

Genotoxic Response and Histological Alterations in Rat Lungs Exposed to Gasoline Generator Exhaust

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ABSTRACT

The epileptic power supply in Nigeria has caused a surge in the production of energy from other sources, including gasoline generator sets. The air pollution caused by the exhaust from these engines has been implicated in a variety of metabolic disorders and diseases, including cancer. This study aims at understanding the genotoxic and histopathological effects of gasoline generator exhaust exposure on the DNA and lungs of adult male wistar rats. Forty-eight (48) adult wistar rats were divided into four categories, each with 12 rats. The control rats were not exposed to gasoline generator exhaust while the test groups were exposed at 2, 4, and 8-hour intervals for a duration of 4, 8, and, 12 weeks respectively. Animals were euthanized using cervical dislocation and blood samples were obtained for genotoxic analysis via cardiac puncture while the lungs were preserved for 72 hours in 10% neutral buffered formalin and further processed for histological studies. The Olive Tail Moment (OTM) of the exposed rats showed significant variation across the exposure time points when compared to the unexposed control, indicating that exposure to gasoline generator exhaust negatively impacts the DNA. Additionally, histological investigations also indicated some cytopathic characteristics, such as peribronchiolar and perivascular infiltration by inflammatory cells, congestion, and thickening of blood vessels. The findings of this study showed that exposure to gasoline generator exhaust induces genotoxic damage with the severity of the damage occurring in a time/exposure-dependent manner.

Keywords: Air pollution; Nigeria; Gasoline; Generator; Genotoxicity.

الاستجابة الجينية والتغيرات النسيجية في رنتي الجرذان المعرضة لعوادم مولدات الجازولين

أكينبيلو مورونكيجيا و فريدريك أكينبوب

المخلص: تسببت انقطاعات التيار الكهربائي في نيجيريا في زيادة إنتاج الطاقة من مصادر أخرى، بما في ذلك مجموعات مولدات البنزين. وقد تم ربط التلوث الهوائي الناتج عن عوادم هذه المحركات بمجموعة متنوعة من الاضطرابات الأيضية والأمراض، بما في ذلك مرض السرطان. تهدف هذه الدراسة إلى فهم التأثيرات الجينية والنسجية لتعرض أبخرة محركات مولدات البنزين على الحمض النووي والرنيتين للذكور البالغين من جرذان الويستر. تم تقسيم ثمانية وأربعين (48) جرذاً ويستار بالغة إلى أربع فئات، كل فئة بها 12 جرذاً. لم تتعرض الجرذان الضابطة لأبخرة مولدات البنزين بينما تم تعريض مجموعات الإختبار لفترات زمنية تبلغ 2 ، 4 ، و 8 ساعات لمدة 4 ، 8 ، و 12 أسبوعاً على التوالي. تم القتل الرحيم للحيوانات باستخدام خلع عنق الرحم وتم الحصول على عينات الدم للتحليل الجيني عن طريق الثقب القلبي في حين تم الاحتفاظ بالرنيتين لمدة 72 ساعة في فورمالين مخفف بنسبة 10% معقم للدراسات النسيجية فيما بعد. أظهرت لحظة الذيل الزيتونية (OTM) للجرذان المعرضة اختلافاً ملحوظاً عبر نقاط زمن التعرض مقارنة بالمجموعة الضابطة غير المعرضة، مما يشير إلى أن التعرض لأبخرة مولدات البنزين يؤثر سلباً على الحمض النووي. بالإضافة إلى ذلك، أشارت نتائج التشريح النسيجي أيضاً إلى بعض الخصائص النسيجية، مثل الاعتلال الخلوي حول الشعب الهوائية والأوعية الدموية والاحتقان وتضخم الأوعية الدموية. أظهرت نتائج هذه الدراسة أن التعرض لأبخرة مولدات البنزين يسبب ضرراً جينياً وأن شدة الضرر تتزايد بناءً على الزمن والتعرض.



1. Introduction

Short-term exposure to certain air pollutants is associated with human mortality and is a major global source of morbidity and mortality [1]. It remains one of the most pressing environmental health risks facing our global population, with the hazardous impact on the increase, especially in developing nations where the majority of people still generate their electricity supply through gasoline-derived power generator engines [2]. Indoor or outdoor air pollution are the two categories of air pollution, however, outdoor pollution can affect indoor quality and vice-versa [3]. About 98% of children under five breathe contaminated air in less developed countries. As a result, air pollution kills 600,000 children under the age of 15 annually, making it the leading cause of mortality in this age group [4]. In 2019, 500,000 new born fatalities in the first month of life were attributed to air pollution [5,6]. Fine particulate matter are complex substances and mixtures which can be formed from combustion or chemical reactions [3]. Earlier reports by Brook *et al.*, [7] and Miller *et al.*, [8] indicated that particulate matter (PM) of less than 2 μ m in diameter can transverse the lungs or enter blood circulation. The interaction of nanoparticles with the DNA can cause genetic damage via DNA breaks, chromosomal damage, or alteration in bases as well as microtubule distortion during mitosis thereby leading to a clastogenic effect [9]. Globally, 2.2 million people die yearly as a result of anthropogenic PM_{2.5} with land transportation contributing about 16% of the global death burden concerning respiratory mortality while the residential and commercial sector contributes about 30% of PM_{2.5} related premature mortality [10]. Several metropolitan areas especially megacities exceed the standards of air pollution, with reports from the Health Effects Institute indicating that over 93% of the global population currently resides in regions that exceed the WHO's recommendations according to 2017 data on safe levels of air pollution [5]. Furthermore, the WHO estimates that about a quarter of diseases occurring today can be, in part, due to environmental pollutants. According to the country's GDP and current worldwide trends, Nigeria's grid-based energy consumption is far behind that of other emerging countries, and it ought to be four to five times greater. Additionally, approximately 95 million Nigerians lack access to electricity, and those who do must deal with lengthy power outages or sporadic power supply [11] thus, making it necessary for households and industry to obtain

