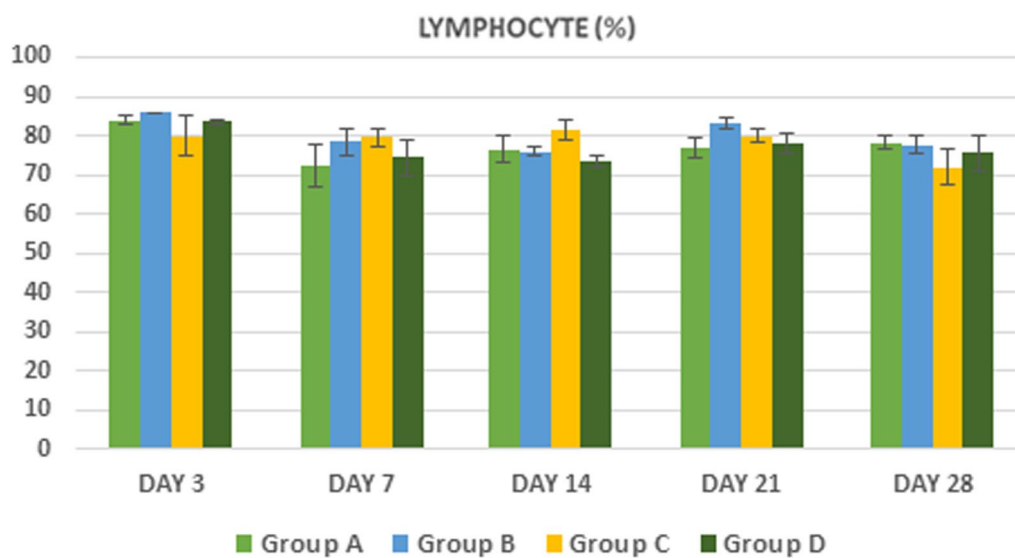
Fig. 26 The Lymphocyte volume

significant reduction in the red blood cell count (RBC) from Day 3 to Day 28 ($P < 0.05$).

White blood cell count (WBC) in Group D followed a similar trajectory, registering a declining trend from Day 3 to Day 28.

An intriguing dynamic was observed in the Neutrophil percentages, which showed a significant increase in the experimental groups from Day 3 to Day 28 ($P < 0.05$). In contrast, the lymphocyte percentage showed a significant and consistent decrease across the experimental groups over the same timeframe ($P < 0.05$).

Taken together, these findings suggest a complex interplay of haematological changes following exposure to soot, with potential implications for immune function and oxygen-carrying capacity. The data emphasise the need for further research to elucidate the mechanisms underlying these observed changes and their potential health impacts.

