



Original article

Assessment of Cardiac Histology and Hematological Parameters in Adult Male Wistar Rats Exposed to Exhaust Emissions from Gasoline Generators.

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ABSTRACT

Background and Aim: In Nigeria, the lack of a reliable supply of electric power has necessitated the use of gasoline-powered generators to generate electricity. This study investigated the effect of gasoline generator exhaust exposure on the haematological parameters and cardiac histoarchitecture of adult wistar rats. **Materials and Methods:** Adult male wistar rats subdivided into four groups were exposed to exhaust emissions at time points of 2, 4, and 8 hours for 4, 8, and 12 weeks, respectively. The rats in Group I were the unexposed control rats, while Groups II-IV were the test groups exposed at time points of 2, 4, and 8 hours over a period of 4, 8, and 12 weeks, with haematological and cardiac histopathology performed at the end of the experiment. **Results:** The results showed that exposed rats had elevated white and red blood cell counts compared to the control, while platelet counts were significantly lower (p -value <0.05), with cardiac histology revealing degenerative myocardial lesions such as inflammatory responses, hemorrhagic intermyocytic spaces, vascular congestion, and fibrosis at various exposure time points. **Conclusion:** Exposure to gasoline generator exhaust emissions adversely impacts the cardiac tissues, with the most pronounced deleterious effect observed at 12 weeks.

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1. Introduction

Exposure to combustion particles may increase the risk of cardiovascular disease (CVD), including atherosclerosis, hypertension, thrombosis, and myocardial infarction [1]. Additionally, the generation of reactive oxygen species (ROS) as a result of particulate matter (PM) components of polluted air plays a role in the development or aggravation of CVD caused by exposure to combustion particles and polycyclic aromatic hydrocarbons (PAHs) [2]. Fossil fuel combustion is the largest driver of air pollution, greenhouse gas emissions, and climate change [3] and remains a primary contributor of nitrogen oxide (NOx) [4]. The epileptic power supply in Nigeria has led to the generation of electricity through the use of gasoline generators. Despite the global transition to cleaner energy sources and less environmental pollution, the rate of gasoline-powered generator importation keeps rising due to the instability of the main power grid [5,6]. The emissions from gasoline generator engines are extremely high and exceed the allowable limits for PM₁, PM_{2.5}, and PM₁₀ specified by the US Environmental Protection Agency and the World Health Organization, exposing humans to health hazards [7]. Despite concerted efforts to develop greener and more cost-effective energy sources to replace petroleum, petroleum utilization keeps increasing in both developed and developing countries [8]. Studies have indicated that petroleum

fumes lower haematological parameters and these effects worsen with prolonged exposure [9-12]. The cytopathic and haemotoxic effect of gasoline generator exhaust is still been studied. Against this background, we aim to investigate the haematological and histological effects of gasoline generator exhaust exposure.

MATERIALS AND METHOD

Animal husbandry

Adult male wistar rats weighing an average of 180g were obtained from the University of Benin's animal holding facility in Benin City, Nigeria. Before commencing the experiments, the rats were acclimatized for two weeks. The International Humane Animal Care Standards were followed when handling experimental animals.

Ethical considerations

The Biomedical Research and Ethics Committee of the Ministry of Agriculture, Benin City, Edo State, Nigeria, approved the experimental protocol used in this study and granted it an ethical clearance registration number V.1040/77.

Exposure technique to generator exhausts

A control group (A) and three test groups, labelled B, C, and D, were split into four groups of mature male wistar rats. Rats in the test groups were exposed to gasoline generator exhaust for 12 weeks at time intervals of 2 hours, 4 hours, and 8 hours respectively, while rats in the control group were not exposed at all. The exposure method was adapted from a study by Henz & Maeder. Experimental animals were placed two meters away from an AC output of 220 volts, and a DC output of 12 volts/8.

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from the exhaust of a brand-new gasoline generator set (Elepaq Yaofeng constant 1.5KVA model SV2500) with a dimension of (L x W x H) mm 460 x 380 x 330, and a noise level of 7 meters 66 dB, fuel capacity of 6.0 lit

Grouping and animal exposure

Four adult male Wistar rats in Group A served as controls; they were not exposed to gasoline generator emissions and were euthanised after the experiments.

Group B comprised 12 adult Wistar rats divided into three subgroups of four rats each, who were exposed to gasoline generator exhaust for 2, 4, and 8 hours daily for one month before being euthanised.

Twelve adult Wistar rats from Group C were divided into three groups of four and exposed to gasoline generator exhaust fumes for 2 hours, 4 hours, and 8 hours daily for two months before being euthanized.