2. Materials and Methods.

2.1 Animals and Experimental Design

Forty-eight (48) adult male Wistar rats weighing an average of 180 \pm 20g were procured from the vivarium at the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were placed in well-ventilated cages at the Animal House and allowed access to water and a

the majority of their power from privately owned generators. Air pollution's adverse health effects are caused by a variety of intricate mechanisms that are still poorly understood however since the nasal cavity is the primary pathway through which pollutants enter the body, both indoor and outdoor environments that contain mixtures of gases, fine particles, and chemical compounds may also have a negative impact on health. Exhaust tar activity is mostly due to the presence of four, five, and six-ring condensed and other aromatic hydrocarbons, with polycyclic aromatic hydrocarbons (PAHs) and carbon monoxide (CO) being particularly significant due to their respective carcinogenic and acute CO intoxication properties [2]. The principal organ that PM targets is the lung, and its metabolism is comparable to that of other insoluble foreign substances. The macrophages that take up the particles subsequently trigger inflammatory reactions and the production of reactive oxygen species (ROS) [12]. The first substance to interact chemically with inhaled PM component is the pulmonary epithelial lining fluid [13]. Inflammation, fibrosis, and vascular wall disruption are the most prominent injuries to pulmonary tissue, and the lung accurately reflects the severity of induced oxidative stress [14]. In the 1930s, researchers initially looked at the genotoxicity of gasoline engine exhaust [15], and more recent studies employing the Salmonella mutagenicity assay have demonstrated the mutagenicity of PM from the exhaust of gasoline engines [16]. Exposure can result in DNA damage, DNA adducts, amplified micronucleus formation, and promoted chromosome aberration, all of which have been associated with carcinogenesis [17]. Mutagenicity assay is also used as a biomarker for human biomonitoring studies in biological effects as well as dosing of occupational and environmental exposure [18]. Individuals of all ages are susceptible to the influence of the environment on health, with the deterioration of lung structure, function, and responsiveness known to be induced in part by oxidative stress, which is an indisputable factor in these processes. How this stress is induced can be significant in the later development of disease pathogenesis [19]. The genotoxic response to gasoline generator exhaust fumes is still being studied. Against this background, this study was conducted to determine the genotoxic response to gasoline generator exhaust fumes in adult male Wistar rats.

standard rodent diet obtained from Edo Feed Mills, Benin City, and water ad libitum. They were acclimatized for two weeks before commencing the experiment. The International Humane Animal Care Standards were followed when handling the experimental animals [20]. The Biomedical

Research and Ethics Committee of the Ministry of Agriculture, Benin City, Edo State, Nigeria, approved the

2.2 Mode of exposure to generator exhausts

A control group (A) and three test groups, denoted by B, C, and D, respectively, were separated into four groups of adult male Wistar rats. The rats in the control group were not exposed to gasoline generator exhaust while rats in the test groups were exposed at a time point of 2, 4, and 8 hours for 12 weeks. The rats in the test group were briefly exposed in a fabricated glass exposure chamber with an inlet and an

2.3 Grouping and exposure of animals

Group A consisted of 12 adult male Wistar rats that served as the control and were not exposed to gasoline generator exhaust fume.

Group B consisted of 12 adult Wistar rats shared into 3 subgroups of 4 rats that were exposed to gasoline generator exhaust fume for 2, 4, and 8 hours respectively daily for 1 month.

2.4 Tissue Preparation and Histopathological Analysis

The rats were euthanized by cervical dislocation and blood samples were obtained via cardiac puncture for genotoxic assays, while the lungs were excised and immediately fixed in 10% neutral-buffered formalin for 24 hours. The lung tissues were histologically processed as described by Bancroft *et al.* [21]. Briefly, specimens were dehydrated with graded alcohol, infiltrated in two changes of Xylene, and embedded in paraffin wax. Sections of 4 mm thickness were obtained using a rotary

2.5 Genotoxic studies (Comet assay)

The alkaline comet assay was used to assess DNA damage. Blood samples obtained were used for this assay following a modified previously described method by Marino *et al.*, [22]. The lymphocytes were isolated from blood obtained from the rats using Ficoll histopaque 1077 and diluted in a ratio 1:1 Roswell Park Memorial Institute medium (RPMI-1640) and layered over 600 μ l of Ficoll histopaque, spun at 800g for 20 min to isolate the lymphocytes. The buffy coat was aspirated into 3 ml of RPMI, and the lymphocytes were pelleted by centrifuging at 250 g for 10 min and resuspended in 1000 μ l of RPMI and counted using a haemocytometer. About, 100 μ l of the harvested lymphocytes were mixed with 1000 μ l of the media and centrifuged at 3000 rpm for 5 min to pellet the lymphocyte. The pellet was then resuspended in 100 μ l of PBS. Onto the base slide earlier precoated with 1% normal melting agar (NMA), a suspension of 100 μ l of 1% low melting point agar (LMPA) was added to 80 μ l of the suspension layered onto the base slides. For the agarose layer to solidify, the slides were carefully placed on the ice packs. The coverslip was carefully removed after solidification and a third layer of 90 μ l LMPA was applied to the slide. Upon

experimental procedure adopted in this study, issuing it the ethical clearance registration number V.1040/77.

outlet valve with dimensions of (L x W x H) mm 710 x 480 x 370 for 30 seconds before being placed two meters away from the exhaust from a brand-new manual start yellow gasoline generator set (Elepaq Yaofeng constant 1.5KVA model SV2500) with gasoline capacity of 6.0 litres and AC output of 220 V and DC output of 12V/8.

Group C consisted of 12 adult Wistar rats shared into 3 subgroups of 4 rats that were exposed to gasoline generator exhaust fume for 2, 4, and 8 hours respectively daily for 2 months.