Discussion
This study aimed at analysing the effect of carbon soot from artisanal refinery activities on the lungs and testis of adult male rats. This is in response to
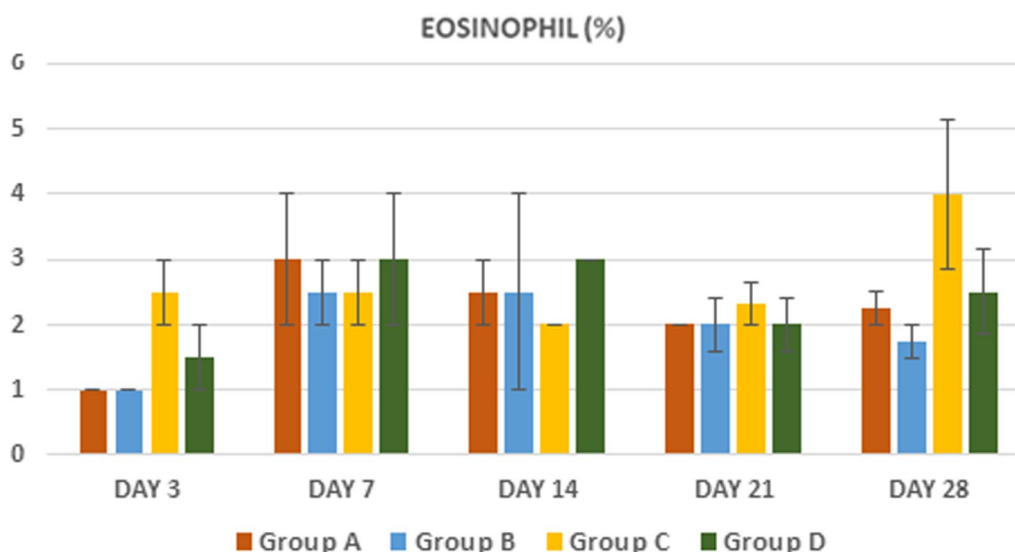
Fig. 27 The Eosinophil volume following soot exposure
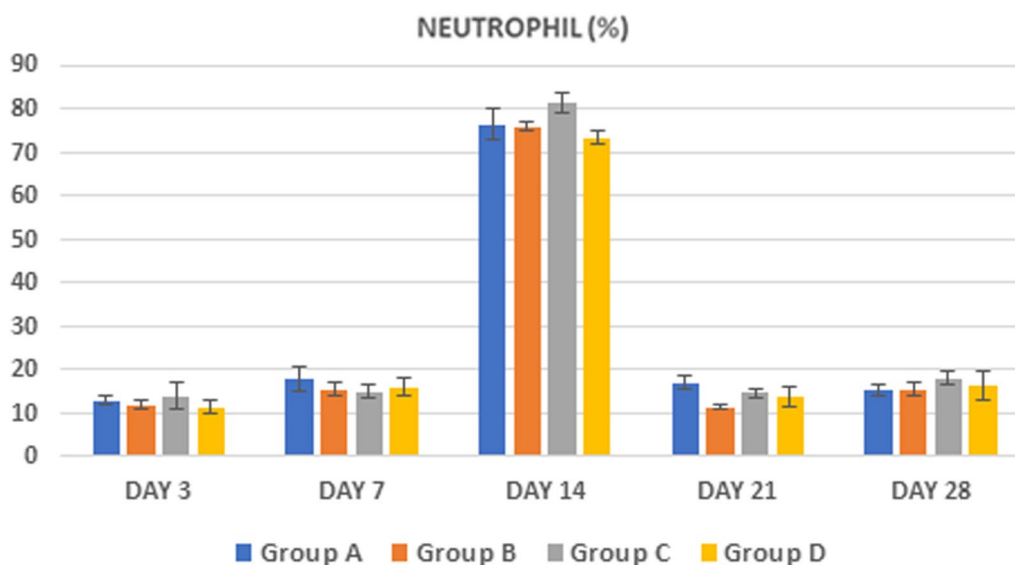
Fig. 28 The Neutrophil volume following soot exposure

the proliferation of artisanal refineries across the Niger Delta region of Nigeria, which has given rise to an increase in environmental pollution (Goodnews and Wordu 2019; Obenade and Amangabara 2014). To ascertain the health effects of artisanal workers exposure to this hazard, raw light crude was obtained (Fig. 1), after which an artisanal combustion chamber was designed (Fig. 2). Analysis was conducted on both exposed male rats and the control, to identify the presence of soot in exposed lungs by Prussian blue stain, changes in the weight, immunohistochemistry and

histology of the lungs and testis, haematological analysis, and determination of oxidative stress in the lungs.

Effects of soot inhalation on the final mean body weight of rats

Exposure of the rats to carbon soot affected body weight and induced pulmonary inflammation. Both exposed and controlled rat group continued to gain weight steadily throughout the duration of the experiment (Table 2). However, the mean weight values of the rats exposed to carbon soot show a significant increase in weight when
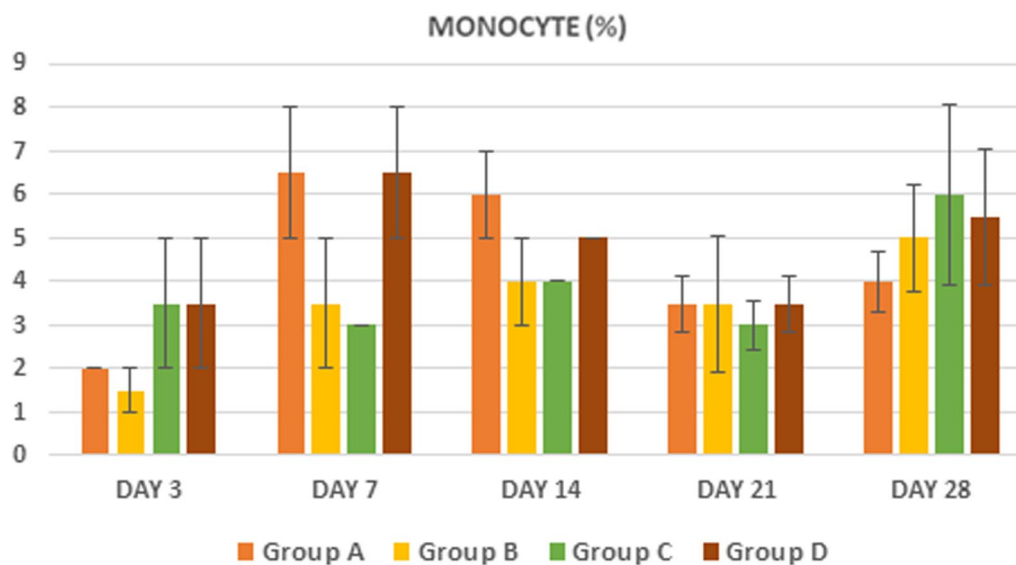
Fig. 29 The Monocyte volume following soot exposure

