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Histopathological and Biochemical Investigations of Gasoline Generator Exhaust Exposure in Adult Male Wistar Rats.

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Abstract.
The use of gasoline generator sets to generate electricity has increased as a result of Nigeria's epileptic power supply. The exhaust from these engines pollutes the air and has been linked to a variety of ailments, including cancer and metabolic disorders. This study aims to investigate oxidative stress and inflammatory responses as well as histopathological changes in the liver and kidneys of exposed rats. Adult male rats were divided into four groups and exposed for 4, 8, or 12 weeks at time points of 2, 4, and 8 hours respectively. While nitric oxide (NO) was evaluated spectrophotometrically, enzyme-linked immunosorbent assay (ELISA) was used to analyse superoxide dismutase (SOD), glutathione peroxidase (GPx), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF- α). The organs were also processed for histopathological examinations. Findings revealed elevated IL-6 and TNF-α values ($p<0.05$) with a significant decline in the GPX values of the exposed rats ($p<0.05$), with a substantial increase in the NO values and SOD activity seen ($p<0.05$). Additionally, the livers of the exposed rats showed histological alterations such as cytoplasmic vacuolation and sinusoidal congestion, while the kidneys revealed poor architecture, congested interstitial spaces, and tubular degeneration. Exposure to gasoline generator exhaust induces oxidative stress, elevates proinflammatory markers IL-6 and TNF- α, and alters the histoarchitecture of the liver and kidney.

Keywords: Air pollution, Nigeria, gasoline generator, oxidative stress.

Introduction

The greatest hazard to human health is air pollution, which annually results in millions of deaths and years of healthy life loss (World Health Organization, 2021). The primary causes of atmospheric pollution include energy production, transportation, including gasoline and diesel vehicles, domestic energy consumption, such as gasoline generators, agriculture, and industry. The particulate components of air pollution are connected to the activation of an inflammatory response which has been associated with various diseases, including cancer, cardiovascular, neurological, and pulmonary disorders (Lewtas, 2007; Arias-Pérez *et al.,* 2020). Fuel combustion from diesel and gasoline accounts for more than 75% of atmospheric pollutants, making it a significant cause of air pollution in urban areas (Libalova *et al.,* 2018; Ribeiro *et al.,* 2018). With approximately 250 distinct hydrocarbons, gasoline has the largest proportion of all transportation fuels. The hydrocarbon composition alters the emission profile, and the resulting exhaust has a detrimental effect on the cell (Mueller *et al.,* 2021). Petrol exhaust nanoparticles constitute vital components of PM2.5 (Bayram *et al.,* 1998) and it is generated when petrol undergoes combustion in automobile engines thus giving rise to combustion-derived nanoparticles (Donaldson *et al.,* 2005). Currently, 43% of the total population, lacks access to electricity, most of them in sub-Saharan Africa (International Energy Agency, 2022). Generally, gasoline and diesel generators are run using fossil fuels which release pollutants such as Carbon monoxide (CO), oxides of Nitrogen (NOx),

Carbon dioxide (CO2,), Oxides of Sulphur (SOx), PM such as PM2.5 and PM10, etc. when burnt (Giwa *et al.,* 2023). These generators constitute a major pollution source around residential areas and workplace and it emits pollutants that contaminate the air we breathe causing adverse health effect (Oguntoke & Adeyemi, 2017). In addition to attacking biomembranes and causing lipid peroxidation chain events, Reactive oxygen species (ROS) is a crucial component of apoptosis and autophagy and can consequently cause a variety of cell death (Su *et al.,* 2019). Studies have shown that exposure to PM 2.5 increases the risk of COVID-19 as well as the likelihood of developing more severe symptoms (Yao *et al.,* 2020; Zhu *et al.,* 2020). Also, it has been documented that PM can induce oxidative damage in addition to the production of ROS either via non-enzymatic processes or by cytochrome p-450 catabolic enzymatic reactions (Bai *et al.,* 2001, Ma & Ma, 2002, Siegel *et al.,* 2004). Furthermore, PM 2.5 has also been reported to increase the degree of lipid peroxidation in many organs, including the liver, lungs, testicles, and heart (Bourdon *et al.,* 2012). The oxidative stress response to various air pollutants is still being studied. Against this background, we investigated the oxidative stress and inflammatory response, as well as histologically analysed the liver and kidneys of rats chronically exposed to gasoline generator exhaust.

Materials and methods.

Animals

Adult male Wistar rats weighing between 180-200g procured from the animal holding of the University of Benin, Benin City, Nigeria were acclimatised for two weeks and fed with a standard rodent diet sourced from Edo Feed Mills, Benin City, and water ad libitum. The experimental animals were handled following the International Humane Animal Care Standards (Hau & Van Hoosier, 2002).

Ethical considerations

This study was approved by the Biomedical Research and Ethics Committee of the Ministry of Agriculture, Benin City, Edo State, Nigeria with an ethical clearance registration number V.1040/77.

Mode of exposure to generator exhausts

We modified an exposure method adapted from a study by Henz & Maeder (2005). The rats were subdivided into four groups that consisted of a control group (A) and three test groups labelled groups B, C, and D respectively. 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. They were briefly exposed in a locally fabricated glass exposure chamber with an inlet and an outlet valve with dimensions of (L x W x H) mm 710 x 480 x 370 for thirty 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 with AC output of 220v and DC output of 12V/ 8.3A.