Group D consisted of 12 adult Wistar rats distributed into 3 subgroups of 4 rats that were exposed to gasoline generator exhaust fume for 2, 4, and 8 hours respectively daily for 3 months.

microtome (Leica RM2125 RTS, by Leica Biosystems, Buffalo Grove, United States of America) and floated out on a water bath. Sections were picked, labelled, dried on a hot plate, and stained in Hematoxylin and Eosin (H&E) staining solutions. Stained tissues were studied microscopically for histopathological changes using the Olympus CX33 trinocular microscope and photomicrographs were obtained using Kodak PIXPRO A2527 with a megapixel of 20.68MP digital camera with a 1/2.3" BSI CMOS Sensor.

solidification of the third layer, the slides were immersed in cold, freshly prepared lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, 8 gm NaOH, 1% Triton X-100, and 1% DMSO, pH 10) and incubated for four hours and protected from light. Slides were gently removed from the lysing solution following incubation and placed in the electrophoresis chamber (300 mM NaOH / 1 mM EDTA). The slides were incubated in a chilled electrophoresis buffer of pH >13 for 20 min to allow for the unwinding of the DNA and the expression of alkaline-labile damage. Electrophoresis was conducted using the same buffer for 30 min, after which the slides were neutralized with Tris buffer (pH 7.5) for 5 min. After three rounds of neutralisation, the slides were stained with 75 μ l of 20ug/ml ethidium bromide and stored in a dark, humid chamber to prevent the gel from drying up. Stained slides were analysed and scored within 24 hours using a fluorescent microscope. To assess the extent of DNA damage in cells, the olive tail moment (OTM) was measured and images were examined using an image software suite (Komet 5 image analysis software developed by Kinetic Imaging, Limited, Liverpool, UK).

2.6 Statistical analysis

The data were presented as the mean and standard deviation of the OTMs. Each experiment was performed in triplicate slides. Using one-way analysis of variance (ANOVA) and the Statistical Package for Social Science (SPSS), Version 25, the effects of gasoline generator exhaust at different time points of 2 hours, 4 hours, and 8 hours of exposure in the test group at 12 weeks were compared with

the unexposed control groups. Additionally, the control groups' mean values were statistically compared to those of the OTMs at the different time points of exposure, at 4 weeks, 8 weeks, and 12 weeks. The least significant difference test was used to assess variations from the mean values with p-values <0.05 considered statistically significant.

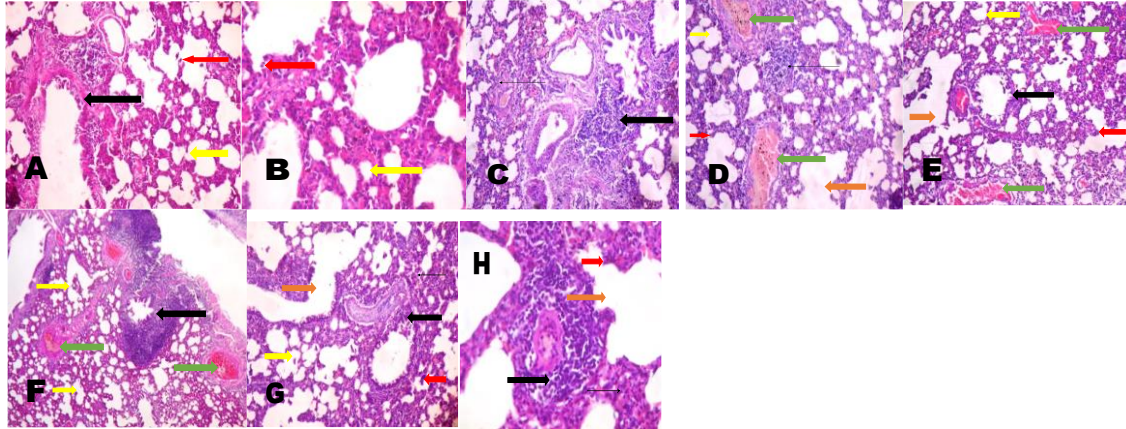


Figure 1. H and E-stained section of rat lungs at 4 weeks. (A) The unexposed control group showed normal bronchiole with no lymphocytic follicles (black arrow), the alveoli sacs are devoid of inflammation, (yellow arrow) and the alveoli epithelium appears normal (red arrow) (H&E x 100). (B) Higher magnification of the control rats' lungs showed a normal alveoli epithelium (red arrow), while the alveoli sacs appeared normal (yellow arrow) (H&E x 400). (C) Rats exposed at 2 hours daily for 4 weeks had mild lymphocytic proliferation within the bronchiole (black arrow), coupled with mildly congested and inflamed intra alveolar spaces (slender arrow) (H&E x 100). (D) Moderate vascular congestion (green arrow) with mild inflammation within the intra alveoli spaces is evident (slender arrow), while the alveoli ducts (orange arrow), alveoli sacs (yellow arrow), and alveoli epithelium (red arrow) are normal in the rats exposed for 2 hours daily at 4 weeks (H&E x 100). (E) Mild lymphocytic follicles in the bronchiole (black arrow), coupled with mild vascular congestion (green arrow) were evident in rats exposed for 4 hours daily at 4 weeks while the alveoli sacs (yellow arrow), alveoli ducts (orange arrow), and alveoli epithelium (red arrow) appear normal (H&E x 100). (F) Also, there is an observable peribronchiolar infiltration by inflammatory cells (black arrow) with mild vascular congestion (green arrows), and the alveoli sacs (yellow arrows) appear normal in the lungs of rats exposed at 4 hours daily for 4 weeks (H&E x 100). (G) Exposure for 8 hours daily at 4 weeks revealed that intra alveolar spaces were mildly inflamed (slender arrow), there are mild lymphocytic follicles in the bronchiole (black arrow), the alveoli sac (yellow arrow), alveoli duct (orange arrow) and alveoli epithelium (red arrow) appear normal (H&E x 100). (H) Exposure for 8 hours daily at 4 weeks revealed the presence of lymphocytic aggregates (black arrow) with mildly inflamed intra alveolar spaces (slender arrow), the alveoli duct (orange arrow), and alveoli epithelium (red arrow) appears normal (H&E x 400).