compared to the control. This is in line with studies, which has established a direct correlation between rats exposed to PM and increased weight gain and the risk of obesity (Wang et al. 2018; Shi et al. 2022).

Determination of soot particulate matter in the lungs using Prussian blue stain

Determining the presence of particulate matter in the lungs by Prussian blue stain (Figs. 8, 9, 10, 11), revealed macrophages with blue granules or ferruginous bodies as they are referred, within the cell after the stain. These ferruginous bodies are bronchoalveolar lavage (BAL), which when insoluble particles like carbon soot are inhaled, are phagocytosed by lung macrophages, and could be coated either partially or completely, with an iron-containing protein at the interface forming a ferruginous body (Ghio and Roggli 2021). This is in line with studies conducted on the analysis of the presence of insoluble particles in the lungs (Olsen et al. 2022; Slesinski and Turnbull 2009). Moreover, the presence of insoluble particulate matter has proven to be associated with decrease in lung function, and various lung diseases such as Fibrosis, COPD and others (Chen et al. 2019; Kyung and Jeong 2020).

Histopathology of soot on exposed lungs

In the present study, the histopathologic effects of soot inhalation on the lungs was evaluated for Day 3 and Day 28 post exposure. The result of exposure to soot inhalation for Day 3 showed Normal pulmonary architecture consisting of alveoli (A) alveolar ducts, alveolar sacs, and the bronchiole (B) for Group A which is the general control group (Fig. 6). However, mild emphysematous air spaces in the alveoli were observed in the lungs of Group B, C and Group D animals that were exposed to soot for Day 3 (Fig. 6). In addition, result of exposure to soot inhalation for Day 28 showed normal histology of the lungs of alveoli (A) alveolar ducts and the bronchiole (B) for the general Control group (Fig. 7). In contrast, inflammatory cell infiltration, thickened alveolar walls, diminished alveolar spaces, hyperaemia, and bronchial epithelial hyperplasia, were found in the lungs of Group B, C and Group D animals that were exposed to soot (Fig. 7).

Similarly, a study conducted by Jheng et al. (2021) on prolonged exposure to traffic-related particulate matter and gaseous pollutants for 3–6 months, rats showed gradual but significant damage to of rat lung tissues with thickened airway walls, infiltration of immune cells within the alveolar and bronchial walls and damage within the bronchial wall.

In another study conducted by Busso et al. (2017) seeking to analyse the effects of mammals' sub-chronic exposure to $PM_{2.5}$ on the lower respiratory tract, by addressing realistic exposure conditions to normal urban air. Exposed Wistar rats were placed under controlled settings to the same normal urban air, with and without particles. Histological analysis showed a mild to moderate infiltration of the lungs.

These results provide strong evidence that exposure to particulate whether acute or chronic, is harmful to lung tissues and could result in various pulmonary related diseases.

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Histopathologic effects of soot inhalation on the testis

The adverse effect caused by exposure particulate matter resulting in damage to the spermatogenesis system, which in turn causes reduction in sperm density, decreased sperm number and viability, considerable damage to testicular tissues, disruption of the blood-testis barrier integrity, toxic effects on germ cells, and promotion of apoptosis have been shown by various studies (Longhin et al. 2013).

The above statement has been observed in our study evaluation of the effects of soot inhalation on the testis after Day 3 and Day 28. For the control (Group A), the testes showed normal cellular architecture, no distortion of the seminiferous tubules, Leydig cells, Sertoli cells, sperm cells, spermatid layers, and flagella in the lumen on Day 3 (Fig. 13). However, in the soot-exposed rats, slight distortions were observed in the Leydig cells, mild vacuolations within the spermatid layer, and a partial loss of flagella in the lumen on Day 3 of the exposed rats (Fig. 13). These distortions became more pronounced after 28 days of soot exposure with the testis tissue showing Significant distortions in the Leydig cells, deformation in the Seminiferous tubule, vacuolations and mild vacuolations within the spermatid layer and loss of flagella in the lumen (Fig. 14).

