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Exposure of dogs to exhaust fumes from petrol fuelled electricity generator: Effects on blood and selected body organs

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Abstract

The use of petrol fuelled electric power generators (PFEPGs) is common in electric power-deficient regions, and contributes significantly to increase in ambient air pollution, morbidity and mortality. This study investigated the effects of exposure of dogs to exhaust fumes from PFEPG on blood cellular elements, serum biochemistry and heart, lungs, bronchial lymph nodes (BLN), spleen, liver and kidney tissues. Sixteen dogs, randomly assigned to four groups of four dogs each were used for the study. Group A served as the unexposed control, while Groups B, C and D dogs were exposed to exhaust fumes from PFEPG for 1, 2 and 3 hours daily, respectively, for 90 days. Haematology and serum biochemistry tests were done on blood samples collected from the dogs on days 0, 30, 60 and 90 of the study. Afterwards, the dogs were humanely sacrificed and tissues from selected organs were processed for histopathology. Results showed that exposure to exhaust fumes from PFEPG led to significantly ($p < 0.05$) higher red blood cell counts and packed cell volume and significantly ($p < 0.05$) lower haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total white blood cell, neutrophil and lymphocyte counts. Serum activity of alkaline phosphatase and levels of albumin, urea and creatinine were significantly ($p < 0.05$) higher in the exposed dogs, while their serum globulin levels were significantly ($p < 0.05$) lower. These recorded effects were related to the duration and degree of exposure. Histopathology revealed carbon particle deposits in the lungs and BLN, necrotic and inflammatory changes in the heart, lungs, liver, and kidneys. These pathophysiological changes highlight the health risks of such exposure of animals and possibly humans to exhaust fumes from PFEPGs.

Keywords: Petrol fuelled electricity generator; Exhaust fumes; Dogs; Blood; Organ toxicity.

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Introduction

Air pollutants are a complex combination of gaseous, solid and liquid pollutants suspended in the air, which can vary extensively in chemical and physical composition, depending on the source and/or location (Afsar *et al*., 2019). The threat of air pollution has long been recognized since 1952 in London, when increased mortality was associated with smog (Bell and Davis, 2001; Park, 2017). Since then, air pollution has been recognized as one of the major causes of several diseases globally (Global Burden of Disease, 2015; Brook *et al*., 2017). Air pollution constitutes a major health risk factor and has been associated with increased mortality from cardiovascular, respiratory, and other life-threatening diseases (Lelieveld *et al*., 2019). Significant increases in death rates have been associated with emission from the combustion of fossil fuels in industrial machineries, motor vehicles and electric power generating equipment (Global Burden of Disease, 2015).

Exhaust fumes generated from diesel or petrol-powered engines are important sources of air pollutants in major cities and towns. Diesel and petrol generators are very useful machines that produce electricity by combustion of diesel and petrol, respectively. Burning diesel or petrol leads to the production of exhaust gases. Diesel and petrol generators produce carbon dioxide (CO_2), nitrogen oxide (NO_2), hydrogen sulphide, sulphur dioxide, volatile organic compounds and particulate matter (Åberg *et al*., 2015). These generators release the gases into the atmosphere and thus significantly adversely affect air quality in the environment. Hydrocarbon emission is a consequence of partial or complete combustion of fuel in the engine. These emitted hydrocarbons react with sun light and nitrogen oxides to form ground-level ozone; a prominent constituent of smog. Ozone has reportedly been known to irritate the eyes, negatively affect the lungs, and worsen respiratory tract health issues (Pope and Dockery, 2006).

Population growth and industrialization have put immense pressure on the electricity assets in many parts of the world, particularly in Africa and the other developing countries. This has led to massive increase in the ownership and use of diesel and petrol-powered electricity generating sets, thus making these electric power deficient nations the highest users of private electric power generators across the globe (Omaye, 2002). According to the International Finance Corporation Report (IFC, 2019), Africa and indeed other electric power deficient developing nations are the leading markets in terms of demand and usage of diesel, petrol and gas-powered electricity generators in the world.

A common practice in densely-populated areas such as the cities in these electric power deficient nations is the sharing of accommodation between man and animals, with these electricity generating sets in close proximity, due to limited space. Previous studies on air pollution in humans were largely based on surveys and use of retrospective data which are subject to the limitations of all observational studies.

The close relationship between humans and dogs in the exposure environment may imply that findings in these animals may provide valuable information on the pathophysiological changes and disease mechanisms caused by exposure to air pollutants in both humans and animals. The possible health implications of air pollution in dogs are also, at most, speculative. Earlier reports showed that exposure of dogs to petrol generator exhaust fumes caused increased levels of troponin 1, C-reactive protein and oxidative stress and adverse effects on canine reproductive indices (Eze *et al*., 2021; Oguejiofor *et al*., 2024). The current study investigated the effects of graded exposure duration to exhaust fumes of petrol-

powered electricity generator on the haematological parameters, serum biochemical biomarkers of organ health and the histology of the heart, kidney, liver, spleen, lungs and bronchial lymph node of exposed dogs.

Materials and Methods

Experimental Animals: The animals used for the study are already described in our earlier report (Eze *et al*., 2021). A total of sixteen (16) male Basenji dogs, obtained from Dynasty Pet Shop Plus, Enugu, Nigeria, were used for the study. At the beginning of the study, the animals were 7 to 12 months of age and 5.8 to 8.0 kg in weight. The study was conducted within the Experimental Animal Unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The kennels housing the dogs were at a temperature of 24 – 29 °C, and humidity of 45 – 85% within a 12-hour light/dark cycle. The dogs were fed approximately 300 g commercial pelletized dog food (Jock; Afrique Pet Food Ltd., Gauteng, South Africa) per dog every day. Drinking water was also provided *ad libitum* throughout the study period. The kennels and dog cages were cleaned daily and sanitized once a week.

The dogs were acclimatized for a period of three weeks to allow for stabilization, mastery of handling and associated drills before the beginning of the experiment. They were confirmed to be apparently healthy after thorough physical evaluation and clinical examination for blood and gastrointestinal parasites. They were routinely given a broad spectrum anthelmintic (Wormrid; Hebei Kexing Pharm. Co. Ltd., China), anti-rabies vaccine (Bioveta, Czech Republic) and distemper, hepatitis, leptospirosis, parainfluenza and parvoviral enteritis (DHLPP) vaccine (Bioveta, Czech Republic).

Ethical approval: Standard experimental animal care and welfare was observed as recommended in the revised version of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 2011). The animals were treated humanely and with regards to alleviation of suffering. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka (FVM-UNN-IACUC-2019-0710).

Petrol-powered electric generator: As described in our previous report (Eze *et al*., 2021), the PFEPG exhaust fumes to which the experimental dogs were exposed to was from a new portable KEMAGE petrol generator, KM1500 (Chongqing Kema Industrial and Trading Co. LTD, China). The tank capacity was 9.6 litre, and it was operated using standard petrol fuel (Rain Oil and Gas Ltd., Nsukka, Enugu State, Nigeria). Other details of the petrol fuelled generator included automatic voltage regulator (AVR), maximum output power (1.5 KVA), rated power (1.3 KVA), and voltage (230 V), and frequency (50 Hz), rated RPM (3000 rpm), COS ϕ r: 1 and size (505 × 385 × 455 mm).

Experimental environment: The housing kennel for the dogs measured 64 m^2 floor space × 2.9 m^2 height and was spacious, illuminated and adequately ventilated. The dogs were housed individually in well-ventilated and evenly-spaced metal cages (0.85 m^2 floor space × 1.2 m^2 height). The generator was positioned outside the kennel, 12 m from the kennel's centre, with the exhaust pipe facing towards the kennel. An air circulator (Vornado 660B, Singapore) was used to assist in the distribution of exhaust fumes within the kennel. Temperature and humidity of the kennel were monitored with a temperature and moisture meter (Extech 45170, USA). Before and after the introduction of exhaust fumes, the kennel's air quality was monitored using air quality sensor heads (Aeroqual 88, Series 200 Portable Monitor, New Zealand) positioned at the centre of the

kennel. The recorded air quality data are presented in Table 1 as previously reported (Eze *et al*., 2021).