Experimental design

The control group consisted of 12 rats which were unexposed and labelled as group A, while rats in group B were subdivided into 3 subgroups consisting of 4 rats each and exposed at time points of 2, 4, and 8 hours respectively. Rats in Group C were subdivided into 3 subgroups and exposed at time points of 2, 4, and 8 hours respectively for 2 months while group rats in Group D were equally divided into 3 subgroups and exposed at time points of 2, 4, and 8 hours respectively for 3 months. The rats were euthanized upon completion of the experiments across the various months of exposure.

Tissue Preparation and Histopathological Analysis

The rats were euthanized by cervical dislocation, and blood samples were collected for biochemical analysis via cardiac puncture, while the liver and kidneys were excised and immediately fixed in 10% neutral-buffered formalin for 24 hr. The tissues were histologically processed as described by Bancroft *et al.* (2019). The stained sections obtained were viewed using an Olympus CX33 microscope for histopathological changes, and photomicrographs were taken using a Kodak PIXPRO A2527 digital camera.

Biochemical studies.

The plasma concentrations of SOD, GPx, IL-6, and TNF-α were measured using ELISA kits

purchased from Elabscience Biotechnology Inc. USA, while NO was analysed using the conventional spectrophotometric method as described by Ridnour *et al.* (2000).

Statistical analysis

Statistical Package for Social Science Version 25 (SPSS, Cary, NC, USA) was used for the analysis of data obtained and one-way analysis of variance (ANOVA) was performed on the data acquired. A student t-test was used to assess the data collected at various time intervals of 2 hours, 4 hours, and 8 hours of exposure throughout the study. Data were presented as mean ± standard deviation (mean ± SD). The data obtained across the weeks were statistically compared to the control, and differences between the mean values were evaluated by the least significant difference test with p-values $\leq$ 0.05 considered to be statistically significant.

Results

Histopathological findings

Histological evaluation of the liver and kidneys of the exposed rats revealed various cytopathic features at various exposure time points (2 hours, 4 hours, and 8 hours) in relation to the duration (4, 8 weeks, and 12 weeks) respectively. There was no pathological lesion in the liver of the unexposed control rats (Fig. 1a). Also, rats exposed for 2 hours daily for 4 weeks did not exhibit any pathological lesions, with normal-appearing hepatocytes and sinusoids devoid of inflammation or congestion (Fig. 1b). Rats exposed for 4 hours daily at 4 weeks had a mild sinusoidal haemorrhage (Fig. 1c), whereas exposure for 8 hours daily revealed a congested central venule and sinusoids with hepatocyte degeneration and cytoplasmic vacuolation (Fig. 1d). Rats exposed for 2 hours daily at 8 weeks, showed a moderately congested central venule (Fig. 2a), while congestion in the sinusoidal spaces and portal vein was evident at 4 hours exposure (Fig. 2b) also 8 hours daily exposure at 8 weeks was consistent with the 4-hour exposed group (Fig. 2c). At 12 weeks, the 2 hours exposed group had a moderately congested central vein (Fig. 2d), which is consistent with the exposure time points of 4 hours (Fig. 2e), and 8 hours respectively (Fig. 2f).

The kidneys of the unexposed control rats were devoid of pathological lesions (Fig. 3a). The exposed rats' kidneys exhibited no abnormalities after 2 hours of exposure at 4 weeks (Fig. 3b) however, a few collapsed tubules with reduced luminar space were observed at exposure time points of 4 hours (Fig. 3c). Additionally, a poor renal histoarchitecture with a significantly dilated interstitial space, coupled with tubular degradation and inflammation, was seen after 8-hour exposure at 4 weeks (Fig. 3d). Exposure at 8 weeks revealed mild vascular congestion at a 2-hour exposure time point (Fig. 4a), whereas a daily exposure at 4 hours revealed poor histoarchitecture along with mesangial hyperplasia in the glomerulus and congested interstitial space (Fig. 4b). Rats exposed at a time point of 8 hours at 8 weeks showed a focal degenerating area and collapsed renal tubules (Fig. 4c). Kidneys of rats exposed at a time point of 2 hours daily at 12 weeks had congested interstitial spaces, (Fig. 4d) while exposure at 4 hours revealed a poor architecture evident with collapsed renal tubules and congestion within the interstitial spaces (Fig. 4e). 12 weeks of exposure for 8 hours daily resulted in mild vascular congestion and collapsed tubules lacking luminar space (Fig. 4f).