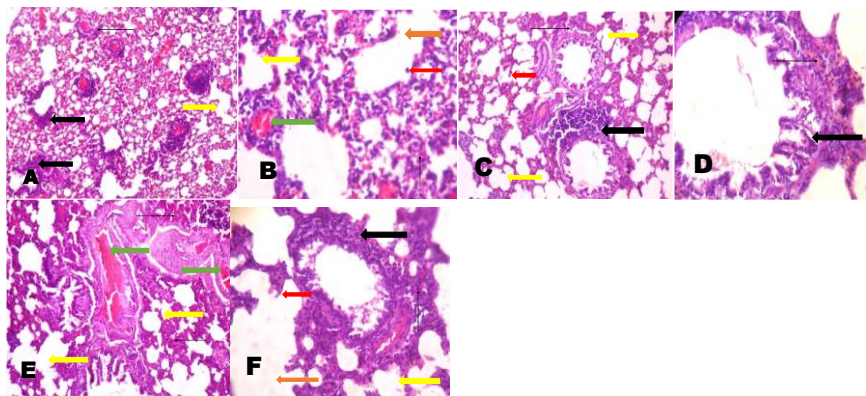


Figure 2. H and E-stained section of rat lungs at 8 weeks (A) Rats exposed for 2 hours daily at 8 weeks reveal a mildly inflamed bronchiole (black arrow), the intra alveolar spaces are mildly infiltrated by inflammatory cells (slender arrow), and the alveoli spaces (yellow arrow) appear normal (H&E x 100). (B) Higher magnification at a time point of exposure of 2 hours daily at 8 weeks revealed a moderate perivascular infiltration of inflammatory cells coupled with vascular congestion (green arrow), the intravascular space is mildly inflamed (slender arrow) while the alveoli duct (orange arrow) and alveoli sacs (yellow arrow) appear normal (H&E x 400). (C) Exposure for 4 hours daily at 8 weeks showed a peri bronchiolar inflammation (black arrow), with moderately inflamed intra alveolar spaces (slender arrow). The alveoli sacs (yellow arrow) and alveoli epithelium (red arrow) appear normal (H&E x 100). (D) Higher magnification in the rats exposed for 4 hours daily at 8 weeks revealed the presence of lymphocytic follicles within the bronchiole (black arrow), also peri bronchial inflammation was evident (slender arrow) (H&E x 400). (E) The lungs of rats exposed for 8 hours daily at 8 weeks revealed a mildly congested thick-walled vessel (green arrow), the intra alveoli spaces are mildly infiltrated (slender arrow) while the alveoli sacs appear normal (yellow arrow) (H&E x

100). (F) A higher magnification at 8 hours daily at 8 weeks shows a moderate peribronchiolar lymphocytic infiltration (black arrow) coupled with moderate inflammation in the alveolar spaces (slender arrow), the alveoli duct (orange arrow), and alveoli sacs (yellow arrow) appear normal (H&E x 400).

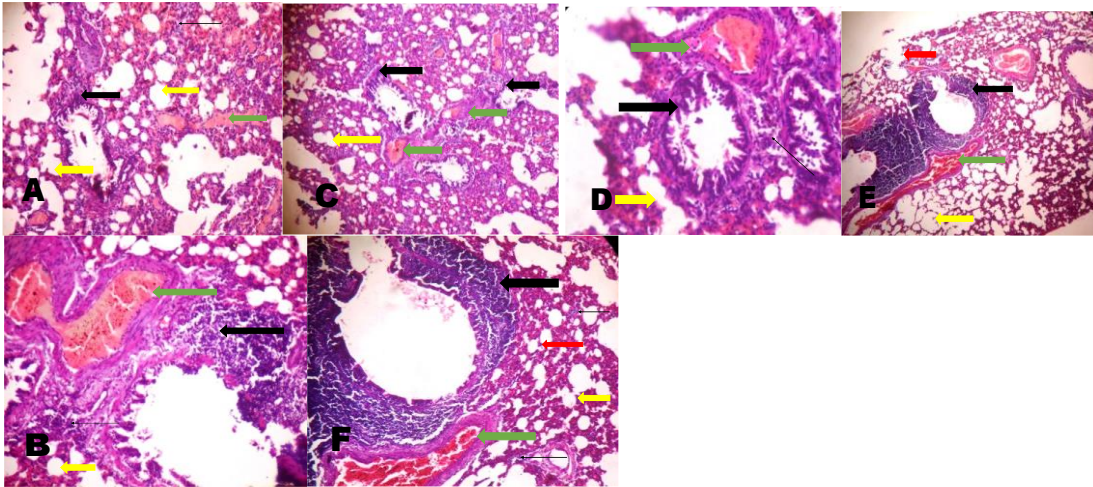


Figure 3. H and E-stained section of rat lungs at 12 weeks. (A) Exposure for 2 hours daily at 12 weeks showed the bronchiole with lymphocytic follicles (black arrow), with the intra alveolar spaces moderately inflamed (slender arrow), and congested (green arrow) with some of the alveoli sacs appearing normal (yellow arrow) (H&E x 100). (B) Higher magnification in rats exposed for 2 hours daily at 12 weeks showed severe vascular congestion (green arrow) coupled with peri bronchiolar infiltration of inflammatory cells (black arrow), the intra alveolar spaces are moderately inflamed (slender arrow) while the alveolar sacs appear normal (yellow arrow) (H&E x 400). (C) Exposure for 4 hours daily at 12 weeks showed moderate bronchiole inflammation (black arrow), with some regions of the intra alveolar spaces mildly inflamed and congested (green arrow) with the alveolar sacs appearing normal (yellow arrow) (H&E x 100). (D) Higher magnification in rats exposed for 4 hours daily at 12 weeks shows the presence of lymphocytic follicles in the bronchiole (black arrow), the intra alveolar spaces are moderately infiltrated by inflammatory cells (slender arrow), and severe vascular congestion is seen (green arrow) while alveolar sacs appear normal (yellow arrow) (H&E x 400). (E) Exposure for 8 hours daily at 12 weeks shows moderate lymphocytic aggregation (black arrow) and severely congested and thickened blood vessel walls (green arrow), the alveoli sacs (yellow arrow) and the alveoli epithelium (red arrow) appear normal (H&E x100). (F) The intra alveolar spaces are moderately infiltrated by inflammatory cells (slender arrow), also observed are congested blood vessels with thickened walls (green arrow), the alveoli sacs (yellow arrow) and the alveoli epithelium (red arrow) appear normal in the rats exposed for 8 hours daily at 12 weeks (H&E x 100).