Experimental design: The 16 dogs used for the study were randomly assigned to four groups (A, B, C and D) of four dogs each. Group A dogs served as the unexposed control and were kept in a kennel different from that of those that were exposed to PFEPG exhaust fumes. The PFEPG exhaust fumes-exposed groups (B, C and D) were exposed for 1, 2 and 3 hours per day (hpd), respectively, for a total period of 90 days. All the twelve exposed dogs (B, C and D) were housed in the same PFEPG exhaust fumes-exposed kennel. They were randomly positioned daily within the kennel. During the daily exposure, group B was exposed for one hour and then transferred to an unexposed holding kennel, group C was transferred after two hours of exposure whereas group D was exposed for three hours before transfer. A period of one hour was allowed for kennel ventilation before returning all the exposed dogs. All the dogs were monitored weekly for changes in food and water intake, body weight and physiologic indices (body temperature, heart rate and respiratory rate). Physical and behavioural signs were also monitored daily in the dogs during and after PFEPG exhaust fumes exposure.

Blood sample collection: Baseline (Day 0) blood samples were collected from all the groups one day before the beginning of exposure of Groups B, C and D to PFEPG exhaust fumes. Subsequent blood samples post-exposure to PFEPG exhaust fumes were collected on days 30, 60 and 90. All samples were collected within the same time period (9 – 10 am in the morning) to avoid the effect of diurnal variations. Five millilitres of blood was collected from the cephalic vein of each dog using a sterile 21-gauge needle. One millilitre of the blood was mixed in a bottle containing anti-coagulant (potassium-EDTA) to be used for haematology, while the remaining blood was allowed to clot in a plain sample bottle. Following clotting, blood samples were centrifuged at 1500 × g for 15 min, and the harvested sera were utilized for biochemical analyses.

Evaluation of haematological parameters: Haematological parameters were evaluated using the standard laboratory methods as described previously (Cheesbrough, 2009). Packed cell volume (PCV) was measured with a microhaematocrit centrifuge and reader (Hawksley, England). Haemoglobin (Hb) concentration was determined following the cyanomethaemoglobin method, using a spectrophotometer (CHEM-5V3; Erba, Mannheim, Germany) read at 546 nm against the reagent blank and a standard solution of cyanmethaemoglobin. The red blood cell (RBC) and white blood cell (WBC) counts were determined using the improved Neubauer haemocytometric method. Differential WBC count was determined in Leishman-stained thin blood films by counting individual cells types under oil immersion at × 1000 magnification using a microscope (Motic B3; Motic, Carlsbad, CA, USA). The following RBC indices were calculated: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) by using the PCV, RBC count and Hb values, as appropriate.

Evaluation of serum biochemical markers: Serum biochemical indices that were evaluated included serum activities of enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the serum concentrations of total protein (TP), albumin, urea and creatinine; these were measured using commercially available laboratory test kits sourced from Dialab, Wiener Neudorf, Austria. For each serum sample, total globulins (TG) concentration was calculated by subtracting the value of albumin from the TP (Cheesbrough, 2009). The serum

concentrations of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were also measured using commercially available test kits sourced from Agappe Diagnostics, Cham, Switzerland. All assays were carried out in duplicates while following the specified instructions provided by the manufacturers. Assay absorbances were measured using a spectrophotometer (CHEM-5V3; Erba, Mannheim, Germany) at the specified wavelengths. The absorbance of each sample was read against the reagent blank, and the absolute concentration values were derived using the standards/calibrators for the respective biochemical assay kits.

Histopathological evaluation: Tissue sections from the heart, lungs, bronchial lymph nodes, spleen, kidney and liver were processed for histopathological evaluation by following the methods described previously (Slaoui and Fiette, 2011). Briefly, tissue samples were fixed in 10% neutral-buffered formalin for 72 hours, dehydrated in serial ethanol grades, cleared in xylene, and embedded in paraffin wax. Tissues were sectioned at 5 µm thickness and stained with haematoxylin and eosin (H & E). Histopathological evaluation was done at × 40, × 100 and × 400 magnifications using a light microscope (Motic B3; Motic, Carlsbad, CA, USA).

Statistical analysis: Data obtained were analysed with mixed repeated measures ANOVA through a general linear model using SPSS, version 26 (IBM Corp, Armonk, NY, USA). The effect of PFEPG exhaust fumes exposure level (hpd) and duration of exposure (days), and their interaction on the dependent parameters were determined. The results were reported as simple main effects (exposure level) and main effects (duration of exposure), followed by Fisher's least significant difference (LSD) post-hoc multiple comparison. Values were expressed as mean ± standard deviation (SD) and observed differences were considered significant when $p < 0.05$.

Results

Pollutants associated with the PFEPG exhaust fumes: Pollutants detected in PFEPG exhaust fumes in this study were hydrogen sulphide (H_2S), nitrogen oxide (NO_2), carbon dioxide (CO_2), carbon monoxide (CO), volatile organic compounds (VOC), sulphur dioxide (SO_2), particulate matter 2.5 ($PM_{2.5}$) and particulate matter 10 (PM_{10}). The detected levels of these pollutants are shown in Table 1.

Haematology: The results of PCV, Hb and RBC are presented in Table 2. There was no significant interaction between PFEPG exhaust fumes exposure duration and exposure level on the packed cell volume (PCV). There was a significant main effect of PFEPG exhaust fumes exposure duration on PCV, F (3, 36) = 13.863, $p < 0.001$, partial eta squared (ηp^2) = 0.536. A pairwise comparison for PFEPG exhaust fumes exposure duration showed that the mean PCV of Groups C and D were significantly higher ($p < 0.05$) than the baseline values at day 60, and by day 90, the PCV of Groups B, C and D were significantly ($p < 0.05$) higher. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the PCV. However, pairwise comparison for PFEPG exhaust fumes exposure level showed a significant increase ($p < 0.05$) in PCV levels in groups C and D on day 60, compared with the control. Subsequently (day 90), all the PFEPG exhaust fumes-exposed groups had significantly elevated ($p < 0.05$) PCV than the control.

There was a significant interaction between PFEPG exhaust fumes exposure duration and exposure level on the Hb concentration, F (5.627, 22.510) = 4.011, $p = 0.008$, $\eta p^2 = 0.501$. There was a significant main effect of PFEPG exhaust fumes exposure duration on the Hb concentration, F (1.876, 22.510) = 9.424, $p = 0.001$, $\eta p^2 = 0.440$. Pairwise comparison for

PFEPG exhaust fumes exposure duration showed that Hb concentration in groups C and D were significantly lower ($p < 0.05$) on days 60 and 90, compared to their baseline levels. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the Hb concentration. However, pairwise comparison for PFEPG exhaust fumes exposure level showed a significantly decreased ($p < 0.05$) Hb concentration in group D (day 60) and in all the PFEPG exhaust fumes-exposed groups (day 90), compared with the control.

No significant interaction was recorded between PFEPG exhaust fumes exposure duration and exposure levels on the RBC count. A significant main effect of PFEPG exhaust fumes exposure duration was observed on the RBC count, $F (3, 36) = 16.898$, $p < 0.001$, $\eta p^2 = 0.585$. Pairwise comparison for PFEPG exhaust fumes exposure duration revealed a significant increase ($p < 0.05$) in RBC counts in all the PGEF-exposed groups at day 90, compared to the baseline values. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the RBC count. However, pairwise comparison for PFEPG exhaust fumes exposure level showed a significantly increased ($p < 0.05$) RBC count in group D on day 90, compared with the control.

Table 1. Air quality of the kennel before and after the introduction of petrol fuelled electric power generator (PFEPG).