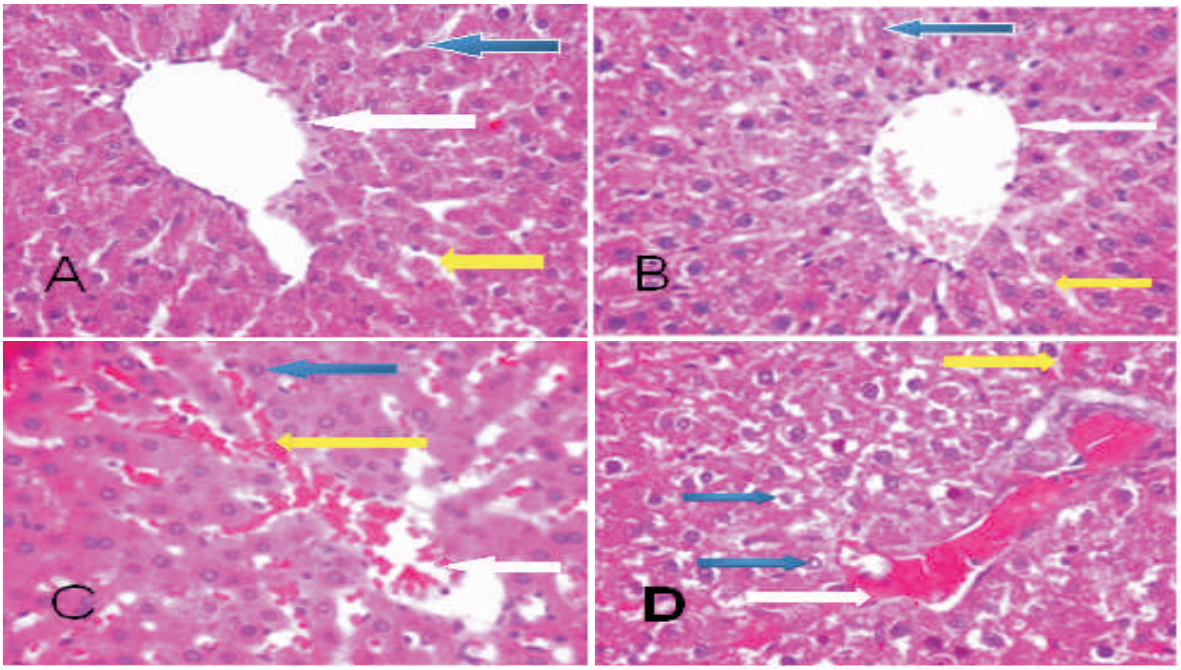

Figure 1. Section of liver stained with H and E **a.** The liver of the unexposed control rats shows normal central venule (white arrow), the hepatocytes appear normal, with the sinusoids devoid of inflammation and congestion **b.** Rats exposed for 2 hours daily at 4 weeks showed normal central venules (white arrow), the morphology of the hepatocytes appears normal (blue arrow), with sinusoid devoid of inflammation (yellow arrow) **c.** Rats exposed for 4 hours daily at 4 weeks showed mildly haemorrhagic sinusoids (yellow arrow) with the central vein (white arrow) and hepatocytes appearing normal (blue arrow). **d.** Rats exposed for 8 hours daily for 4 weeks showed congested central venule (white arrow), with some hepatocyte degeneration while some showed cytoplasmic vacuolation (blue arrow), with mild sinusoidal congestion (yellow arrow) magnification = x400.

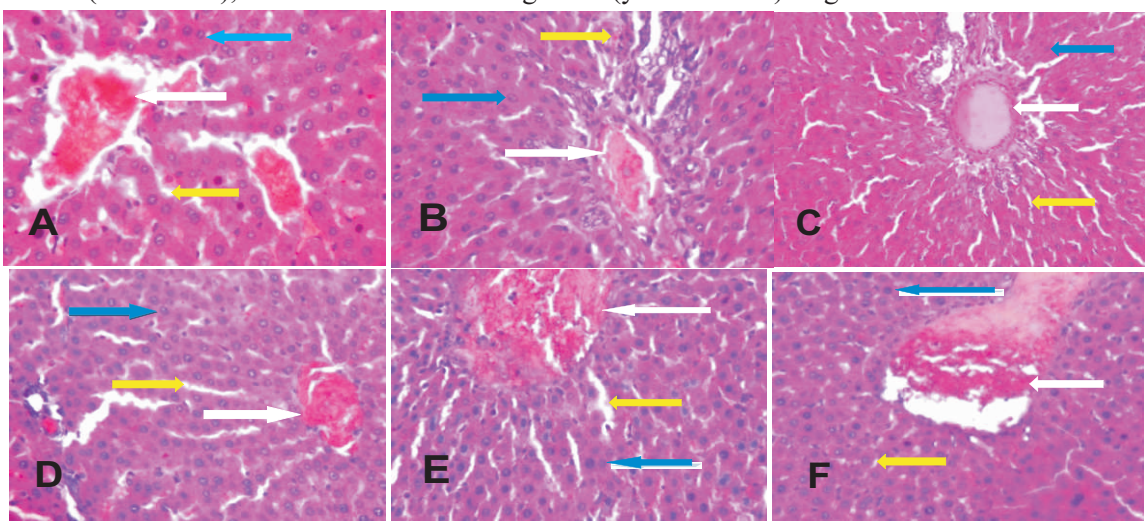

Figure 2. a. The liver of rats exposed for 2 hours daily at 8 weeks showed moderately congested central venules (white arrow) with normal appearing hepatocytes (blue arrow), and un-infiltrated sinusoidal space (yellow arrow) **b.** Exposure for 4 hours daily at 8 weeks showed a mildly congested portal vein (white arrow) with normal hepatocytes (blue arrow), with mildly congested sinusoids (yellow arrow) **c.** 8 hours daily exposure at 8 weeks showed a mildly congested portal vein (white arrow) with normal appearing hepatocytes (blue arrow) and sinusoidal space (yellow arrow) **d**. Exposure for 2 hours daily at 12 weeks showed a moderately congested central vein (white arrow), normal hepatocytes (blue arrow) with non-infiltrated sinusoidal space (yellow arrow) **e.** 4 hours daily exposure at 12 weeks showing a mildly congested central vein (white arrow), with normal hepatocytes (blue arrow) and non-infiltrated sinusoids (slender arrow) **f.** 8 hours daily exposure at 12 weeks showing congested central venules (white arrow) with normal hepatocytes (blue arrow), and non-infiltrated sinusoidal spaces (yellow arrow) magnification= x400.