3. Results

3.1 Light microscopic result (H&E-stained section)

Histopathological evaluation in this study revealed that the lungs of the unexposed control rats were devoid of pathological lesions (Figure 1a, b) while the rats exposed for 2 hours daily at 4 weeks revealed the bronchiole consisting of lymphocytic proliferation with mildly inflamed and moderately congested intra alveoli spaces (Figure 1 c, d). The lung of rats exposed for 4 hours daily at 4 weeks was consistent with the rats exposed for 2 hours daily during the same week (Figure 1e, f), while rats exposed for 8 hours daily at 4 weeks revealed mild lymphocytic follicles in the bronchioles coupled with inflamed intra alveolar spaces (Figure 1 g, h). Exposure for 2 hours daily at 8 weeks revealed a mildly inflamed bronchiole and intra alveolar spaces, additionally, a moderate perivascular inflammation coupled with vascular congestion was also evident (Figure 2 a, b). A peribronchiolar and perivascular inflammation

coupled with moderately infiltrated intra-alveolar spaces were observed in rats exposed for 4 hours daily at 8 weeks (Figure 2 c, d). A thick-walled vessel coupled with mild congestion and inflamed intra-alveolar spaces was observed in rats exposed for 8 hours daily at 8 weeks (Figure 2 e,f). Peribronchiolar inflammation, congested and inflamed interalveolar spaces were observed in rats exposed for 2 hours daily at 12 weeks (Figure 3a, b). Furthermore, at 12 weeks, rats exposed for 4 hours daily had a mildly inflamed bronchiole with lymphocytic follicles present. There was considerable inflammation and significant congestion in the interalveolar regions (Figure 3 c, d). Rats exposed for 8 hours daily for 12 weeks had highly inflammatory intra-alveolar spaces, moderate lymphocytic aggregation, and severely congested blood vessels with thickened walls (Figure 3 e, f).

3.2 Genotoxic studies (Comet assay)

Generally, our study showed that DNA damage in Wistar rats significantly increases with respect to the duration of exposure when compared with the control. The elevated OTM values in the exposed rats indicate that chemical damage had occurred. In addition to this,

comparison within the exposed group at the time point of 2 hours of exposure was less significant when compared with the 8-hour time point exposure. In this study, rats exposed to gasoline exhaust fume for 4 and 8 hours at 4 weeks revealed significant genotoxicity ($P < 0.05$). Furthermore, the rats

exposed for 2 and 4 hours did not show significant variation in the OTMs when compared to the control. However, a significant variation was observed in the 8 hours of exposure at 4 weeks ($P<0.05$) (Figure 4a; Table 1). The OTMs of the exposed rats at a time point of 2, 4, and 8 hours respectively revealed a significant DNA fragmentation when compared with the control at 8 weeks (Figure 4b). In addition, significant variations in OTMs were observed across all the time points of exposure at 8 weeks ($P<0.05$) (Figure 4b; Table 2). Exposure at time point 8 hours for 12 weeks

showed significant variation in the OTMs when compared with the control ($P<0.05$) (Figure 4c; Table 3). Generally, DNA damage was pronounced in the groups exposed at a time point of 8 hours across the different weeks of exposure ($P<0.05$) compared to other time points of exposure at 2-hour and 4-hour time points. In addition, the time point of exposure significantly led to an increase in OTMs when compared with the control (Figure 4d).