S/N	Parameters	Condition of the kennel before fume introduction	Quantity of emission at the exhaust level of the generator	Quantity of emission at 12m at the level of dog's head)
1	Temperature (^{o}C)	25.3	41.5	31.5
2	Relative humidity (%)	28.3	14.4	32.4
3	Foot candle (phots)	2.4 x 10	52.5 x 10	158.4 x 10
4	Ozone (ppm)	0.00	0.00	0.00
5	Hydrogen sulphide (ppm)	0.00	12.495	1.29
6	Nitrogen dioxide (ppm)	0.002	0.00	0.034
7	Carbon dioxide (ppm)	518.00	2390.00	591.00
8	$PM_{2.5}$ (ppm)	0.011	0.014	0.036
9	PM_{10} (ppm)	0.019	0.051	0.049
10	Carbon monoxide (ppm)	0.00	241.00	144.30
11	Volatile organic compounds (ppm)	82.9	8899.00	417.00
12	Sulphur dioxide (ppm)	0.00	84.67	26.07

PM – particulate matter; parts per million (ppm). Foot candle is the unit of illuminance and is defined as one lumen per square foot = 1.076×10^{-3} phots. (Eze *et al*., (2021)

Table 2. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the packed cell volume, haemoglobin and red blood cells of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Packed cell volume (%)	**A**	35.5 ± 6.5	34.5 ± 4.5	35.5 ± 3.7	35.3 ± 2.5
	B	34.3 ± 5.3	32.8 ± 5.7	37.0 ± 3.6	40.2 ± 2.1$^{*\alpha}$
	C	36.2 ± 7.2	34.7 ± 5.7	41.5 ± 3.4$^{*\alpha}$	43.3 ± 3.6$^{*\alpha}$
	D	35.3 ± 8.1	36.5 ± 3.9	43.5 ± 4.0$^{*\alpha}$	43.8 ± 2.5$^{*\alpha}$
Haemoglobin concentration (g/dl)	**A**	12.0 ± 1.7	12.6 ± 0.9	12.4 ± 0.7	12.9 ± 0.7
	B	11.9 ± 1.0	12.1 ± 1.1	11.7 ± 0.9	11.2 ± 0.8*
	C	12.3 ± 1.6	11.8 ± 1.0	11.2 ± 1.0$^{\alpha}$	10.7 ± 1.0$^{*\alpha}$
	D	12.1 ± 1.5	12.0 ± 1.1	10.8 ± 0.8$^{*\alpha}$	9.8 ± 0.8$^{*\alpha}$
Red blood cell counts ($\times10^{12}$/L)	**A**	5.34 ± 0.8	5.09 ± 0.7	5.37 ± 0.4	5.24 ± 0.6
	B	5.03 ± 0.9	4.93 ± 0.8	5.64 ± 0.6	5.92 ± 0.7$^{\alpha}$
	C	5.26 ± 0.7	4.74 ± 0.7	5.87 ± 0.6	6.19 ± 0.8$^{\alpha}$
	D	5.25 ± 0.7	4.97 ± 1.0	5.96 ± 0.3	6.47 ± 0.6$^{*\alpha}$

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

The results of the MCV, MCH and MCHC are presented in Table 3. The values for the MCV in all the experimental groups ranged from 47.5 – 87.3 fl. Exposure to PFEPG exhaust fumes did not significantly alter the MCV when compared with the control. Within each group, there were also no differences from the baseline values.

There was a significant interaction between PFEPG exhaust fumes exposure duration and exposure level on the MCH, F (5.490, 21.960) = 4.627, p = 0.004, ηp^2 = 0.536. There was a significant main effect of PFEPG exhaust fumes exposure duration on the MCH, F (1.830, 21.960) = 31.096, $p < 0.001$, ηp^2 = 0.722. Pairwise comparison for PFEPG exhaust fumes exposure duration showed that the MCH levels of the PFEPG exhaust fumes-exposed groups were significantly lower ($p < 0.05$) than the baseline values at day 60 (groups C and D) and day 90 (groups B, C and D). There was a significant simple main effect of PFEPG exhaust fumes exposure levels on MCH, F (3, 12) = 5.832, p = 0.011, ηp^2 = 0.593. Pairwise comparison for PFEPG exhaust fumes exposure levels showed significantly lower ($p < 0.05$) MCH levels in all the PFEPG exhaust fumes-exposed groups on days 60 and 90, compared with the control.

Significant interaction was recorded between PFEPG exhaust fumes exposure duration and exposure level on the MCHC, F (5.498, 21.993) = 5.589, p = 0.001, ηp^2 = 0.583. A significant main effect of PFEPG exhaust fumes exposure duration was observed on the MCHC, F(1.833, 21.993) = 34.040, $p < 0.001$, ηp^2 = 0.739. Pairwise comparison for PFEPG exhaust fumes exposure duration indicated a significant

decline ($p < 0.05$) in MCHC on day 90 (group B) and on days 60 and 90 (groups C and D), compared to their baseline values. There was a significant simple main effect of PFEPG exhaust fumes exposure levels on the MCHC, $F(3, 12) = 17.463$, $p < 0.001$, $\eta p^2 = 0.814$. Pairwise comparison for PFEPG exhaust fumes exposure levels showed a significant decline ($p < 0.05$) in MCHC in all the PFEPG exhaust fumes-exposed groups on days 60 and 90, compared with the control.

Table 3. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Mean corpuscular haemoglobin (pg)	**A**	22.5 ± 2.2	24.9 ± 1.7	23.0 ± 0.8	24.7 ± 1.7
	B	24.1 ± 4.1	24.7 ± 2.5	20.7 ± 0.9*	19.0 ± 1.3*α
	C	23.5 ± 1.9	25.1 ± 3.1	19.1 ± 1.2*α	17.3 ± 1.1*α
	D	23.2 ± 2.0	24.5 ± 3.4	18.0 ± 0.5*α	15.2 ± 0.9*α
Mean corpuscular haemoglobin concentration (g/dl)	**A**	34.1 ± 5.4	36.9 ± 2.6	34.9 ± 1.8	36.6 ± 1.1
	B	35.0 ± 3.0	37.2 ± 3.3	31.5 ± 1.0*	27.8 ± 0.9*α
	C	34.2 ± 2.7	34.2 ± 3.4	26.9 ± 1.1*α	24.7 ± 0.9*α
	D	35.1 ± 4.9	32.8 ± 1.3	24.7 ± 0.7*α	22.4 ± 0.7*α
Mean corpuscular volume (fl)	**A**	72.7±22.8	68.6 ±12.9	63.6 ± 5.1	67.7 ± 10.1
	B	76.1 ±11.3	66.8 ± 7.0	66.1 ± 14.4	71.9 ± 8.4
	C	69.8 ±18.7	73.7 ± 8.7	71.4 ± 11.0	70.3 ± 9.1
	D	69.1 ±8.4	78.1 ± 15.7	71.9 ± 8.1	66.2 ± 10.1

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; αsignificant ($p < 0.05$) compared with day 0.

Table 4 shows the results of WBC, neutrophils and lymphocyte counts. There was a significant interaction between PFEPG exhaust fumes exposure duration and exposure level on the WBC count, $F(9, 36) = 14.684$, $p < 0.001$, $\eta p^2 = 0.786$. There was also a significant main effect of PFEPG exhaust fumes exposure duration on the total WBC count, $F(3, 36) = 114.946$, $p < 0.001$, $\eta p^2 = 0.905$. Pairwise comparison for PFEPG exhaust fumes exposure duration indicated a significant decrease ($p < 0.05$) in total WBC count in group D on day 60 and in all the PFEPG exhaust fumes-exposed groups on day 90, compared to the baseline values. There was no significant simple main effect of PFEPG exhaust fumes exposure levels on the total WBC count. However, a pairwise comparison of PFEPG exhaust fumes exposure levels revealed a significant decrease ($p < 0.05$) in WBC count in group D (day 60), and in all the PFEPG exhaust fumes-exposed groups (day 90), compared with the control.

With regards to the neutrophil counts, there was a significant interaction of PFEPG exhaust

fumes exposure duration and exposure levels, F (9, 36) = 4.592, $p < 0.001$, $\eta p^2 = 0.534$. A significant main effect of PFEPG exhaust fumes exposure duration was also observed on the neutrophil count, F (3, 36) = 30.955, $p < 0.001$, $\eta p^2 = 0.721$. Pairwise comparison for PFEPG exhaust fumes exposure duration revealed a significant decrease ($p < 0.05$) in neutrophil count in groups C and D (day 30), group D (day 60) and in all the PFEPG exhaust fumes-exposed groups (day 90), compared to their baseline values. There was also a significant simple main effect of PFEPG exhaust fumes exposure levels on the neutrophil count, F (3, 12) = 6.591, $p = 0.007$, $\eta p^2 = 0.622$. Pairwise comparison for PFEPG exhaust fumes exposure levels indicated a significant decrease ($p < 0.05$) in neutrophil count in all the PFEPG exhaust fumes-exposed groups on days 30 and 90, compared with the control.

For lymphocyte counts, there was a significant interaction between PFEPG exhaust fumes exposure duration and exposure levels on the lymphocyte count, F (9, 36) = 10.011, $p < 0.001$, $\eta p^2 = 0.715$. There was also a significant main effect of PFEPG exhaust fumes exposure duration on the lymphocyte count, F (3, 36) = 65.737, $p < 0.001$, $\eta p^2 = 0.846$. Pairwise comparison of PFEPG exhaust fumes exposure duration indicated an initial significant increase ($p < 0.05$) in lymphocyte count in all the PFEPG exhaust fumes-exposed groups on day 30, followed by a significant decline on day 90, compared to their baseline values. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the lymphocyte count. However, pairwise comparison for PFEPG exhaust fumes exposure levels showed that all the exposed groups had an initial significant increase ($p < 0.05$) in lymphocyte count on day 30 followed by a significant decrease on day 90, compared with the control.