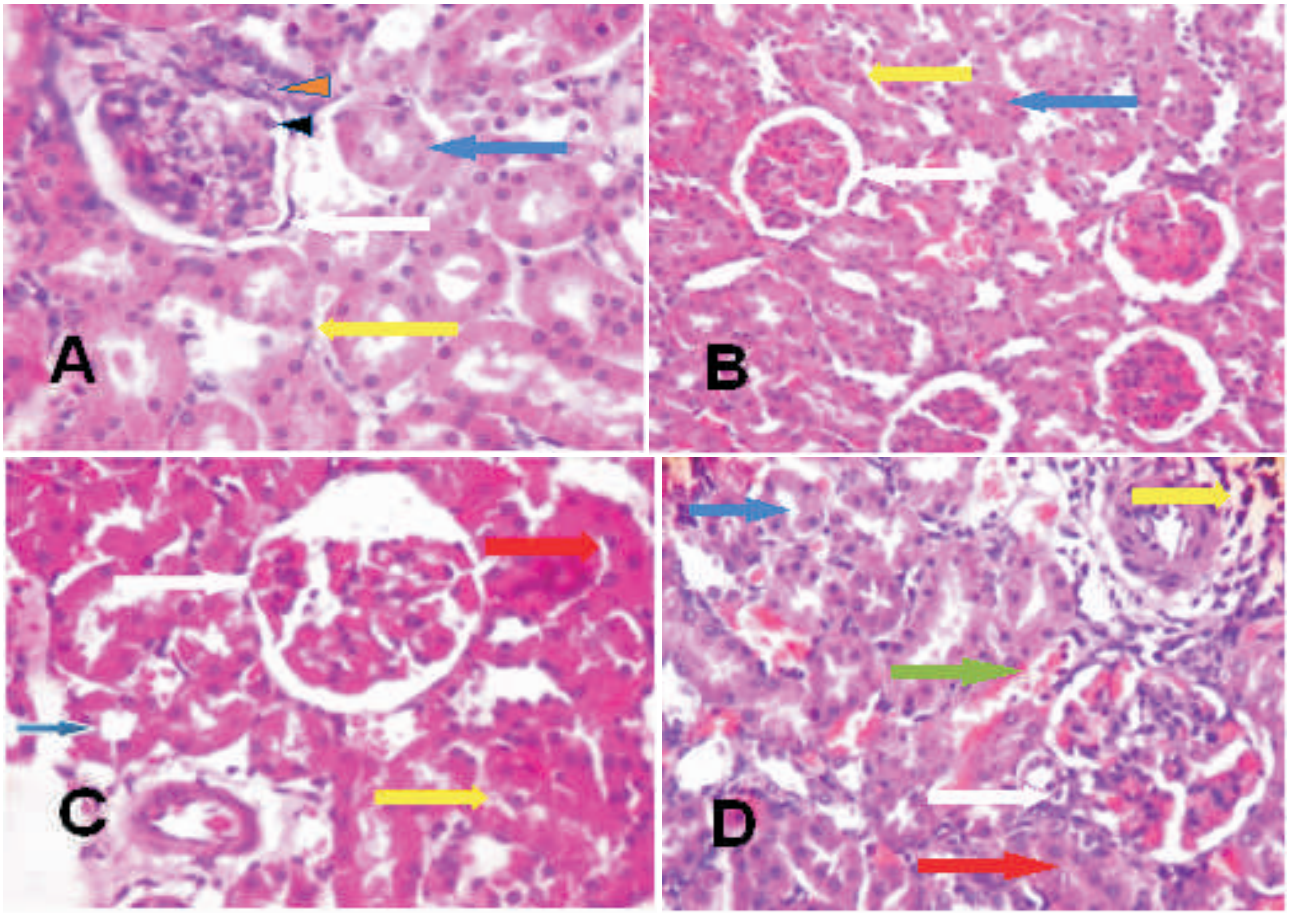

Figure 3. Kidney section of rats stained with H and E. **a.** Unexposed control rats had normal histoarchitecture with the renal cortex showing normal glomeruli with normal mesangial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), The juxtaglomerular apparatus shows the juxtaglomerular cells and macula densa (orange arrowhead), podocytes are seen within the renal corpuscles (black arrowhead) and the interstitial spaces appear normal (yellow arrow) **b.** Exposure for 2 hours daily at 4 weeks showed normal kidney histoarchitecture with the renal cortex showing consisting of glomeruli (white arrow), the renal tubules (blue arrow), and the interstitial spaces (yellow arrow) appearing normal. **c**. Exposure for 4 hours daily at 4 weeks showed normal glomeruli (white arrow), most of the renal tubules appear normal (blue arrow), few tubules appear collapsed with reduced lumina spaces (red arrow), the interstitial spaces appear normal (yellow arrow). **d.** Exposure for 8 hours daily at 4 weeks shows normal glomeruli (white arrow) with few renal tubules appearing normal (blue arrow), while others show renal tubular epithelial degeneration with collapsed or reduced lumina spaces (red arrow), the interstitial spaces are moderately dilated and mildly congested (green arrow) and infiltration of inflammatory cells evident (yellow arrow) magnification= x400.