Twelve adult Wistar rats from Group D were divided into three groups of four and exposed to gasoline generator fumes for 2 hours, 4 hours, and 8 hours daily for three months before being euthanized.

Tissue Preparation and Histopathological Analysis

The hearts of rats euthanized by cervical dislocation were removed and immediately placed in 10% neutral-buffered formalin for 24 hours, while blood samples were collected through cardiac puncture and put into an ethylenediaminetetraacetic (EDTA) anticoagulant bottle for haematological analysis. The tissues were histologically processed as described by Bancroft et al. while the Hematoxylin and Eosin (H and E) stained sections were examined for histopathological changes using an Olympus CX33 trinocular microscope, and photomicrographs were obtained with a Kodak PIXPRO A2527.

Haematological analysis

At 12 weeks, the haematological parameters were evaluated using an automated Sysmex XN-350 five-part differential haematology analyzer. Additionally, the red blood cell count (RBC), haematocrit (HCT), haemoglobin (Hb), platelet levels, and red blood cell indices such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also measured.

Statistical analysis.

The Statistical Package for Social Science (SPSS, Cary, NC, USA). Version 25 was used to perform one-way analysis of variance on the acquired haematological data. A student t-test was used to compare the effects of exposure at different time points at 12 weeks. All quantitative data were reported as mean standard deviation (mean SD). The data from the various exposure periods were statistically compared to the unexposed control, and differences in mean values were evaluated using the least significant difference test, with p-values < 0.05 considered statistically significant.

RESULTS

Histopathological findings

Histoarchitectural evaluation of the cardiac tissues across the exposed groups at varying time points revealed cytopathic features ranging from congestion and inflammation evident by the presence of mononuclear inflammatory infiltrate in the exposed rats. The cardiac histology of the control rats was devoid of pathological lesions. However; 2 hours of daily exposure for 4 weeks revealed a

haemorrhagic intermyocytic space while 4 hours of daily exposure showed vascular congestion within the epicardial and myocardial layers, as well as mild fibrosis in the myocardium. Daily exposure for 8 hours at 4 weeks revealed congested epicardial and myocardial layers (Figure 1) while exposure at 8 weeks resulted in myocardial congestion and inflammatory response evident by the presence of infiltrating inflammatory cells at the 2-hour exposure period. The features in the 2-hour time point at 8 weeks were consistent with the 4-hour exposed group while exposure at 8 hours revealed a severely congested myocardial layer (Figure 2). The 12-week exposure revealed varying cytopathic features across the various time points of exposure. While the rats exposed for 2 hours daily had congested myocardium, exposure at 4-hour time points revealed a severely congested myocardium with a focal area of inflammation. The features exhibited in the rats exposed for 8 hours were consistent with the 4-hour exposed group at 12 weeks (Figure 3).

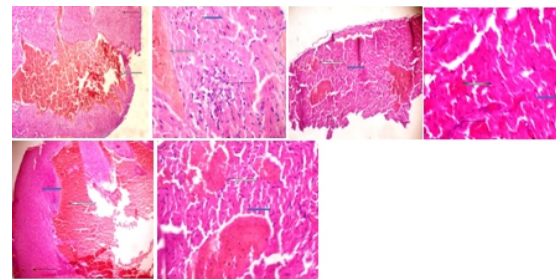


Figure 1. H and E-stained section of rat cardiac tissue a) Normal epicardial layer (white arrow) and myocardial layer (black arrow) of the unexposed control rats (mag=x100). b) In the unexposed control, higher magnification revealed a normal epicardium (white arrow) and myocardium consisting of central nuclei with perinuclear cytoplasm (black arrow). (mag=x400). c) A normal myocardium (black arrow) and epicardium (white arrow) are seen in rats exposed for 2 hours daily for 4 weeks. The inter myocytic spaces appear haemorrhagic (slender arrow) (mag=x100). d) Higher magnification reveals the epicardial layer (white arrow), myocardium with perinuclear cytoplasm and central nuclei (black arrow), haemorrhagic intermyocytic spaces (slender arrow) is evident (mag=x400). e) The epicardial layer of the rats exposed for 4 hours daily for 4 weeks, shows vascular congestion (white arrow), and some areas of the myocardium are congested (yellow arrow) while some regions also exhibit mild fibrosis (black arrow) (mag=x100). f) A higher magnification reveals the epicardial layer with vascular congestion (white arrow), the myocardium consisting of central nuclei with perinuclear cytoplasm (black arrow) appear congested in selected regions (yellow arrow) while some areas appear mildly fibrosed (black arrow) (mag=X400). g) 4 weeks of exposure for 8 hours daily resulted in a congested epicardial layer (white arrow) and a moderately congested myocardial layer (black arrow) (mag=X100). h) A moderately congested myocardial layer (white arrow) and myocytes (blue arrow) (mag=x400).