Table 4. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the total white blood cell, neutrophil and lymphocytes counts of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Total White blood cell counts (×10^9/L)	**A**	15.66 ± 1.6	15.79 ± 2.2	16.88 ± 2.5	15.60 ± 2.3
	B	14.97 ± 3.6	17.32 ± 4.0	13.91 ± 3.7	5.88 ± 1.7$^{*\alpha}$
	C	13.94 ± 2.7	16.48 ± 3.8	12.35 ± 2.9	3.31 ± 1.4$^{*\alpha}$
	D	15.89 ± 2.8	18.21 ± 4.1	9.61 ± 3.0$^{*\alpha}$	2.75 ± 1.0$^{*\alpha}$
Absolute Neutrophil counts (×10^9/L)	**A**	10.53 ± 2.6	11.04 ± 2.1	11.86 ± 3.5	10.83 ± 1.2
	B	10.27 ± 2.1	7.53 ± 1.8*	8.57 ± 3.3	4.04 ± 1.3$^{*\alpha}$
	C	9.09 ± 3.4	5.48 ± 2.4$^{*\alpha}$	7.41 ± 2.7	1.91 ± 0.9$^{*\alpha}$
	D	10.68 ± 2.9	4.65 ± 2.2$^{*\alpha}$	5.31 ± 3.1$^{*\alpha}$	1.84 ± 0.5$^{*\alpha}$
Absolute Lymphocyte counts (×10^9/L)	**A**	4.01 ± 0.8	3.37 ± 1.2	4.39 ± 2.1	3.69 ± 0.9
	B	3.50 ± 1.3	8.76 ± 2.6$^{*\alpha}$	4.07 ± 0.9	1.17 ± 0.4$^{*\alpha}$
	C	3.83 ± 1.1	9.63 ± 2.1$^{*\alpha}$	3.61 ± 0.8	0.74 ± 0.6$^{*\alpha}$
	D	4.51 ± 1.5	12.03 ± 3.4$^{*\alpha}$	2.78 ± 1.5	0.41 ± 0.4$^{*\alpha}$

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

Serum Biochemistry: The results of serum ALP, ALT and AST activities of exposed dogs are presented in Table 5. There were no significant differences (p > 0.05) in the mean serum activities of ALT and AST in the exposed dogs compared with the control. The mean values were also not different from the baseline values, within each group. In all the experimental groups, the ALT activity ranged from 28.5 – 36.2 IU/L while AST ranged from 35.1 – 46.3 IU/L.

There was a significant interaction between PFEPG exhaust fumes exposure duration and exposure level on serum ALP activity, F (9, 36) = 10.883, $p < 0.001$, $\eta p^2 = 0.731$. A significant main effect for PFEPG exhaust fumes exposure duration was observed on the ALP activity, F (3, 36) = 38.844, $p < 0.001$, $\eta p^2 = 0.764$. A pairwise comparison of PFEPG exhaust fumes exposure duration indicated a significant ($p < 0.05$) increase in ALP activity in all the exposed groups from days 30 – 90, compared to their baseline values. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the ALP activity. However, a pairwise comparison of PFEPG exhaust fumes exposure level showed that the ALP activity in all the groups was not significantly different on days 30 and 60. However, on day 90, there was a significant increase ($p < 0.05$) in ALP activity in groups C and D, compared with the control.

The serum total protein, albumin and total globulin concentrations of exposed dogs are presented in Table 6. Exposure to PFEPG exhaust fumes did not significantly alter the serum total protein concentration when compared with the control. There were also no differences from the baseline values within each group. Total protein concentration in all the groups ranged from 6.2 – 6.8 g/dl.

No significant interaction was recorded between PFEPG exhaust fumes exposure duration and exposure level on serum albumin concentration. There was a significant main effect of PFEPG exhaust fumes exposure duration on the albumin concentration, F (3, 36) = 17.900, $p < 0.001$, $\eta p^2 = 0.599$. A pairwise comparison for PFEPG exhaust fumes exposure duration indicated a significant increase ($p < 0.05$) in the albumin concentration as the duration of exposure was increased. Compared to the baseline values, albumin concentration was increased on days 60 and 90 in groups B and C and on days 30 – 90 in group D. There was no significant simple main effect of PFEPG exhaust fumes exposure level on the albumin concentration. However, a pairwise comparison for PFEPG exhaust fumes exposure level showed a significant increase ($p < 0.05$) in the albumin concentration in group D on day 60, compared with the control.

There was no significant interaction between PFEPG exhaust fumes exposure duration and exposure level on the serum globulin concentration. A significant main effect of PFEPG exhaust fumes exposure duration was observed on the globulin concentration, F (3, 36) = 6.329, $p = 0.001$, $\eta p^2 = 0.345$. Moreover, a pairwise comparison for PFEPG exhaust fumes exposure duration showed a significant decrease ($p < 0.05$) in the globulin concentration in groups C and D on day 90, compared to their baseline values. Although there was no significant simple main effect of PFEPG exhaust fumes exposure level on the globulin concentration, a pairwise comparison for PFEPG exhaust fumes exposure level showed a significant decrease ($p < 0.05$) in the globulin concentration in groups C and D on days 60 and 90, compared with the control.

Table 7 shows the serum urea and creatinine concentrations of exposed dogs. A significant interaction was recorded between PFEPG exhaust fumes exposure duration and exposure level on serum urea concentration, F (9, 36) = 2.308, $p = 0.036$, $\eta p^2 = 0.366$. There was a significant main effect of PFEPG exhaust fumes exposure duration on the urea concentration, F (3, 36) = 8.024, $p < 0.001$, $\eta p^2 = 0.401$. A pairwise comparison for PFEPG

exhaust fumes exposure duration showed a significantly increased ($p < 0.05$) urea concentration in group C on day 90 and in group D on days 30–90, compared to their baseline values. There was a significant simple main effect of PFEPG exhaust fumes exposure level on urea concentration, F (3, 12) = 5.805, $p = 0.011$, $\eta p^2 = 0.592$. A pairwise comparison for PFEPG exhaust fumes exposure level showed a significant increase ($p < 0.05$) in the urea concentration in all the exposed groups on days 30 and 90, compared with the control. However, only group D had a higher urea concentration than the control on day 60.

There was no significant interaction between PFEPG exhaust fumes exposure duration and exposure level on serum creatinine concentration. A significant main effect of PFEPG exhaust fumes exposure duration was observed on the creatinine concentration, F (3, 36) = 7.982, $p < 0.001$, $\eta p^2 = 0.399$. Moreover, a pairwise comparison for PFEPG exhaust fumes exposure duration showed a significant increase ($p < 0.05$) in the creatinine concentration in group D from days 30–90, compared to the baseline value. There was also a significant simple main effect of PFEPG exhaust fumes exposure level on the creatinine concentration, F (3, 12) = 3.615, $p = 0.046$, $\eta p^2 = 0.475$. A pairwise comparison for PFEPG exhaust fumes exposure level showed no significant difference in the creatinine concentration in all the groups from days 0 to 60. However, there was a significant increase ($p < 0.05$) in the creatinine concentration in all the exposed groups on day 90, compared with the control.