These results are in line with the study conducted by Liu et al. (2022), on the $PM_{2.5}$ exposure at different concentrations and modes in male rats. The histopathological findings revealed that the interstitium of the seminiferous tubules of rats exposed to $PM_{2.5}$ presented oedema, widening, reduction in cellular levels, distorted or even absence of spermatogenic cells at all levels, with a significant reduction in sperm count, testicular cell damage, and apoptosis.

In addition, Jiang et al. (2023) initiated a study on the adverse effects of prenatal exposure to oxidized black carbon particles on the reproductive system of male, in the study, prenatal female mice were intratracheally instilled with oxidized black carbon particles throughout gestation period, analysis of the male pups testes at postnatal Day 35 and 84, showed degenerative and necrotic changes on the seminiferous tubules, low cellular adhesion of seminiferous epithelia, and vacuolation of some seminiferous tubules.

The findings align with that of Onyeso et al. (2020), after exposure of rats to black soot, the results obtained indicated a statistically significant decrease in some sperm parameters and male sex hormones (follicle stimulating hormone, luteinizing hormone, and testosterone) when compared with the control.

Our findings combined with these studies, indicated that environmental exposure to carbon soot may alter hormonal profile decrease sperm motility, reduce testosterone production, or abnormal sperm production, all of which point towards increased risk of male infertility (Moore Jr and Bertram 2018). Thus, this study reinforces the critical role of regulating particulate pollution to protect male reproductive health.

Effects of soot inhalation on the expression of Tumour-Necrosis-Factor alpha (TNF-α) in the lungs

Existing literature has shown that exposure to soot triggers inflammation of the lungs (Chan et al. 2013; Saber et al. 2012; Sarnat et al. 2012). Inflammation in the lungs involves a complex set of molecular and cellular responses resulting from exposure to external stimuli such as soot. In response, the body induces the release of cytokines, which includes the tumour necrosis factor-alpha (TNF-α) from immune cells and structural airway cells (Ristovski et al. 2011). Previous studies support that the inflammation process causes severe damage to the lung function via varying mechanisms, one of which involves the release of cytokines, which have been identified as a causal factor in lung function damage (Howarth 1998; Morimoto et al. 2013).

In this present study, the effects of soot particulate exposure on TNF-α expression in the lungs was studied. It was observed that the TNF-α expression in the experimental groups were significantly higher than that of the control group for Day 3, 7. 14, and 21, respectively (Fig. 15).

In line with this study, Jalaludin et al. (2014) conducted research on the exposure to indoor pollutants among primary school children in Klang valley, using tumour-necrosis-factor alpha as biomarkers. Sputum samples taken from the children were being analysed in order to get TNF-α concentration levels. According to the statistical analysis, the mean value of TNF-α concentration levels for children in urban was significantly higher than children in rural.

Research has also indicated that a significant proportion of mortality related to air pollution can be attributed to cardiovascular diseases. Exposure to air pollution has been linked with heightened cardiovascular morbidity and mortality due to factors such as myocardial ischemia, arrhythmia, and heart failure (Araujo 2011). Air pollution is known to trigger acute cardiac events through various mechanisms, including systemic inflammation, activation of homeostasis pathways, vascular dysfunction, accelerated atherosclerosis, plaque instability, autonomic control changes, and cardiac arrhythmia (Link and Dockery 2010).

Studies using in vitro, in vivo, and controlled human research have demonstrated that exposure to PM can lead to an increase in interleukin 6 (IL-6) and tumour-necrosis-factor alpha (TNF-α) (Tsai et al. 2012). The

impact of soot particulate exposure on TNF-α expression in relation to cardiovascular disorders has been examined (Suhardi 2012). His study demonstrated a statistically significant difference in TNF-α expression due to 30-day exposure to soot particulates between the control and treatment groups.

Similarly, Hopkins et al. (2018) reported an increase in pro-inflammatory cytokines IL-1b and TNF-α in olfactory bulb homogenates from iron–soot-exposed mice, which supports our observation of elevated TNF-α expression in the lungs of soot-exposed rats.