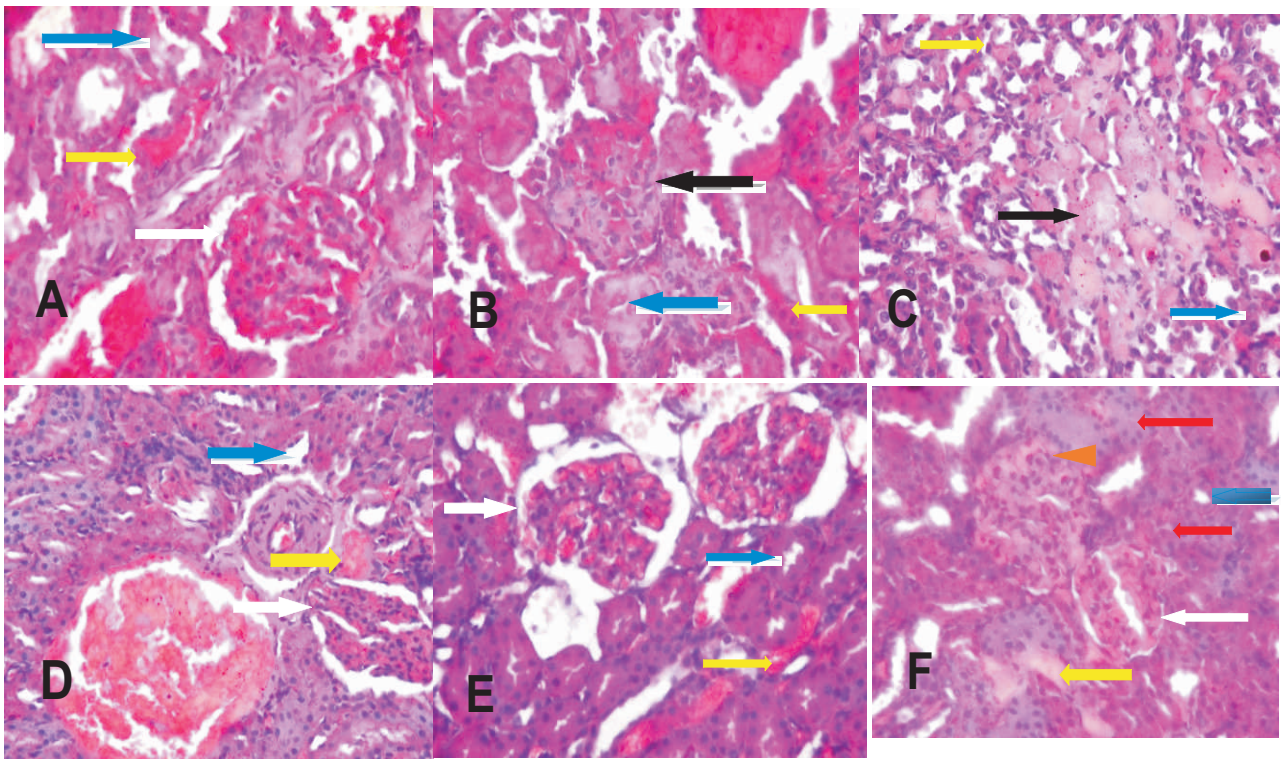

Figure 4. a. Daily exposure for 2 hours at 8 weeks showing the renal cortex (white arrow), and renal tubules (blue arrow) appearing normal with mildly congested interstitial spaces (yellow arrow). **b.** The 4 hours exposure at 8 weeks reveals a poor histoarchitecture characterized by mesangial hyperplasia in the glomerulus (black arrow), the renal tubules appear collapsed with the loss of luminar spaces (blue arrow), and congested interstitial spaces (yellow arrow) **c.** 8 hours daily exposure at 8 weeks showed some collapsed renal tubules (blue arrow), with moderately congested interstitial spaces (yellow arrow), the collecting tubules of the medulla shows a focal area of degenerated tubules (black arrow) **d.** 2 hours daily exposure at 12 weeks showed a mildly congested interstitial space (yellow arrow) with normal glomeruli (white arrow) and renal tubules (blue arrow). **e.** 4 hours daily exposure at 12 weeks showed poor architecture characterized by, collapsed renal tubules with reduced luminar spaces (blue arrow), and mildly congested interstitial spaces (yellow arrow) with normal appearing glomeruli (white arrow) **f.** 8 hours daily exposure at 12 weeks showed poor architecture, with the renal cortex showing the glomeruli consisting of mesangial cells and reduced capsular spaces (white arrow). A glomerulus without capsular space is evident (orange arrowhead), some of the renal tubules are normal (blue arrow) while some collapsed tubules lacking luminar spaces are seen (red arrow), the interstitial spaces are mildly congested (yellow arrow) magnification=x400.

Biochemical studies

By comparing the NO, SOD, GPX, IL-6, and TNF-α values between the exposed rats at different time points with the unexposed control rats, the biochemical findings of this study demonstrated the deleterious effects of exposure to gasoline generator exhaust. After 4 weeks of exposure, the weight of the rats in each group, as well as the oxidative stress parameters, are displayed in Table 1. Comparative analysis of the parameters showed that the NO and SOD values varied significantly between the exposed rats and the unexposed control group at each of the different time points (Table 2). When compared to the control, there was a significant difference in the GPX, IL-6, and TNF-α levels at the various exposure time points ($p< 0.05$) (Table 2). Rat weights at various exposure times relative to the unexposed control were statistically insignificant ($p> 0.05$) (Table 2). The mean and standard deviation of the weight values and oxidative stress parameters for each group at

eight weeks are displayed in Table 3. The NO and SOD values at various time intervals were statistically significant when compared to the control ($p<0.05$) (Table 4). When compared to the control, the exposed rats' levels of IL-6, and TNF-α were elevated (Table 4). As of the twelfth week, Table 5. displays the mean and standard deviation for the weight values and oxidative stress parameters across all groups. In comparison to the unexposed control group, the NO, SOD, GPX, and TNF- α were statistically significant throughout the different time points of exposure (Table 6). When compared to the unexposed control, the weight of the rats exposed for 2 hours and 4 hours significantly decreased ($P<0.05$), however, the other groups remained statistically insignificant ($P>0.05$). The comparative analysis of the weight values and oxidative stress metrics across the various exposure time points relative to the weeks is shown in Table 7.