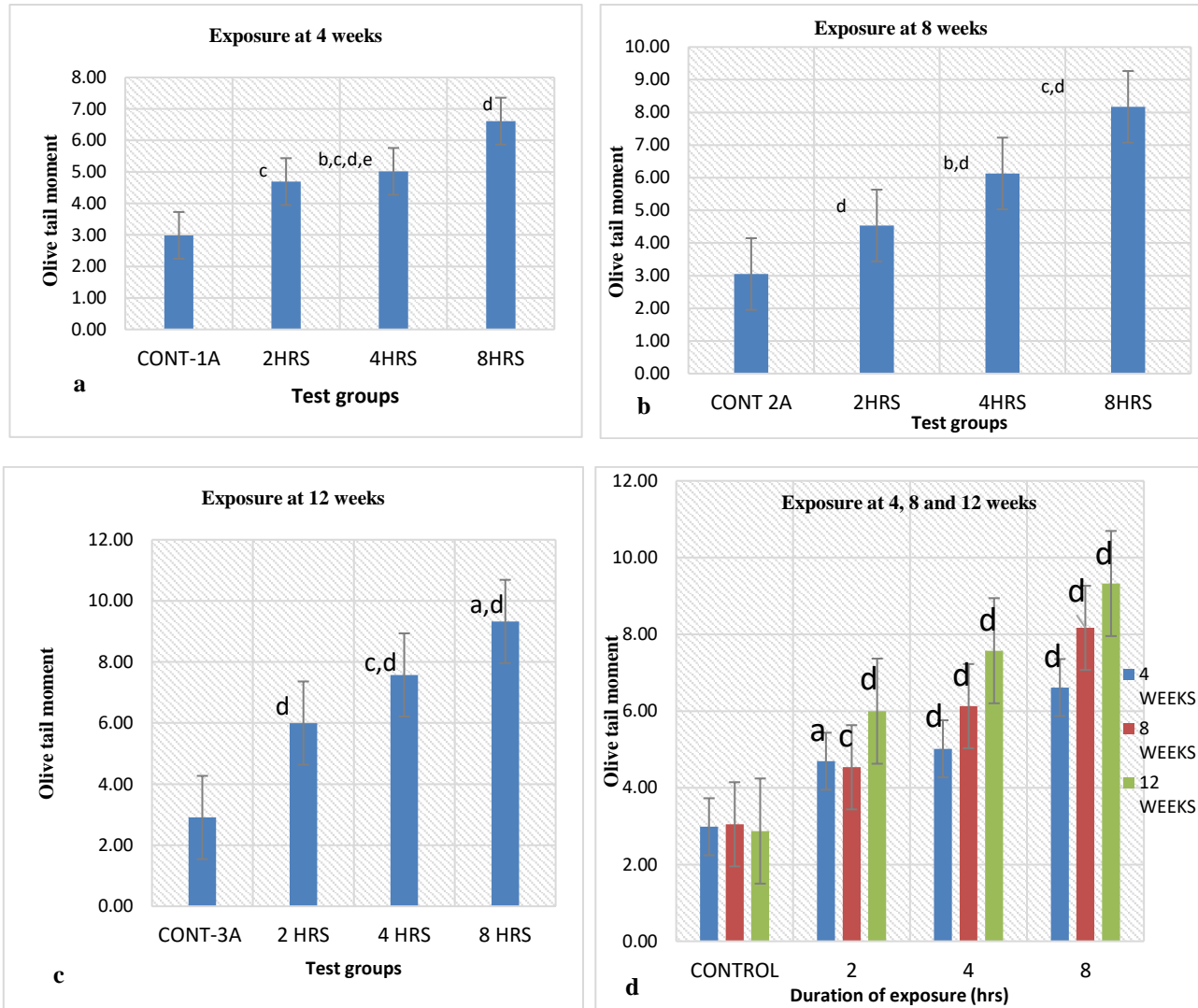


Figure 4. Comparative analysis of the impact of gasoline exhaust fume in Wistar rats across the weeks (4,8, 12 weeks). **Narration:** (a) The OTM analysis in the rats exposed daily at time points of 2, 4, or 8 hours increased significantly ($P<0.05$) in comparison to the unexposed control group after 4 weeks. Graphs show a significant increase in DNA damage between the experimental and the control group (a= *(0.0118); b= *(0.0041), c= *(0.006); d= *(0.0001); e= not significant (0.05). (b) Comparison of the impact of gasoline exhaust in exposed Wistar rats at 2, 4, and 8 hours at 8 weeks shows that inhalation of gasoline generator exhaust fume for 8 weeks damages DNA as detected via OTM analysis in the Wistar rats' blood increased significantly ($P<0.05$), in relation to the unexposed control rats with the figure showing a significant increase in DNA damage between the experimental and the control group. (c) Exposure to gasoline generator exhaust for 12 weeks damages DNA, as indicated by elevated OTM levels in the blood of the exposed Wistar rats at time points of 2, 4, and 8 hours respectively ($P<0.05$), when compared to the unexposed control rats (d) The OTM values in the Wistar rats increased significantly ($P<0.05$), in an exposure-dependent manner, throughout 4, 8, and 12 weeks as a result of the chronic genotoxic effect of inhaled generator exhausts at different time points of exposure (2 hours, 4 hours, or 8 hours). The difference in DNA damage between the experimental and control groups is seen in the figure.

Table 1. Mean and Standard Deviation of the Olive tail moment across the duration of exposure at 4 weeks.

CONTROL MEAN	2HOURS MEAN	4HOURS MEAN	8HOURS MEAN
2.99±1.17	4.18±1.47	5.39±2.03	6.45±3.11
2.76±1.01	4.34±1.67	5.17±1.69	6.46±2.45
3.12±1.01	4.11±1.51	4.99±2.28	6.19±2.21
2.89±1.032	3.78±1.70	4.99±1.65	7.07±2.58
3.14±1.056	4.13±1.59	4.95±1.82	7.46±2.76
3.11±1.10	4.32±1.70	4.69±2.22	6.35±2.55
3.03±0.99	4.12±1.78	5.01±1.78	6.87±2.95
2.86±0.72	3.60±1.18	4.88±1.71	6.69±3.18
2.93±0.94	3.66±1.45	5.05±2.71	5.92±3.49
Average mean 2.99	4.69	5.02	6.61

Values were expressed as mean value ± SD. Mean values were significantly different compared to the unexposed control group at P<0.05.

Table 2. Mean and Standard Deviation of the Olive tail moment across the duration of exposure at 8 weeks.

CONTROL MEAN	2HOURS MEAN	4HOURS MEAN	8HOURS MEAN
2.84±1.11	4.46±1.33	5.85±1.64	8.61±2.27
2.68±0.87	4.96±1.34	5.63±1.39	8.57±2.51
2.93±1.03	4.51±1.20	5.83±1.35	8.54±3.08
3.14±1.15	4.39±1.28	6.29±1.41	7.61±2.49
3.29±1.01	4.52±1.38	5.96±1.27	7.96±2.37
3.20±1.06	4.54±1.30	6.14±2.28	7.84±2.71
3.20±0.97	4.18±1.29	6.24±1.97	8.24±3.61
3.27±1.10	4.76±1.25	6.48±2.63	8.38±3.89
2.85±1.00	4.46±1.20	6.70±2.67	7.71±3.58
Average mean 3.05	4.54	6.13	8.17

Table 3. Mean and Standard Deviation of the Olive tail moment across the duration of exposure at 12 weeks.

CONTROL MEAN	2HOURS MEAN	4HOURS MEAN	8HOURS MEAN
2.81±0.94	6.18±1.58	7.77±2.42	9.15±2.81
2.82±1.02	6.67±2.02	8.27±2.60	9.24±2.66
2.87±0.86	5.98±1.53	7.36±2.09	8.65±2.50
2.78±0.95	5.72±1.47	8.06±2.37	9.11±2.93
2.93±1.04	6.03±1.37	8.07±2.17	9.69±3.13
2.82±0.99	5.94±1.63	7.26±1.77	9.64±3.28
3.01±0.97	5.38±1.24	6.91±1.92	9.53±3.08
2.94±0.93	6.00±1.54	7.10±2.08	9.97±3.14
3.14±0.93	6.02±1.82	7.31±2.26	8.89±2.19

Average mean

2.91 6.00 7.57 9.32

Values were expressed as mean value ± SD. Mean values were significantly different compared to the unexposed control group at P<0.05.