Effects of soot on reactive oxidative species

The respiratory epithelium of the lungs is the first tissue to get constant exposure with different kinds of soots present in the environment. Soot toxicity causes the interruption of respiratory process by altering lung functions (Borm et al. 1996). This result is an inflammatory response which consist of the direct contact-mediated dysfunctions of lung cells that include generating Reactive Oxidative Species (ROS), cell hyperplasia, cell death, or apoptosis of lung airway epithelium and other adjacent cells (Hussain et al. 2010).

Inflammation is initially a protective mechanism, which removes the injurious stimuli and produces reactive oxygen species (ROS) able to induce cell killing. However, continuous production of ROS will result in Oxidative stress, this potentially does lead to damage of lipids, proteins, and macromolecules such as DNA and RNA (Risom et al. 2005).

In response to oxidative stress, superoxide dismutase (SOD) is produced in order to remove O_2, scavenge for ROS and its precursors, inhibit ROS and chelate metal ions. Research shows that continuous exposure to particulate matter will result in SOD being overwhelmed, and its production decreased, giving rise to free radicals known as Malondialdehyde (MDA).

This is in line with the findings of this present study, where it was observed that the expression of SOD in the lungs in the experimental group was significantly lower when compared to the control group in Day 21 of exposure to soot (Fig. 18). There was a reduction of the expression of SOD in the experimental groups for Day 3 to Day 28 (Fig. 18), and an increase in Malondialdehyde (MDA) (Fig. 17). This result implies the presence of oxidative stress which is due to the exposure of rats to carbon soot.

Similarly, in a study conducted by Al Housseiny et al. (2020), on the Identification of Toxicity Parameters Associated with Combustion Produced Soot Surface Chemistry and Particle Structure by in Vitro Assays, through the exposure of lung epithelial cell line to different soot at a range of concentrations and assess cell viability, inflammation, and oxidative stress. The results showed a significant decrease in SOD expression with each increase in concentration and a significant increase in MDA.

In another study, Sun et al. (2020), exposed rats to $PM_{2.5}$ for 1 month (10 times), followed by normal feeding for 18 months. Pulmonary oxidative stress, manifested by increase of MDA and decrease of GSH and SOD, was induced during exposure but disappeared in later post-exposure duration.

In the same vein, Ezzat et al. (2011), conducted a study on rats exposed to gasoline emissions for 30 min each day for 6 weeks. The result was a marked decrease in SOD activity, their conclusion based on the observed result that, gasoline vapour inhalation induced lung tissue injury and cellular damage concomitant with impairment of the lung antioxidant defence system.

These findings and ours show that oxidative stress occurs with exposure to soot, which in turn could lead to decline in lung function and increase in susceptibility to pulmonary diseases.

Effects of soot inhalation on the haematological parameters of the animals

The immediate pulmonary and systemic effects of inhaling Coal Fly Ash (CFA) have been studied in rats (Smith et al. 2006). CFA, a by-product of high-temperature combustion, is a type of particle pollution often released from power plants alongside soot. Given the wide usage of coal as an energy source, particularly in developing nations like China and India, exposure to such particulate matter poses a significant health risk, with associated increases in respiratory and cardiac diseases (Smith et al. 2006).

In Smith's study, rats underwent exposure to either filtered air or aerosolized CFA for four hours per day over three days. They were then examined 18 or 36 h after the final CFA exposure. Following this, the rats were euthanised, and their blood was collected for analysis. Various haematologic parameters, such as red and white blood cell counts and percentages of different types of white blood cells, were measured.

Smith et al. (2006) reported statistically significant changes in all bronchoalveolar lavage fluid (BALF), blood, and lung tissue parameters after exposure to CFA. These included substantial increases in neutrophils in both the lung BALF and blood, as well as elevated levels of cytokines. Other significant changes noted were increases in transferrin, lung tissue total antioxidant potential, plasma protein, and blood complement.

In our study, we found a significant decrease in red blood cell counts (RBC) due to the cytotoxic effects of soot particulate matter on the erythropoietic tissue, the bone marrow (Fig. 18). This disturbance can alter the

cell cycle and reduce erythropoiesis (Sharma et al. 2007). There was also a notable decrease in packed cell volume (PCV) in the experimental group from Day 3 to Day 28 (Fig. 19), which aligns with previous studies reporting a significant decrease in PCV (Sheikh et al. 2013).