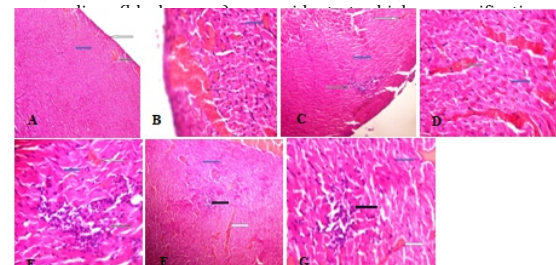


Figure 2. H and E-stained section of rat cardiac tissue exposed daily at 8 weeks a) Exposure for 2 hours daily for 8 weeks resulted in congestion in the myocardial layer (white arrow), some regions of the myocardium are mildly infiltrated by mononuclear cells (slender arrow) (mag=X100). b) Higher magnification revealed the congested myocardium (white arrow), with a mild mononuclear infiltration (slender arrow), (mag=x400). c) Exposure for 4 hours daily for 8 weeks revealed a congested myocardial layer (white arrow) with a normal appearing myocyte (blue arrow) (mag=x100). d) Higher magnification reveals a focal area of inflammation and mild congestion within the myocardium (black arrow) (mag=x400). e) the myocardium was moderately congested (white arrow) after 8-hour daily exposure at 8 weeks (mag=x100) f) Higher magnification of 8-hour daily exposure at 8 weeks revealed a severely congested myocardial layer (white arrow), with apparently normal myocytes (blue arrow) (mag=x400).

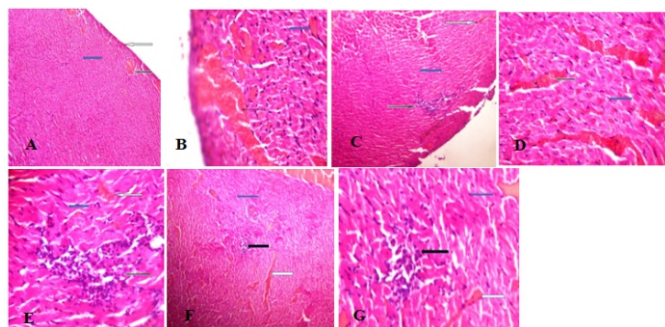


Figure 3. H and E-stained section of rat cardiac tissue exposed daily at 12 weeks a) 12 weeks of exposure for 2 hours daily revealed a normal epicardium (white arrow), a mildly congested myocardium (black arrow), and normal-looking myocytes (blue arrow) (mag= x100). b) At a higher magnification, note the congested myocardial layer (slender arrow) containing normal appearing myocytes (blue arrow) (mag = x400). c) exposure for 4 hr daily at 12 weeks reveals mild congestion within the myocardial layer (white arrow), note the focal area of inflammation (black arrow), with the myocytes appearing normal (blue arrow). d) The myocardial layer is congested (black arrow) with the myocytes appearing normal (blue arrow). e) mild congestion within the myocardial layer (white arrow) and focal area of inflammatory cell infiltration (black arrow), are seen at a higher magnification with the myocytes appearing normal (blue arrow) (mag= x400). f) Exposure for 8 hours daily for 12 weeks resulted in mild congestion of the myocardial layer (white arrow) and a focal area of inflammation (black arrow), although the myocytes appear normal (blue arrow) (mag = x100). g) Higher magnification reveals the presence of inflammatory mononuclear infiltrate within the myocardium (black arrow), normal-appearing myocytes (blue arrow), and myocardial congestion (white arrow) (mag= x400).

Table 1. Mean and Standard deviation of Hematology parameters at twelve (12) weeks.

Parameter(s)	Control	2 hours	4 hours	8 hours
WBC (10 ³ / μl)	5.62±0.78	8.9±2.4*	8.87±1.45*	8.44±2.52*
Neutrophil (10 ³ / μl)	1.36±0.26	1.36±0.25	2.11±0.44*	1.96±0.49*
Lymphocyte (10 ³ / μl)	4.07±0.83	7.03±1.81*	5.95±0.267*	5.02±1.46
Monocyte (10 ³ / μl)	0.08±0.09	0.13±0.067	0.1±0.06	0.23±0.13
Eosinophil (10 ³ / μl)	0.004±0.005	0.004±0.005	0.006± 0.005	0.004±0.005
Basophil (10 ³ / μl)	0.00±0.00	0.006±0.005	0.006±: 0.008	0.0018±0.007*

Data expressed as mean value ± SD. *Mean values were significantly different compared to the unexposed control group at p-value <0.05.