Table 5. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the serum alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) activity of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Alkaline phosphatase activity (IU/L)	**A**	372.1 ± 24.1	418.6 ± 30.9	423.6 ± 32.6	385.7 ± 44.1
	B	355.7 ± 20.9	441.4 ± 74.1$^{\alpha}$	437.3 ± 60.0$^{\alpha}$	430.5 ± 55.3$^{\alpha}$
	C	355.3 ± 20.4	431.9 ± 53.9$^{\alpha}$	474.7 ± 52.6$^{\alpha}$	508.2 ± 80.8$^{*\alpha}$
	D	349.1 ± 25.3	453.2 ± 62.0$^{\alpha}$	473.2 ± 89.8$^{\alpha}$	682.5 ± 99.6$^{*\alpha}$
Alanine aminotransferase activity (IU/L)	**A**	34.41 ± 4.88	32.00 ± 4.67	31.99 ± 7.33	34.92 ± 5.74
	B	36.21 ± 7.42	29.92 ± 3.02	31.14 ± 1.48	36.42 ± 5.04
	C	29.21 ± 7.65	28.50 ± 4.76	30.26 ± 6.38	32.45 ± 3.05
	D	31.10 ± 7.87	30.40 ± 3.57	33.64 ± 7.77	34.92 ± 3.50
Aspartate aminotransferase activity (IU/L)	**A**	39.61 ± 5.32	37.94 ± 2.65	38.06 ± 4.11	39.27 ± 3.44
	B	36.64 ± 3.31	35.07 ± 4.85	37.91 ± 3.87	42 .44 ± 8.83
	C	38.47 ± 10.17	39.74 ± 3.31	40.80 ± 7.44	44.36 ± 5.65
	D	39.65 ± 6.87	38.95± 5.95	41.89 ± 3.01	46.30 ± 4.28

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

Table 6. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the serum levels of total proteins, albumin and globulins of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Total proteins (g/dl)	**A**	6.50 ± 0.52	6.55 ± 0.31	6.77 ± 0.34	6.41 ± 0.45
	B	6.22 ± 0.17	6.45 ± 0.10	6.52 ± 0.17	6.45 ± 0.57
	C	6.42 ± 0.20	6.25 ± 0.37	6.42 ± 0.17	6.30 ± 0.34
	D	6.15 ± 0.66	6.35 ± 0.41	6.22 ± 0.55	6.25 ± 0.49
Albumins (g/dl)	**A**	3.34 ± 0.33	3.50 ± 0.26	3.69 ± 0.13	3.57 ± 0.45
	B	3.14 ± 0.39	3.48 ± 0.31	3.64 ± 0.18$^{\alpha}$	3.76 ± 0.20$^{\alpha}$
	C	3.23 ± 0.33	3.54 ± 0.47	3.85 ± 0.29$^{\alpha}$	4.07 ± 0.37$^{\alpha}$
	D	3.19 ± 0.42	3.78 ± 0.43$^{\alpha}$	4.06 ± 0.26$^{*\alpha}$	4.12 ± 0.51$^{\alpha}$
Globulins (g/dl)	**A**	3.16 ± 0.71	3.05 ± 0.45	3.06 ± 0.22	3.08 ± 0.43
	B	3.08 ± 0.43	2.97 ± 0.31	2.89 ± 0.14	2.70 ± 0.45
	C	3.19 ± 0.39	2.71 ± 0.29$^{\alpha}$	2.57 ± 0.15*	2.23 ± 0.22$^{*\alpha}$
	D	2.97 ± 0.96	2.57 ± 0.72	2.34 ± 0.28*	2.13 ± 0.65$^{*\alpha}$

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

Table 7. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the serum levels of urea and creatinine of the dogs.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Urea (mg/dl)	**A**	22.9 ± 6.9	18.3 ± 3.0	24.0 ± 6.7	20.5 ± 2.5
	B	25.5 ± 8.8	31.6 ± 2.7*	29.9 ± 5.5	32.6 ± 5.9*
	C	24.6 ± 4.6	30.6 ± 5.2*	30.5 ± 6.9	37.3 ± 7.7$^{*\alpha}$
	D	21.0 ± 4.7	32.0 ± 8.5$^{*\alpha}$	36.7 ± 8.0$^{*\alpha}$	38.5 ± 4.7$^{*\alpha}$
Creatinine (mg/dl)	**A**	0.82 ± 0.18	0.93 ± 0.09	0.93 ± 0.05	0.85 ± 0.16
	B	0.86 ± 0.16	0.91 ± 0.08	0.94 ± 0.07	1.00 ± 0.07*
	C	0.89 ± 0.12	0.94 ± 0.08	0.99 ± 0.07	1.06 ± 0.05*
	D	0.79 ± 0.18	1.01 ± 0.11$^{\alpha}$	1.06 ± 0.15$^{\alpha}$	1.18 ± 0.05$^{*\alpha}$

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

The total cholesterol, HDL, LDL and triglyceride concentrations of PFEPG exhaust fumes-exposed dogs are presented in Table 8. There was a significant interaction between PFEPG exhaust fumes exposure duration and exposure level on serum total cholesterol concentration, F (9, 36) = 2.553, $p = 0.022$, $\eta p^2 = 0.390$. There was no significant main effect of PFEPG exhaust fumes exposure duration on the total cholesterol concentration. However, a pairwise comparison for exposure duration showed that total cholesterol level decreased significantly ($p < 0.05$) in group D dogs on day 90, compared with the baseline value. There was also no significant simple main effect of PFEPG exhaust fumes exposure level on the total cholesterol levels. A pairwise comparison for PFEPG exhaust fumes exposure level showed a significant decrease ($p < 0.05$) in total cholesterol level in group D on day 90, compared with the control.

There was no significant interaction between PFEPG exhaust fumes exposure duration and exposure level on serum HDL concentration. There was no significant main effect of PFEPG exhaust fumes exposure duration on the HDL concentration. Pairwise comparison for PFEPG exhaust fumes exposure duration showed a significant decrease ($p < 0.05$) in the HDL concentration in group D on day 90, compared to the baseline value. There was also no significant simple main effect of PFEPG exhaust fumes exposure level on the HDL concentration. However, pairwise comparison for PFEPG exhaust fumes exposure level showed a significant decrease ($p < 0.05$) in the HDL concentration in group D on day 90, compared with the control.

There were no significant differences in the mean serum LDL and triglycerides concentrations in the exposed dogs, compared with the control. Within each group, the mean values were also not different from the baseline values. In all the groups, the serum concentrations of LDL ranged from 60.2–66.0 mg/dL while that of triglycerides ranged from 71.4–87.3 mg/dL.

Histopathology of the heart, lungs, bronchial lymph nodes, spleen, liver and kidney: The heart tissues of the PFEPG exhaust fumes-exposed dogs showed various degrees of degeneration and necrosis of myofibres, with severity dependent on the duration of exposure to the PFEPG exhaust fumes. The heart tissues of dogs exposed for 3 hours in addition had moderate infiltration of mononuclear inflammatory cells in the interstitial spaces (Figure 1). In the lungs of the exposed groups, the inter-alveolar connective tissues were thickened due to severe infiltration of mononuclear inflammatory cells, hyperaemia, fibroplasia and deposition of carbon particles (Figure 2). Black pigments, which were also being phagocytosed by macrophages, were seen in the cortex and follicles of the bronchial lymph nodes in dog groups exposed for 2 and 3 hours, and this was associated with lymphoid depletion (Figure 3). Lymphoid depletion was also observed in the splenic tissues of the exposed dogs, but erythrophagocytosis was more pronounced in the dogs exposed for 2 hours (Figure 4). Lesions in the kidneys of the exposed dogs comprised mainly of coagulative necrosis of renal tubules, which was more severe in the dogs exposed for 3 hours (Figure 5). The liver of the exposed dogs also showed fragmentation of the hepatic cords and hepatocellular necrosis dependent on degree of exposure to PFEPG exhaust fumes. The liver of dogs exposed for 2 hours and 3 hours also had mild to moderate perivascular infiltration of mononuclear inflammatory cells (Figure 6).

Clinical Observations: The feeding and drinking patterns of the dogs in all the groups did not change, though there were increase in respiratory and heart rates in the PFEPG exhaust fumes-exposed groups.

Table 8. Effects of exposure to petrol fuelled electric power generator (PFEPG) exhaust fumes on the serum levels of total cholesterol, high density lipoproteins, low density lipoproteins and triglycerides.

Parameters	Groups	Duration of Exposure (Days)			
		0	30	60	90
Total Cholesterol (mg/dl)	**A**	137.83 ± 16.30	150.46 ± 12.99	147.66 ± 6.36	150.06 ± 17.15
	B	148.00 ± 18.38	155.35 ± 16.61	151.05 ± 25.80	149.10 ± 4.94
	C	143.06 ± 18.25	153.03 ± 12.73	145.60 ± 22.64	139.66 ± 25.60
	D	151.13 ± 15.08	146.53 ± 16.25	146.16 ± 8.86	125.73 ± 6.15$^{*\alpha}$
High density lipoproteins (mg/dl)	**A**	76.16 ± 10.85	82.18 ± 5.38	87.10 ± 9.59	83.38 ± 5.07
	B	72.56 ± 5.91	73.17 ± 7.37	86.60 ± 6.62	83.33 ± 11.85
	C	84.72 ± 10.88	85.73 ± 15.54	83.19 ± 16.16	81.25 ± 9.80
	D	82.60 ± 7.77	79.60 ± 17.52	77.46 ± 12.86	69.09 ± 6.08$^{*\alpha}$
Low density lipoproteins (mg/dl)	**A**	63.11 ± 19.67	65.06 ± 15.84	63.97 ± 7.13	65.96 ± 14.21
	B	61.02 ± 8.76	64.46 ± 16.09	65.57 ± 11.43	63.64 ± 6.14
	C	61.28 ± 14.99	63.17 ± 10.95	63.20 ± 7.98	60.83 ± 12.24
	D	65.52 ± 13.27	64.39 ± 31.59	63.10 ± 9.00	60.22 ± 17.07
Triglycerides (mg/dl)	**A**	77.18 ± 15.77	72.73 ± 12.54	74.81 ± 15.60	71.43 ± 18.57
	B	76.41 ± 10.51	75.59 ± 16.69	77.63 ± 9.55	79.36 ± 13.20
	C	73.72 ± 7.99	73.80 ± 12.31	77.39 ± 19.06	81.63 ± 8.17
	D	81.56 ± 7.03	79.05 ± 11.86	81.39 ± 13.84	87.32 ± 19.18

Groups and their level of exposure to PFEPG exhaust fumes in hours per day (hpd): Group A – Unexposed Control; Group B – Exposure for 1 hpd; Group C – Exposure for 2 hpd; Group D – Exposure for 3 hpd. Values represent mean ± SD (n = 4). *Significant ($p < 0.05$) compared with group A; $^{\alpha}$significant ($p < 0.05$) compared with day 0.