Table 1. Mean and standard deviation of the oxidative stress parameters and weight values across the various groups in the fourth week of exposure.

Parameter	Control	2 hours	4 hours	8 hours
NO (µmol/L)	208±4.64	248±6.04	266.75±1.299	279.75±0.43
SOD (ng/mL)	1.88±0.04	2.63±0.178	3.10±0.12	3.18±0.04
GPx (pg/mL)	60.75±0.829	19.50±0.866	20.00±0.00	41.00±1.00
IL-6 (pg/mL)	5.00±0.71	12.00±2.449	11.75±1.30	14.00±1.00
TNF-a (pg/mL)	43.25±0.43	62.50±1.66	65.00±0.71	63.75±1.30
Weight (gram)	217.2±12.75	206.4±7.23	202.4±10.56	202.6 ±12.34

Table 2. Comparative analysis of the oxidative stress parameters and weight values across the various groups in the fourth week.

Parameter	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
NO (µmol/L)	**"p"**	0.00005*	0.00001*	0.00001*	0.001*	0.00005*	0.00001*
SOD (ng/mL)	**"p"**	0.0002*	0.00001*	0.00001*	0.0044*	0.001*	0.177
GPx (pg/mL)	**"p"**	0.00001*	0.00001*	0.00001*	0.177	0.00001*	0.00001*
IL-6 (pg/mL)	**"p"**	0.002*	0.0001*	0.00001*	0.440	0.119	0.03
TNF-α (pg/mL)	**"p"**	0.00001*	0.00001*	0.00001*	0.03*	0.17	0.10
Weight (g)	**"p"**	0.09	0.05	0.07	0.27	0.30	0.49

Table 3. Mean and standard deviation of the oxidative stress parameters and weight values across the various groups in the eighth week.

Parameters	Control	2 hours	4 hours	8 hours
NO (µmol/L)	211±1.00	256±2.5	268.25±2.05	282.25±2.2
SOD (ng/mL)	1.95±0.09	2.78±0.083	3.05± 0.087	3.35±0.087
GPx(pg/mL)	60.25±1.30	20.5±1.66	20.25±1.79	43.25±1.79
IL-6(pg/mL)	4.75±0.43	12.5±1.66	11.75±1.30	18.75± 1.30
TNF-α(pg/mL)	44.00±1.00	60.75±1.30	65.50±1.66	66.5±1.66
Weight (g)	231±13.02	200.4±3.2	201.8±9.95	216.4±16.19

Table 4. Comparative analysis of the oxidative stress parameters and weight values across the various groups at eight weeks of exposure.

Parameters	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
NO (µmol/L)	"p"	0.00001*	0.00001*	0 .00001*	0.0003*	0.00001*	0.00009*
SOD (ng/mL)	"p"	0.00001*	0.00001*	0.00001*	0.004*	0.00008*	0.003*
GPX(pg/mL)	"p"	0.00001*	0.00001*	0.00001*	0.43	0.00001*	0.00001*
IL-6 (pg/mL)	"p"	0.0001*	0.00006*	0.00001*	0.28	0.001*	0.0003*
TNF-α (pg/mL)	"p"	0.00001*	0.00001*	0.00001*	0.004*	0.002*	0.24
Weight (g)	"p"	0.0009*	0.004*	0.04*	0.40	0.04*	0.08

Table 5. Mean and standard deviation of the oxidative stress parameters and weight values across the various groups at the twelfth week of exposure.

Parameters	Control	2 hours	4 hours	8 hours
NO (µmol/L)	211±: 1.22	253±9.35	276±8.746	288±0.43
SOD (ng/mL)	1.88±0.08	2.93±0.108	3.53± 0.29	3.38±0.11
GPx (pg/mL)	61.25±0.83	18.75±2.17	20.00±0.00	42.5± 2.50
IL-6 (pg/mL)	4.50±0.50	12.00±3.08	11.5± 0.866	17.5±2.50
TNF-α (pg/mL)	43.50± 1.12	63.50±1.50	66.5±1.50	68.75±1.10
Weight (g)	223.6±3.34	205.2±3.06	201.4±6.83	216±15.07

Table 6. Comparative analysis of the oxidative stress parameters and weight values across the various groups at the twelfth week.

Parameters	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
NO (µmol/L)	"p"	0.0001*	0.00001*	0.00001*	0.01*	0.0003*	0.03*
SOD (ng/mL)	"p"	0.00001*	0.00004*	0.00001*	0.007*	0.001*	0.21
GPx (pg/mL)	"p"	0.00001*	0.00001*	0.00001*	0.177	0.00001*	0.00001*
IL-6 (pg/mL)	"p"	0.003*	0.00001*	0.00006*	0.397	0.03*	0.004*
TNF-α(pg/mL)	"p"	0.00001*	0.00001*	0.00001*	0.02*	0.001*	0.04*
Weight (g)	"p"	0.00002*	0.0002*	0. 18	0.17	0.10	0.06

Table 7. Comparative analysis of the oxidative stress parameters and weight values across the various groups in relation to weeks and the time point of exposure.