Table 2 Comparative analysis of the white blood cell parameters across the various groups at 12 weeks.

Parameter(s)	P-value	Control vs 2 hours	Control Vs 4 hours	Control Vs 8 hours	2 hours Vs 4 hours	2 hours Vs 8 hours	4 hours Vs 8 hours
WBC (10 ³ / μl)	"p"	0.015*	0.002*	0.03*	0.477	0.39	0.39
Neutrophil (10 ³ / μl)	"p"	0.208	0.009*	0.03*	0.195	0.30	0.33
Lymphocyte (10 ³ / μl)	"p"	0.009*	0.001*	0.14	0.13	0.06	0.12
Monocyte (10 ³ / μl)	"p"	0.21	0.39	0.05	0.25	0.11	0.05
Eosinophil (10 ³ / μl)	"p"	0.50	0.29	0.50	.029	0.50	.289
Basophil (10 ³ / μl)	"p"	0.07	0.086	0.0007*	0.50	0.013#	0.03#

Data expressed as mean value ± SD. *Mean values were significantly different compared to the unexposed control group at P<0.05. #Mean values were significantly different across the exposure time points at P <0.05.

Table 3. Mean and standard deviation of the red blood cell parameters/indices and platelet values at 12 weeks.

Parameter(s)	Control	2 hours	4 hours	8 hours
RBC (10 ⁶ /μl)	3.66±0.25	8.15±0.73*	7.82±1.01*	8.04±0.39*
HGB (g/dl)	12.46±1.77	17.20±1.17*	17.7±0.979*	18.70± 1.08*
HCT (L/L)	42.00±2.00	50.00±4.00*	52.00±1.9*	55.00±4.00*
MCV (fl)	51.25±1.14	60.98±2.60*	57.26± 6.34*	64.42±1.69*
MCH (pg)	17.82±0.20	21.74±0.63*	20.76±1.56*	22.62± 0.19*
MCHC (g/dl)	33.00±0.63	35.80±2.23*	35.20±1.60*	33.80±0.75*
PLT (10 ³ /dl)	52.80±2.60	38.00±5.70*	43.80±3.10*	43.60±1.00*

Data expressed as mean value ± SD. *Mean values were significantly different compared to the unexposed control group at p-value <0.05.

Table 4. Comparative analysis of the red blood cell parameters/indices and platelet values at 12 weeks.

Parameter(s)	P-value	Control vs 2 hours	Control Vs 4 hours	Control Vs 8 hours	2 hours Vs 4 hours	2 hours Vs 8 hours	4 hours Vs 8 hours
RBC (10 ⁶ /μl)	"p"	0.00001*	0.00002*	0.00001*	0.30	0.40	0.35
HGB (g/dl)	"p"	.001*	.0004*	.0002*	0.26	0.04#	0.10
HCT (L/L)	"p"	0.005*	0.0001*	0.0001*	0.31	0.05	0.054
MCV (fl)	"p"	0.00007*	0.04*	0.00001*	0.15	0.03#	0.07
MCH (pg)	"p"	0.00001*	0.003*	0.00001*	0.1387	0.02#	0.02#
MCHC (g/dl)	"p"	0.02*	0.02*	0.04*	0.34	0.06	0.08
PLT (10 ³ /dl)	"p"	0.0007*	0.001*	0.0001*	0.05	0.04#	0.45

Data expressed as mean value ± SD. *Mean values were significantly different compared to the unexposed control group at p-value <0.05. #Mean values were significantly different across the exposure time points at p-value <0.05.

DISCUSSION

The toxic impact of air pollutants and the heterogeneous, complex mixture of gases, liquids, and PM has demonstrated a consistently increased risk for cardiovascular events in both chronic and acute exposure. We observed that gasoline generator exhaust had a deleterious impact on heart tissue, causing congestion, inflammation and fibrotic changes in the epicardial and myocardial layers of the experimental animal with the most severe cytopathic features evident at 12 weeks of exposure. Additionally, haematological changes in the blood of exposed adult wistar rats were detected. The processes by which inhaled PM2.5 causes a deleterious cardiovascular effect are unknown. Endothelial dysfunction and systemic inflammation, however, remain the leading causes of CVD. PAH(s) are widespread environmental pollutants that are mostly produced by human activities such as the incomplete burning of fossil fuels and organic materials and death associated with gasoline-fueled generators and diesel exhaust engines have been reported. In this study, observation at 4 weeks across the various time points of exposure revealed hemorrhagic intermyocytic spaces, along with vascular congestion and fibrotic changes. The pathophysiology of CO which is a component of the product of fossil fuel combustion can be toxic to the heart due to