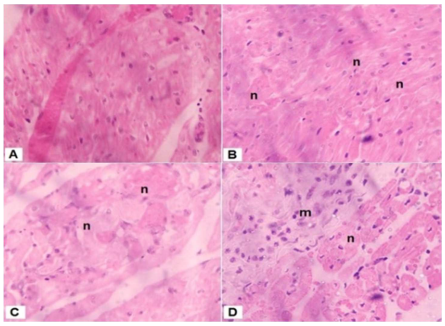

Figure 1. Cardiac tissue sections of dogs exposed to petrol fuelled electric power generator (PFEPG) exhaust fumes for 1 hour (B), 2 hours (C) and 3 hours (D) or unexposed control group (A). Coagulative necrosis and severe fragmentation of cardiac myofibres are seen in B, C and D. Moderate infiltration of mononuclear inflammatory cells (arrows) is observed in the interstitium of D. [H & E stain, ×400].

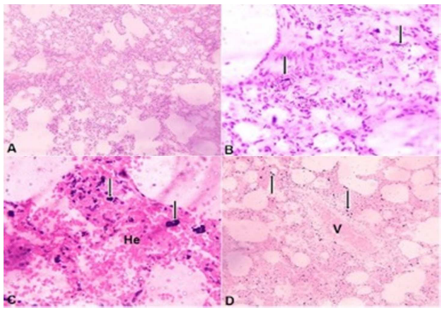

Figure 2. Lung tissue sections of dogs exposed to fuelled electric power generator (PFEPG) exhaust fumes for 1 hour (B), 2 hours (C) and 3 hours (D) or unexposed control dogs (A), showing massive deposition of black carbon particles (soot) in the interalveolar connective tissues (arrows). Note severe thickening of the interalveolar connective tissues in B due to infiltration of mononuclear inflammatory cells, severe haemorrhage (He) and alveolar necrosis in C, and severe alveolar necrosis and vascular congestion (V) in D. [H & E stain; A & D = ×100; B & C = ×400).

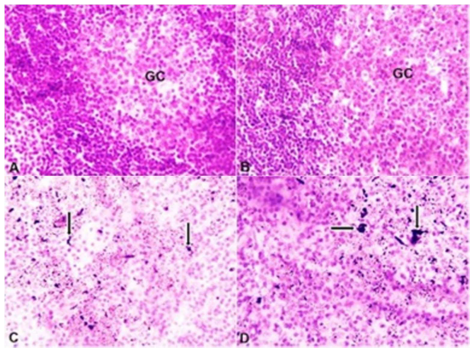

Figure 3. Sections of the bronchial lymph nodes of dogs exposed to fuelled electric power generator (PFEPG) exhaust fumes for 1 hour (B), 2 hours (C) and 3 hours (D) or unexposed control dogs (A). Note depletion of lymphoid cells in C and D and deposition of black carbon pigments (arrows), which are also being phagocytosed by macrophages. GC = Germinal centre. [H & E stain, ×400]

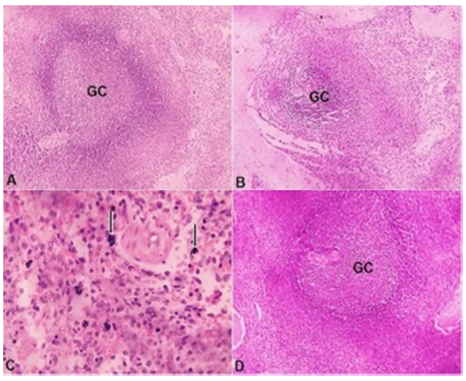

Figure 4. Splenic tissue sections of dogs exposed to fuelled electric power generator (PFEPG) exhaust fumes for 1 h (B), 2 h (C) & 3 h (D) or unexposed control dogs (A) showing moderate lymphoid depletion in the germinal centre of the white pulp. Note severe haemosiderosis in C (arrows). [H & E stain, ×400]

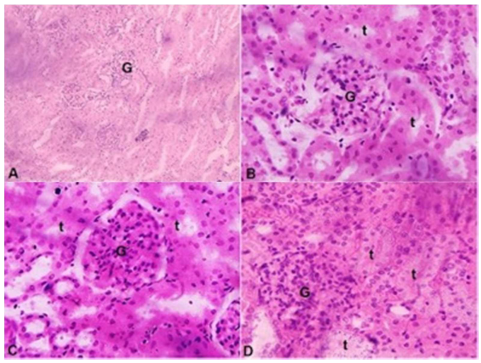

Figure 5. Renal tissue sections of dogs exposed to fuelled electric power generator (PFEPG) exhaust fumes for 1 hour (B), 2 hours (C) and 3 hours (D) or unexposed control dogs (A) showing severe tubular necrosis (t), most severe in D. G = glomerulus. [H & E stain; A = ×100; B – D = ×400)

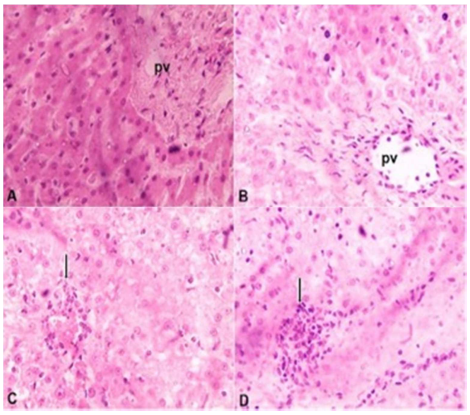

Figure 6. Sections of the liver tissue of dogs exposed to fuelled electric power generator (PFEPG) exhaust fumes for 1 h (B), 2 h (C) & 3 h (D) or unexposed control dogs (A). Note severe hepatocellular necrosis and fragmentation of the hepatic cords in B, C and D, with moderate infiltration of mononuclear inflammatory cells (arrows) in the periportal area, mostly in C and D. PV = portal vein. [H & E stain; ×400].

Discussion and Conclusion

The significantly higher PCV and RBC count on days 60 and 90 in the PFEPG exhaust fumes exposed dogs is believed to be a compensatory physiologic response to hypoxia as a result of the competitive binding of carbon monoxide (CO) to the haeme groups of haemoglobin forming carboxyhaemoglobin (Ghorani-Azam *et al*., 2016). The competitive binding of CO to the haeme groups of haemoglobin obviously led to the reduction in the Hb, MCH and MCHC, as observed in the study. A ten-day acute study on the effect of a maximum of 61 mg/m^3 volatile organic compounds (VOC) on haematology in mice revealed a decrease in the RBC count (Akpan

et al., 2014; Wang *et al*., 2016). In contrast, this study observed a trend for an initial decline in RBC count which was not significant on day 30. The difference may be due to the longer duration of our study in which the body of exposed dogs may have evolved physiologic ways to cope with the chronic hypoxia.