		2hrours			4hrours			8hrours		
Parameters	P-value	4weeks vs 8weeks	4weeks Vs 12weeks	8weeks vs 12weeks	4weeks vs 8weeks	4weeks vs 12weeks	8weeks vs 12weeks	4weeks vs 8weeks	4weeks vs 12weeks	8weeks vs 12weeks
NO (µmol/L)	"p"	0.038*	0.01*	0.305	0.16	0.059	0.09	0.04*	0.00001*	0.002*
SOD (ng/mL)	"p"	0.117	0.02*	0.053	0.29	0.027*	0.016*	0.01*	0.01*	0.38
GPx (pg/mL)	"p"	0.19	0.29	0.15	0.41	0.50	0.41	0.05	0.185	0.34
IL-6 (pg/mL)	"p"	0.39	0.50	0.41	0.50	0.40	0.40	0.001*	0.03*	0.235
TNF-α (pg/mL)	"p"	0.10	0.23	0.03*	0.32	0.08	0.23	0.03*	0.001*	0.04*
Weight (g)	"p"	0.08	0.38	0.03*	0.47	0.44	0.47	0.11	0.10	0.49

Discussion

Nigeria's persistent power supply crisis causes citizens to rely on alternative energy sources, such as gasoline generators, to generate electricity. Exposure to gasoline generator exhaust induces the processes associated with oxidative stress coupled with an inflammatory response by increasing proinflammatory cytokines like IL-6 and TNF-α, as well as an exposure-dependent variation in the NO, GPX, and SOD values in the exposed rats. Selective bioaccumulation of PM in different organs has the potential to cause a variety of diseases, including cardiovascular disease, diabetes mellitus, immunological disorders, asthma, and chronic respiratory disease (Choi *et al.,* 2020). We demonstrate that gasoline exhaust has a detrimental effect on mammalian cells. The heterogeneity of gasoline exhaust has obscured the mechanism; however, the induction of oxidative stress, as well as DNA damage, has been linked to the development of numerous diseases, including cancer (Møller *et al.,* 2008). Histopathological changes in the kidneys and liver indicate that the kidneys were more seriously impacted as compared to the liver. There were no detectable lesions at the time point of exposure of 2 hours in the liver at 4 and 8 weeks of exposure; however, exposure at the time point of exposure of 2 hours in the kidney at 4, 8, and 12 weeks of exposure revealed alteration in the kidney histoarchitecture as well

as congestion within the interstitial space. The liver's sophisticated detoxifying capabilities and nutrient supply may have contributed to the liver's lesser histological changes as compared to the exposed rats' kidneys. After 4 weeks, rats exposed for 2 hours showed no histomorphological changes while exposure for 4 hours at 4 weeks exhibited mildly hemorrhagic sinusoids. Rats exposed for 8 hours at 4 weeks had cytoplasmic vacuolation and mildly congested sinusoids, implying that prolonged exposure to gasoline generator exhausts has a deleterious impact on the liver. According to Siegel *et al.* (2004), the production of ROS is accompanied by the induction of oxidative damage by PM and carbon black. Additionally, non-enzymatic mechanisms or catabolic enzymatic cytochrome p-450 reactions due to PM exposure have been reported (Bai *et al.* 2001; Ma & Ma 2002). Our findings demonstrate apparent histopathological features such as vacuolation and sinusoidal congestion in the liver, as well as epithelial and tubular degradation and mesangial cell hyperplasia in the exposed rats' kidneys. Also, Mauderly *et al.* (2014) previously reported a light microscopic influx of inflammatory cells and epithelial hyperplasia in the liver and kidneys of rats exposed to gasoline exhaust, which aligns with the findings in our study as evidenced by histological alterations in the organ studied coupled with elevated IL-6 levels in the exposed rats. Studies by Gerlofs-nijland *et al.* (2013) also indicated that exposure to PM elevates IL-6 levels in addition to cytotoxicity in human bronchial epithelial cell cultures.

In this study, rats exposed for 2 hours daily for 8 weeks had a moderately congested central venule, while rats exposed for 4 hours daily for 8 weeks had mildly congested sinusoids and portal vein with findings at 8 hours daily for 8 weeks consistent with the 4hours exposed group. Our results are consistent with the findings of Adeegbe *et al.* (2022) who reported that rats exposed to gasoline generator emissions developed histopathological lesions in their liver tissues, including sinusoidal congestion and vacuolar degeneration of the hepatocytes. According to the observations, rats exposed for 2 hours daily for 12 weeks had mildly congested central venule, although the architecture of the hepatocytes and sinusoidal space appeared normal. The findings at the 12-week mark across the 4 hours and 8 hours time points are consistent with the 2 hours of daily exposure at 12 weeks. According to Kim *et al.* (2014), PM increases oxidative stress, damages DNA via strand breaks, and also plays a role in the development of steatohepatitis by modifying lipid metabolism and producing an inflammatory milieu, hence exacerbating non-alcoholic steatohepatitis.

Nephrotoxic substances can impair kidney function in a variety of ways. While Ueng *et al*. (2004) documented a time and dose-dependent increase in cytochrome P-450-dependent monooxygenase and glutathione S-transferase in the visceral organs of rats exposed to PM from motorcycle exhaust, Ali *et al.* (2022) reported possible changes in biochemical and haematological parameters in diesel generator workers.