Table 4. Combined Mean and Standard Deviation of the Olive tail moment across the duration of exposure for 4, 8, and 12 weeks.

	CONTROL MEAN	2HOURS MEAN	4HOURS MEAN	8HOURS MEAN
4 WEEKS	2.98±1.00	4.03±1.56	5.01±1.99	6.61±2.81
8 WEEKS	3.04±1.03	4.53±1.29	6.12±1.85	8.16±2.94
12 WEEKS	2.90±0.96	5.99±1.58	7.57±2.19	9.32±2.86
Average mean				
4 WEEKS	2.99	4.69	5.02	6.61
8 WEEKS	3.05	4.54	6.13	8.17
12 WEEKS	2.87	6.00	7.57	9.32

Values were expressed as mean value ± SD. Mean values were significantly different compared to the unexposed control group at P<0.05 across the weeks of exposure.

4. Discussion

The large number of respiratory problems is greatly influenced by environmental factors. Environmental factors affect the development of asthma, chronic obstructive pulmonary disease (COPD), lung cancer, and several interstitial lung diseases [24, 25]. Chronic exposure to environmental toxicants such as ozone (O₃) and nitrogen dioxide (NO₂) has been linked to several respiratory mortalities [25]. The effect of gasoline engine emissions on pulmonary dysfunction is less studied than that of diesel engine emissions [26], and particles' potential to cause cancer in the lung under varied conditions may be explained by several genotoxic modes of action. The ability of the PAH produced from gasoline engines to interact with PM, which are carcinogens with the capacity to induce DNA damage, is consistent with the interaction of mutagenic and carcinogenic chemicals with DNA [17]. To our knowledge, there is a dearth of information on gasoline generator exhaust fumes in the lungs of exposed wistar rats. The persistent inflammation observed across the varying time points of exposure as well as duration indicated that consistent exposure to gasoline generator exhaust remains unlimiting and chronic exposure could trigger the development of a more severe lung reaction and damage. Findings in this study at four weeks revealed an inflammatory response with the presence of inflammatory cells within the interalveolar spaces, coupled with mild lymphocytic follicles in the bronchioles and vascular congestion. This is in tandem with Temneanu *et al.*, [14] who reported the effect of pulmonary oxidative stress in rats to include inflammation, thickening of alveolar walls, and fibrosis; however, the latter was not evident in this study after 12 weeks of exposure. Histopathological and ultrastructural alterations which include the detachment and necrosis of epithelial cells, as well as clogging of the bronchioles with neoplastic cells in rats exposed to gasoline vehicle exhaust for 30 minutes daily for six consecutive weeks with an evident accumulation of inflammatory cells, fibrosis, and congestion of blood vessels, has been documented by Ezzat [27]. The biological pathways linking PM-induced oxidative stress have been reported by Corsini *et al.*, [28]. PM depletes antioxidants, resulting in the production of ROS, which is linked to oxidative damage. Activating upstream stress-related mitogen-activated protein kinases (p38, JNK) and redox-sensitive transcription factors (NF-B and AP-1), causes the lung cells to express proinflammatory cytokines and promote inflammation. The alteration in the cellular redox reaction coupled with the genotoxic substance present in the PM can cause DNA damage [28]. This study highlights the risks associated with exposure to gasoline generator exhaust with the sections of the lungs of the exposed rats revealing varying cytopathic effects ranging from inflammation to vascular congestion at various time points of exposure at 12 weeks. Our findings indicate that people who are frequently exposed to gasoline

engine exhaust and live in polluted locations run a higher chance of contracting a wide range of illnesses ranging from chronic metabolic disorders to various forms of malignancy. Studies have linked exposure to PM with various diseases ranging from cardiovascular morbidity Miller *et al.*, [8]; Manisalidis *et al.*, [29] asthma, chronic respiratory disease Choi *et al.*, [24] altered lung function Owumi and Oladimeji [30], lung cancer Lewtas, [31], neuroinflammation, and central nervous diseases Calderón-Garcidueñas *et al.* [32]. The severity of cytopathic features observed in this study was increased with respect to the duration of exposure. At 4 weeks after the varied time points of exposure, the bronchioles and interalveolar spaces had mild lymphocyte infiltration along with apparent moderate vascular congestion. This finding agrees with Arias-Pérez [33] who indicated that PM can penetrate the airways and trigger allergic and inflammatory responses with ultrafine particulate matter entering the terminal bronchioles and alveoli to enter the bloodstream and affect other organs.

Exposure at different time points at 8 weeks also revealed features such as perivascular infiltration with mildly infiltrated interalveolar spaces. Additionally, exposed rats showed bronchioles with mild lymphocytic follicles along with mild vascular congestion. Lecureur *et al.*, [34] also indicated that rats subjected to diesel emissions-derived PM experienced histological alterations in their airways that ranged from mild to moderate inflammation. The role of alveolar macrophages (M1 and M2) in the pathogenesis of pulmonary inflammation has been suggested by Koltermann-Jully *et al.*, [35]. The induction of oxidative stress and inflammation, impairment of phagocytosis, dysregulation of cell immunity, epigenetic modification, and disruption of the cellular signalling pathway are all components of the process by which environmental toxicant (PM_{2.5}) causes lung injury [36]. Histopathological studies of the lungs of the exposed rats at a time point of 4 hours and 8 hours at 8 weeks showed infiltration of the interalveolar spaces by inflammatory cells with the bronchiole showing a mild peri lymphocytic infiltration at a time point of 8 hours. A more severe response was seen at 12 weeks with the bronchiole revealing a peri lymphocytic inflammation and vascular congestion. The inflammatory reactions at the time point of exposure of 4 hours and 8 hours were also consistent with the pathological features earlier observed in weeks 4 and 8. However, thickened walled vessels with congestion of blood vessels were pronounced at a time point exposure of 8 hours at 12 weeks. Pulmonary inflammatory mediators spurred on by PM can transverse the bloodstream and induce oxidative stress, systemic inflammation, and damage to distant organs [7]. The activation of the inflammasome complex, necrosis through the activation of PARP-1 and proteases (calpains), interference with mitochondrial function, ensuring apoptosis by pathways (intrinsic and extrinsic), leading to the activation of caspase-3, and