There was an initial increase in the total WBC count on day 30, followed by a decline on day 90 in all the exposed groups. This initial increase in total WBC may be a leukocytic response to components of PFEPG exhaust fumes such as particulate matter (PM). Fine PM is known to trigger the white blood cells proliferation, particularly monocytes and T-lymphocytes (Popell *et al*., 2016). It has been reported that through cytokine release by the neutrophils or the independent mobilization of inflammatory monocytes, macrophages are recruited which eventually release the Fas-ligand, thus, causing apoptosis of white blood cells (Brown and Savill, 1999; Henderson *et al*., 2003). On the other hand, earlier reports of the exposure to ambient PM decreased WBC counts in human patients (Ghio *et al*., 2003). In this study, the increase in ambient PM ($PM_{2.5}$ and PM_{10}) and other pollutants may have triggered inflammatory response, which eventually resulted in the initial increase in total WBC followed by a subsequent reduction, possibly due to macrophage induced apoptosis. Differential WBC counts showed that the initial rise in WBC was as a result of an increase in lymphocytes. The subsequent decrease in total WBC count on day 90 explains the severe depletion of the lymphoid cells in the BLN observed in the study. The possible consequence of a marked reduction in white blood cells is that the animal may become immuno-compromised, and therefore prone to infections by opportunistic microorganisms.

Serum ALP activity significantly increased in the exposed dogs in direct proportion to level and duration of exposure to PFEPG exhaust fumes. Alkaline phosphatase catalyses the hydrolysis of inorganic pyrophosphate, which is an inhibitor of vascular calcification (Schoppet and Shanahan, 2008). This leads to vascular stiffening and loss of elasticity and hastens the atherosclerotic process (O'Neill, 2006). Increased ALP activity and calcification have been reportedly observed in advanced atherosclerotic lesions (Tang *et al*., 2006). Our previous report showed that the exposure of dogs to PFEPG exhaust fumes led to increased expression of troponin 1 and C-reactive protein; two important markers of cardiovascular disease (Eze *et al*., 2021). It has also been reported that some components of exhaust fumes such as VOC, SO_2 and NO_2 can trigger increased inflammatory response (Li *et al*., 2017). This may explain the inflammatory reactions observed histologically in the heart, kidney and liver tissues of the PFEPG exhaust fumes-exposed dogs.

Moreover, there was a progressive increase in serum albumin concentrations of PFEPG exhaust fumes-exposed dogs on days 30, 60 and 90 from their baseline values. This could be a response to increased oxidative stress associated with some components of PFEPG exhaust fumes, such as CO, known to cause hypoxia. Since albumin represents a very abundant and important antioxidant (Roche *et al*., 2008), it has been reported that an increase in its levels is expected in conditions of hypoxia induced by exhaust fumes toxicity (Debevec *et al*., 2017). On the other hand, toxic components of fossil fuel combustion are known to decrease serum globulin levels (Marieb, 1995). Our study demonstrated a significant dose- and time-dependent decrease in serum globulin concentration in the PFEPG exhaust fumes-exposed dogs. Notably, an increase in albumin concentration without a concurrent increase in the TP concentration, as was observed in this study, would ultimately be due to decrease in the serum globulin concentration. Furthermore, the reduction in serum globulin could be related to the significant reduction in

lymphocyte counts due to a decrease in immunoglobulin production (Shenton and Rebelatto, 2015). From the foregoing, it is evident that components of PFEPG exhaust fumes are potential immune-suppressants, since the measurement of globulin levels provides an assessment of the level of circulating immunoglobulins (Shenton and Rebelatto, 2015).

Several studies have demonstrated that urea is a direct and indirect toxin, with regard to cardiovascular disease. Also, elevated serum urea concentrations are common in moderate or advanced chronic kidney disease (Laville *et al*., 2023). Urea is also associated with protein carbamylation which is linked with the progression of chronic kidney disease and exacerbation of vascular calcification (Kalim *et al*., 2014). Our study demonstrated a dose- and time-dependent elevation of serum urea levels in all the PFEPG exhaust fumes-exposed dogs, which also coincided with renal tubular necrosis. This could be an indication of impaired renal urea excretion, thus leading to a build-up of urea.

It has also been reported that renal insufficiency, marked by elevated creatinine levels, was associated with an increased risk of cardiovascular events and mortality (Fried *et al*., 2003). Our results also showed an elevated serum creatinine level in the PFEPG exhaust fumes-exposed dogs which was both dose- and time-dependent. The elevated serum creatinine levels could be a consequence of increased muscle catabolism, leading to a massive release of creatinine beyond renal clearance capacity and/or as possible renal insufficiency.

A significant decline in the total cholesterol and HDL concentrations in group D (exposed to PFEPG exhaust fumes 3 h/day) was observed on day 90, compared with the control. The decrease in total cholesterol concentration may suggest an impairment of liver cholesterol synthesis. The histopathological findings in the study agree with other previous reports in earlier animal studies. Acute and chronic exposure to diesel exhaust reportedly caused a decrease in alveolar air space volume, thickening of interalveolar septa, alveolar necrosis and haemorrhage, elevated tumour necrosis factor alpha levels in lung tissue, impairment of lung function and the penetration of the particles into the circulation through the lungs (Hiramatsu *et al*., 2003; Nemmar *et al*., 2015; De Souza Xavier Costa *et al*., 2020). The impairment of lung function and PFEPG exhaust fumes entry into the circulation may result in decreased oxygen supply to tissues and increased oxidative stress. Moreover, tissue necrosis observed in the liver, kidney, lungs and heart, as well as the depletion of the lymphoid cells seen in the spleen and bronchial lymph nodes may be associated with PFEPG exhaust fumes-induced tissue oxidative damage.

$PM_{2.5}$ is considered the most toxic of all the components of the exhaust fumes because it triggers reactive oxygen species (ROS) which have been established as the most important mediators of particle toxicity (Leni *et al*., 2020). The prolonged exposure to the exhaust fumes may have exposed the dogs to large doses of $PM_{2.5}$ which may have mediated the inflammatory responses observed in the hearts, livers, lungs and kidneys of exposed dogs. Furthermore, prolonged exposure to CO, SO_2 and $PM_{2.5}$ has been reportedly associated with a consistent increase in heart rate and hypoxia (Peter *et al*., 1999). The increase in the Hb concentration with a concurrent decrease in the PCV was thought to be evidence of hypoxia and the body's response to hypoxia respectively. Sulphur dioxide, nitrogen oxide and volatile organic compounds have also been associated with increased inflammatory responses (Li et al., 2017) and these pollutants may have played a role in the inflammatory responses observed in the organs of exposed dogs.

The study has demonstrated that the exposure of dogs to PFEPG exhaust fumes led to haematological disturbances and the increased expression of some biomarkers of heart, liver and kidney diseases. These are consistent with the earlier report that exposure of dogs to PFEPG exhaust fumes led to increased expression of troponin 1 and C-reactive protein; two biomarkers of cardiovascular disease (Eze *et al*. 2021). Our earlier findings also suggest that the altered expression of these biomarkers is cumulative. Thus, as the exposure dose and the duration of exposure increased, the changes in the expression of these biomarkers were amplified. Altogether, the findings portend a great danger to dogs and possibly humans, who stay in close proximity to these petrol electric generators during their operation. Since electric power supply is a great problem in many developing and electric power deficient regions, haematological disturbances and immunosuppression, heart, liver, lung and kidney diseases due to exposure to the exhaust emissions from these generators may constitute a significant public health problem.

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Conflict of Interest

The authors declare that they have no known competing interests financially or personally that could have influenced the work reported in this paper.

References

Åberg A, Hansen TK, Linde K, Nielsen AK, Danborg R, Widd A, Abildskov J, Jensen AD and Huusom JK (2015). A framework for modular modeling of the diesel engine exhaust gas cleaning system. *Computer Aided Chemical Eng*ineering, 37: 455 – 460. https://doi.org/10.1016/B978-0-444-63578-5.50071-2

Afsar B, Afsar RE, Kanbay A, Covic A, Ortiz A and Kanbay M (2019). Air pollution and kidney disease: review of current evidence. *Clinical Kidney Journal*, 2(1): 19 – 32. DOI: 10.1093/ckj/sfy111.

Akpan KV, Sogbanmu TO and Otitoloju AA (2014). Effects of Volatile Organic Solvents' Inhalation on Haematological and Histological Indices of *Mus Musculus*. *Current Advances in Environmental Science*, 2(2): 46 – 51.

Bell ML and Davis DL (2001). Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. *Environmental Health Perspectives*, 109(Suppl 3): 389 – 394. https://doi.org/10.1289%2Fehp.01109s3389

Brook RD, Newby DE and Rajagopalan S (2017). The global threat of outdoor ambient air pollution to cardiovascular health: time for intervention. *JAMA Cardiology*, 2: 353 – 354.

Brown SB and Savill J (1999). Phagocytosis triggers macrophage release of Fas ligand and induces apoptosis of bystander leukocytes. *Journal of Immunology*; 162: 480 – 485.