Furthermore, our results showed a gradual and significant reduction in lymphocytes in the experimental group from Day 3 to Day 28 (Fig. 20). This decrease in lymphocytes points to the toxic effect of soot particulate matter, likely due to lysis (the disintegration or rupture of a cell) and depletion of lymphocytes in the spleen, causing a reduction in total leucocyte count in the experimental groups.

Systemic responses in animals following exposure to combustion source or ambient particles have been scarcely reported. However, a minor yet significant increase in circulating neutrophils may hint at their release from the bone marrow into the bloodstream. Changes in bone marrow stimulation and the appearance of band cells in circulation have been reported in animals exposed to particles (Mukae et al. 2001). Our study also found an increase in neutrophil count in the experimental group compared to the control group, potentially due to the lung's inflammatory response to soot particulate matter deposition.

Conclusions

In summary, our research reveals compelling evidence of the detrimental health impacts of artisanal refinery activity, particularly highlighting its effects on the respiratory and reproductive systems. The exposure to carbon soot particulate matter, as demonstrated through our animal model, led to significant alterations in the cytoarchitecture of both lungs and testis, and oxidative stress markers. The notable increase in TNF-α and MDA, coupled with a decrease in SOD levels, signifies an elevated inflammatory response and oxidative stress. Furthermore, haematological findings, including changes in PCV, RBC count, WBC count, and the distribution of neutrophils and lymphocytes, substantiate the systemic physiological stress induced by the exposure. Continuous exposure to artisanal refinery emissions can potentially lead to chronic health implications on the workers, such as decrease in lung function, COPD, and complications with the male reproductive organ. Furthermore, there is need for all stakeholders to invest in the revamping of existing refineries in the country to meet with the demand for the finished product. greater emphasis on monitoring and regulating such activities. To better understand the full extent of health risks and explore possible mitigation measures, we recommend further investigations into the impact on other vital organs, application of advanced histological and molecular techniques, and the study of neurobehavioral correlates. This study underscores the importance of health surveillance and prevention measures for individuals and communities involved in or living near artisanal refineries.

Abbreviations

ANOVA	Analysis of Variance
BALF	Bronchoalveolar Lavage Fluid
BP	British Petroleum
CFA	Coal Fly Ash
CH_4	Methane
CO	Carbon Monoxide
CO_2	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease
DL	Distortion of Leydig cell
DS	Distortion of Seminiferous Tubule
EV	Excessive Vacuolation
GPX1	Glutathione Peroxidase 1
GST	Glutathione S-Transferases
GSH	Glutathione
H&E	Haematoxylin and Eosin (Staining Technique)
H&E	Haematoxylin and Eosin
HB	Haemoglobin
H_2S	Hydrogen Sulphide
hs-CRP	High-sensitivity C-reactive protein
MAP	Mild Emphysematous Air Spaces
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
MV	Mild Vacuolation
NOx	Nitrogen Oxides
O_3	Ozone
OSNs	Olfactory Sensory Neurons
PAHs	Polycyclic Aromatic Hydrocarbons
PBS	Prussian Blue Stain reaction
PLT	Platelet count
PM	Particulate Matter
PM_{10}	Particulate Matter 10 microns and lower
$PM_{2.5}$	Fine Particulate Matter (particles that are 2.5 μm in diameter and smaller)
ppm	Parts per million
ppb	Parts per billion
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
SOD	Superoxide Dismutase
SOD	Superoxide Dismutase
SO_2	Sulphur dioxide
TNF-α	Tumour-Necrosis-Factor alpha
TRAP	Traffic-Related Air Pollution
VOC	Volatile Organic Compounds
WBC	White Blood Cell count

Author contributions
PGS was responsible for the conceptualization and design of the study, devised the project methodology, conducted the research experiments, validated the results, managed data curation, and drafted the original manuscript. EU assisted in the study's conceptualization and methodology design, supervised the research project as PGS' PhD supervisor, and contributed to manuscript review and editing. OFO conducted the statistical analysis and interpreted the data, created data visualizations for the study, and participated in manuscript review and editing. DREE reviewed the work for intellectual content, ensuring the scientific rigor and merit of the work, and contributed to manuscript review and editing. All authors gave final approval of the

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version to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Availability of data and materials
Not applicable.

Declarations

Ethics approval
Ethical approval (UPH/CEREMAD/REC/MM87/105), was obtained from the University of Port Harcourt at the 87th meeting of the Research Ethics Committee (REC), held on the 31st of January 2023.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests, financial or non-financial, that could be perceived as influencing the content or conclusions of this paper.

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