rapid diffusion into the bloodstream and binding to haemoglobin. It can also modify platelet function to boost nitric oxide (NO) production, which interacts with oxygen free radicals to form peroxynitrite (ONOO⁻), inhibiting mitochondrial function and activating platelets and neutrophils further. Several possible molecular mechanisms have also been proposed, including platelet activation, endothelial dysfunction, a proclivity for coagulation/thrombosis, an inclination for arrhythmias, acute arterial vasoconstriction, systemic inflammatory responses, and chronic promotion of atherosclerosis. Except for fibrotic changes, the cytopathic characteristics detected at week 4 were still evident at 8 weeks. Ankwue & Otamiri, documented histological abnormalities associated with gasoline exposure to include cellular degradation, congested myocardium, and varied degrees of mononuclear myocardial infiltration in exposed rats. In our study, inflammation was most prominent in the 12th week of exposure, implying that chronic exposure consistently elicits inflammatory responses within cardiomyocytes. According to Krishnan et al., there is a strong correlation between long-term residential PM_{2.5} concentration and NO-mediated endothelial dysfunction, which has been linked to the development of CVD. It is worth noting that the exhaust from fossil fuel combustion contains hundreds of pro-inflammatory and hazardous substances such as hydrocarbons, carbon particles, SOX, and NOX, all of which can exacerbate cardiac tissue survival and function. A congested epicardial and myocardial layer was observed at 12 weeks, in addition to the substantial mononuclear infiltration reported across the exposure time points. Chu et al. earlier indicated that hypoxia-induced as a result of carboxyhemoglobin (COHb) formation and the generation of ONOO⁻ inhibits cytochrome enzymes thus leading to myocardial injury. The haematotoxic effect of gasoline generator exhaust has been reported. We observed elevated WBC values across the various time points of exposure compared to the control. Anyiam et al. documented a decrease in RBC, MCH, MCHC, and HCT levels among gasoline-stationed workers while Okoro et al. reported that exposure to petroleum fumes lowers RBC in a time-dependent manner. These reports contradict the findings of this study; however, our findings are consistent with Sirdah et al. who reported increased RBCs in individuals exposed to gasoline vapour. The increase in the inflammatory cell counts in the exposed rats could be attributed to the inflammatory response induced by oxidative stress with reports by Bai et al., indicating that PM-induced vascular cytotoxicity and elevates ROS generation. Additionally, Choi et al. also documented the adverse effects of PM and their role in disease development coupled with alteration in the oxidant-antioxidant homeostasis and elevated proinflammatory indicators. Elevated RBC parameters across the various time points of exposure were observed in this study with significant platelet reduction when compared to the control which aligns with the reports of Anyiam et al. who documented a significant reduction in the platelet count in gasoline stationed workers in Anambra state, Nigeria. Ajugwo et al. reported that RBC, Hb, MCH and MCHC are more reduced in fuel attendants than in auto mechanics further suggesting this could be due to the effects of benzene and xylene, as reported by d'Azevedo et al. While Opute et al. observed no significant variation in the MCH values of exposed individuals, a significant increase in the MCH was observed in our study. We observed that the RBC was significantly elevated at 2, 4, and 8-hour time points of exposure relative to the control which is also consistent with other parameters such as HGB and HCT and

other red cell indices (MCV, MCH and MCHC). Our findings further align with Onyeka et al. Who reported a significant increase in the PCV, HB, MCV and MCH of rats exposed to gasoline vapour. Elevated Hb levels were observed in the experimental groups compared to the control. The increase in the red cell indices, Hb and HCT values observed in this study may be due to dysfunction in the secretion of erythropoietin caused by the exposure to the noxious elements and PM content of the gasoline generator exhaust. Comparative analysis of the platelet values revealed a significant reduction at various time points of exposure relative to the control thus agreeing with the reports of Elnabi et al. Conclusively, exposure to gasoline generator exhaust negatively impacts the cardiac tissue with the most pronounced damage occurring at 12 weeks.

CONCLUSION

This study provides insight into the deleterious effects of gasoline generator exhaust exposure. The findings revealed the negative impacts on the cardiac tissue evident with a focal area of inflammation, congestion and fibrosis across various time points of exposure. Additionally, elevated WBC, RBC, and red cell indices were observed with the platelet considerably decreased across the experimental group. It is pertinent that awareness to further enlighten people about the potential hazards associated with gasoline generator exhaust exposure be encouraged.

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