Cheesbrough M (2009). Clinical chemistry tests. In: District laboratory practice in tropical countries. Part 1. 2nd ed. Cambridge, UK: Cambridge University Press, pp. 358.

de Souza Xavier Costa N, Ribeiro Júnior G, dos Santos Alemany AA, Belloti L, Schalk AS, Cavalcante MF, Ribeiro S, Veras MM, Kallas EG, Saldiva PHN, Dolhnikoff M and da Silva LFF (2020). Air pollution impairs recovery and tissue remodelling in a murine model of acute lung injury. *Science Reports*, 10: 15314. https://doi.org/10.1038/s41598-020-7213

Debevec T, Millet GP and Pialoux V (2017). Hypoxia-induced oxidative stress modulation with physical activity. *Frontiers of Physiology,* 8: 84.

Eze UU, Eke IG, Anakwue RC, Oguejiofor CF, Onyejekwe OB, Udeani IJ, Onunze CJ, Obed UJ, Eze AA, Anaga AO and Anene BM (2021). Effects of controlled generator fume emissions on the levels of troponin I, C-Reactive protein and oxidative stress markers in dogs: Exploring air pollution-Induced cardiovascular disease in a low-resource country. *Cardiovascular Toxicology*, 21: 1019 – 1032. https://doi.org/10.1007/s12012-021-09693-8

Fried LF, Shlipak MG, Crump C, Bleyer AJ, Gottdiener JS, Kronmal RA, Kuller LH and Newman AB (2003). Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. *Journal of American College of Cardiologists*, 41(8): 1364 – 1372.

Ghio AJ, Hall A, Bassett MA, Cascio WE and Devlin RB (2003). Exposure to concentrated ambient air particles alters hematologic indices in humans. *Inhalation Toxicology*, 15: 1465 – 1478.

Ghorani-Azam A, Riahi-Zanjani BM and Balali-Mood M (2016). Effects of air pollution on human health and practical measures for prevention in Iran. *Journal of Research in Medical Sciences*, 21: 65. https://doi.org/10.4103/1735-1995.189646.

Global Burden of Disease 2015 (2016). Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet,* 388(10053): 1659 – 1724. https://doi.org/10.1016/S0140-6736(16)31679-8

Henderson RB, Hobbs JA, Mathies M and Hogg N (2003). Rapid recruitment of inflammatory monocytes is independent of neutrophil migration. *Blood,* 102: 328 – 335. https://doi:10.1182/blood-2002-10-3228

Hiramatsu K, Azuma A, Kudoh S, Desaki M, Takizawa H and Sugawara I (2003). Inhalation of diesel exhaust for three months affects major cytokine expression and induces bronchus-associated lymphoid associated tissue formation in murine lungs. *Experimental Lung Research*, 29: 607 – 622.

IFC (International Finance Corporation) (2019). The Dirty Footprint of the Broken Grid; The Impacts of Fossil Fuel Back-up Generators in Developing Countries. International Finance Corporation, www.ifc.org

Kalim S, Karumanchi SA, Thadhani RI and Berg AH (2014). Protein carbamylation in kidney disease: pathogenesis and clinical implications. *American Journal of Kidney Disease*, 64: 793 – 803. DOI: 10.1053/j.ajkd.2014.04.034

Laville SM, Couturier A and Lambert O (2023). Urea levels and cardiovascular disease in patients with chronic kidney disease. *Nephrology Dialysis Transplantation*, 38(1): 184 – 192.

Lelieveld J, Klingmüller K, Pozzer A, Pöschl U, Fnais M, Daiber A and Münze T (2019). Cardiovascular disease burden from ambient air pollution in Europe reassessed using novel hazard ratio functions. *European Heart Journal,* 40(20): 1590 – 1596. https://doi.org/10.1093/eurheartj/ehz135.

Leni Z, Kunzi L, Geiser M (2020). Air pollution causing oxidative stress. *Current Opinion in Toxicology,* 20: 1 – 8. https:// doi.org/10.1016/j.cotox.2020.02.006

Li, W, Dorans KS, Wilker EH, Rice MB, Ljungman PL, Schwatz JD, Coull BA, Koutrakis P, Gold DR, Keaney Jr JF, Vasan RS, Benjamin EJ and Mittleman MA (2017). Short-term exposure to ambient air pollution and biomarkers of systemic inflammation. The Framingham Heart Study. *Arteriosclerosis Thrombosis and Vascular Biology*, (7): 1793 – 1800.

Marieb EN (1995). Human Anatomy and Physiology. 3rd ed. California: Benjamin and Cummings Pub, Co, 585 – 611.

NRC (National Research Council) (2011). Institute for Laboratory Animal Research. The Guide for the Care and Use of Laboratory Animals. 8th ed. The National Academies Press.

Nemmar A, Al-Salam S, Yuvaraju P, Beegam S and Ali BH (2015). Emodin mitigates diesel exhaust particles-induced increase in airway resistance, inflammation and oxidative stress in mice. *Respiratory Physiology and Neurobiology*, 215: 51 – 57.

O'Neill WC (2006). Pyrophosphate, alkaline phosphatase, and vascular calcification. *Circulation Research*, 99(2): e2. https://doi.org/10.1161/01.RES.0000234909.24367.a9

Oguejiofor CF, Eze UU, Eke IG, Eze AA, Onyejekwe OB and Anene BM (2024). Adverse effects of exposure to petrol-generator exhaust fumes on the reproductive hormones, testis and spermatozoa in male dogs. *Reproductive Toxicology*, 123: 108516. https://doi.org/10.1016/j.reprotox.2023.108516

Omaye ST (2002). Metabolic modulation of carbon monoxide toxicity. *Toxicology*, 180(2):139-150. http://dx.doi.org/10.1016/S0300-483X(02)00387-6

Park SK (2017). Ambient air pollution and type 2 diabetes: do the metabolic effects of air pollution start early in life? *Diabetes*, 66: 1755 – 1757. https://doi.org/10.2337%2Fdbi17-0012

Peters A, Per, S, Dfiring A, Stieber J, Koenig W, Wich-mann SH (1999). Increases in heart rate during an air pollution episode. *American Journal of Epidemiology,* 150: 1094 – 1098.

Pope CA and Dockery DW (2006). Health effects of fine particulate air pollution: Lines that connect. *Journal of Air and Waste Manage*, 56(6): 709 – 742.

Popell CA, Bhatnagar IA, McCracken JP, Abplanalp W, Conklin DJ and O'Toole T (2016). Exposure to Fine Particulate Air Pollution Is Associated With Endothelial Injury and Systemic Inflammation. *Circulation Research*, 119: 1204 – 1214. https://doi.org/10.1161/CIRCRESAHA.116.309279

Roche M, Rondeau P, Singh NR, Tarnus E and Bourdon E (2008). The antioxidant properties of serum albumin. *FEBS Letters*, 582: 1783 – 1787. https://doi.org/10.1016/j.febslet.2008.04.057

Schoppet M and Shanahan CM (2008). Role for alkaline phosphatase as an inducer of vascular calcification in renal failure? *Kidney International*, 73(9): 989 – 991. https://doi.org/10.1038/ki.2008.104

Shenton JM and Rebelatto MC (2015). Consequences and indicators of test article-related immune suppression in non-human primates In: Bluemel J, Schenck E, Korte S, Weinbauer GF (Ed.), The Non-human Primate in Nonclinical Drug Development and Safety Assessment. Elsevier Inc. DOI: https://doi.org/10.1016/C2013-0-04736-6

Slaoui M and Fiette L (2011). Histopathology procedures: from tissue sampling to histopathological evaluation. In: Gautier JC (Ed.), Drug Safety Evaluation: Methods in Molecular Biology (Methods and Protocols). Totowa, New Jersey: Humana Press, pp. 69 – 82. https://doi.org/10.1007/978-1-60761-849-2_4

Tang FT, Chen SR, Wu XQ, Wang TQ, Chen JW, Li J Bao LP, Huang HQ and Liu PQ (2006). Hypercholesterolemia accelerates vascular calcification induced by excessive vitamin D via oxidative stress. *Calcified Tissue International*, 79: 326 – 339. DOI: 10.1007/s00223-006-0004-8

Wang W, Liu F, Liu H, Chen W, Si X and Ma X (2016). Effects of immunological and hematological parameter in mice exposed to mixture of volatile organic compounds. *Inhalation Toxicology*, 28(4): 164 – 169. http://dx.doi.org/10.3109/08958378.2016.1148087.