autophagy induced by stress signals that inhibit mTOR signalling lead to the formation of the autophagosome are all possible outcomes of PM exposure-induced inflammation [33]. According to Homaei and Hadizadeh, [37] air pollution has been linked to the development of invasive breast cancer in premenopausal and postmenopausal women. Additionally, anthropogenic ozone causes about 493,000 deaths annually, while premature mortality from COPD, stroke, ischemic heart disease, and lung cancer accounts for about 675,000 of these deaths [10]. Additionally, Kasai *et al.*, [38] indicated that multiwalled carbon nanotube which has important industrial use induces lung cancer in adult rats. Acute heart attacks [7], chronic lung development deficiencies in children between the ages of 10 and 18 years old [39], and ovarian cancer [40] are a few of the negative impacts of exhaust pollution that have been documented with reports by Dehghani *et al.*, [41] revealing a correlation between the variation of pollutant concentration in air to lungs and blood cancer mortality with a significant correlation of CO and NO_x in the air. Particles can induce genotoxicity by transferring lung cancer-causing substances that have adhered to their surfaces [42]. In this investigation, persistent inflammation observed in the exposed rats may have contributed to the reported genotoxicity. Owagboriaye *et al.* [43] had previously described the genotoxic effects of gasoline exhausts in rats, and Choi *et al.* [24] suggested PM 2.5's ability to trigger oxidative damage and trigger an inflammatory response, which has been shown to increase the risk of diseases like asthma, chronic respiratory disease, diabetes mellitus, cardiovascular diseases, and immunological disorders. An altered cellular redox reaction and the genotoxic substance contained in the particulate matter can both lead to DNA damage [28]. According to a previous summary by Knaapen *et al.* [42] on potential genotoxic mechanisms of micro and nanoscale particles *in vivo*, these mechanisms may involve both directly particle-related (primary) genotoxic mechanisms as well as indirect (secondary) mechanisms that are driven by inflammation and/or phagocytosis [44]. In this study, we found that prolonged exposure to gasoline generators' exhaust causes DNA damage since significant DNA fragmentation was observed with increasing exposure time points and duration, supporting reports by Bisig *et al.* [45], who observed that exposure to gasoline exhaust induces oxidative stress and that the condensate from gasoline combustion exhaust may be mutagenic [46]. The findings in this study revealed that the OTM levels in the exposed rats varied significantly over time relative to the unexposed control rats. Furthermore, when compared to both the unexposed control group and other exposed groups, the extent of DNA damage was pronounced in the rats exposed for 8 hours at 12 weeks. The mechanism for particle-induced carcinogenesis has been

5. Conclusions

Exposure to gasoline generator exhaust induces genotoxic damage and alters the histoarchitecture of the lungs of exposed wistar rats. It is pertinent that regulations be put in place to decrease the use of fossil fuels in generating electricity and to increase the production of

power from non-fossil fuels. Further research, such as transcriptomic analysis of Rats' RNA to study the gene expression pattern to better understand how gasoline generator exhaust regulates the expression of selected genes, is encouraged.

reported by Knaapen *et al.* [42], and the severe damage seen at 12 weeks suggests that chronic exposure to gasoline generator exhaust causes oxidative stress, incites inflammatory responses, and damages the DNA of exposed rats. This suggests that chronic exposure in humans can result in an incorrect repair, which can result in mutagenesis, which leads to cancer. This further buttresses the reports of Møller *et al.*, [47] who suggested that PM components can damage human and animal biomolecules and cause diseases like cancer. In animal studies, particulate matter was linked to higher levels of oxidised guanines in different tissues, including the lung, and chronic exposure was said to impair the DNA repair system. Air pollution's detrimental effects at the molecular level, coupled with damage to cellular macromolecules that result in DNA or protein adducts, as well as strand breaks and rearrangements of nucleic acids, may predispose to mutations that might initiate carcinogenesis [48]. In this study, the degree of DNA damage significantly increased with exposure time and durations, with statistically significant DNA damage at various time points of exposure at 2 hours, 4 hours, and 8 hours at 4 weeks and 8 weeks as compared to the unexposed control rats. The ability of particulate matter to translocate the alveolar barriers into the circulatory system is one of the proposed mechanisms. This would result in the production of pulmonary inflammatory cytokines and other mediators that could negatively impact the cardiovascular system as well as other bodily systems [49]. Additionally, vascular toxicity may be indicated by a combustion emission component [50]. Inhaling petroleum hydrocarbons has been linked by Azeez *et al.* [51] to the development of oxidative stress, which may be a risk factor in the pathogenesis of pulmonary dysfunction. Hallare [52] also suggested that workers at petrol stations and law enforcement agencies are more likely to suffer chromosomal damage due to exposure to potential genotoxic gaseous and particulate pollutants from internal combustion engines utilised in cars. According to Azqueta and Dusinska, [9], the genotoxic potential of nanomaterials could cause pre-mutagenic lesions to develop into mutation and possibly cancer by depleting antioxidant materials, the antioxidant defence system, or altering the DNA repair mechanism. The primary objective of the genotoxic analysis is to ascertain whether variations in exposure period to gasoline generator exhaust have a substantial impact on the OTMs. This current study revealed that damage to DNA increases significantly in an exposure-dependent manner across the various time points as well as duration with more severe damage to the DNA observed at a time point of 8 hours at 12 weeks. Also, histopathological assessment of the lungs of the rats revealed various cytopathic features ranging from the thickening of vessels and pulmonary inflammation and congestion.

Conflict of interest

The authors declare no conflict of interest.

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