The kidneys of the unexposed control group were devoid of pathological lesions, and the 2-hour time point exposure at 4 weeks revealed no significant lesions, with the glomerulus appearing normal and the histoarchitecture of the renal tubules adequately preserved as observed in this study. However, rats exposed at 4 hours daily at 4 weeks exhibited a few collapsed tubules with reduced luminar space, with a more severe impact apparent at 8 hours of exposure evident by dilated vasculature and congested interstitial spaces. According to meta-analyses by Lee 2020, PM can transverse the bloodstream and bioaccumulate in various regions of the body, compromising their function and harming lung function (Xu et al., 2018; Su *et al.,* 2019). Air pollution, according to Rossner *et al*. (2021), has a detrimental effect on cells at the molecular level by causing damage to macromolecules, which can result in DNA or protein adducts, nucleic acid strand breaks, and rearrangements that may be predisposed to mutations and initiate carcinogenesis. Additionally, environmental and occupational exposure to gasoline combustion exhaust in vehicles has been linked to an increased risk of developing cancer (Mueller *et al.,* 2021). PM causes lipid peroxidation in some organs, including the kidneys, and acts as a

systemic toxin (Bourdon *et al.,* 2012). At 8 weeks, rats exposed for 2 hours daily showed mild vascular congestion, whereas rats exposed for 4 hours daily showed impaired renal histoarchitecture, collapsed renal tubules, a loss of luminar space, and moderately congested interstitial spaces. Additional findings included collapsing renal tubules and a focal area of degeneration in the medullar collecting tubules at an exposure time point of 8 hours at 8 weeks. The burden of chronic kidney disease (CKD) has been linked to fine particulate matter air pollution in low- and lower-middle-income countries (Bowe *et al.,* 2020). The renal tubules and interstitial spaces displayed mild vascular congestion 12 weeks after a 2-hour exposure, while the glomerulus exhibited a relatively normal histoarchitecture. In addition, at 12 weeks, rats treated for 4 hours had mild interstitial space congestion and compressed renal tubules with reduced luminal gaps. The final group, which was exposed for 8 hours daily at 12 weeks, had generally poor architecture with moderately congested interstitial spaces. According to reports, particles with an aerodynamic diameter of less than 10 μm deposit primarily in conducting airways while particles with a diameter of less than 2.5 μm transverse the alveolar-capillary background and selectively bioaccumulate in specified organs, with the primary mechanism of injury thought to involve oxidative stress, inflammation, genotoxicity, and cell death (Arias-Pérez *et al.,* 2020). Given that significantly higher NO values were observed in the exposed rats at various time points of exposure in this study, it gives credence to our findings on the deleterious effects of gasoline generator exhaust exposure.

We suggest that all intracellular oxidative stress indicators were dose-dependent since significant variations in antioxidant (SOD and GPX) levels were observed in rats exposed at various time points. The findings of this investigation supported those of Durga *et al.* (2014), who reported that pro-inflammatory cytokines (TNF-α, IL-6) were generated in lung A549 cells and RAW 264.7 macrophages after 12 and 24 hours of exposure to petroleum exhaust nanoparticles (PENPs). This verifies the oxidative stress and inflammatory response identified in our work by measuring proinflammatory mediators in the blood of exposed rats.

Oxidative stress can damage macromolecules by producing ROS, which is harmful to lipids, proteins, and nucleic acids. Lipid peroxidation generates a highly reactive substance that attacks other macromolecules, causing oxidative damage (Su *et al.,* 2019). Furthermore, unlike DNA, oxidized lipid molecules have no mechanisms for repair. The biochemical analyses in our study revealed the effect of gasoline exhaust on the induction of IL-6 and TNF-α, which may cause oxidative damage to macromolecules including the generation of ROS. Furthermore, we observed that exposed rats have significantly higher NO levels at various exposure time points. Since superoxide is the source of the majority of ROS, enzymatic antioxidants have been shown to protect against oxidative stress. SOD is one of these antioxidants that is essential for cell survival because it protects cells from oxidative attack by degrading ROS (He *et al.,* 2017) and catalyses the conversion of superoxides to oxygen and hydrogen peroxide (Wang *et al.,* 2018).

We observed increased SOD activity across all groups, with the greatest significance observed at 4 and 8 hours of exposure as compared to the unexposed control, as well as a decrease in GPX values across all groups. At 4 weeks, IL-6 levels were higher in all exposed groups than in the control at 2 hours, 4 hours, and 8 hours. Furthermore, at different exposure time points, all exposed groups had significantly higher TNF-α values than the control. The pathogenic processes underlying the link between air pollution and kidney disease are not well understood. However, Xu *et al.* (2018) reported a potential mechanism connecting inhaled particles with the spillover of cytokines causing inflammation and oxidative stress, as well as circulating autoantibodies against antiphospholipase A2 receptor (PLA2R), formation of immune complexes, and vascular (endothelial) injury. We found that exposure causes oxidative stress, as evidenced by elevated NO levels at various time points, and stimulates the proinflammatory markers IL-6 and TNF-α, which are chemokines that are released into the

circulation and cause inflammation, which can lead to organ impairment (Xu *et al.,* 2018, Wang *et al.,* 2020). At week 8, the exposed groups' NO, SOD, GPX, IL-6, and TNF- α levels were higher than those of the unexposed control rats. Multiple isoenzymes of GPX exhibit different tissue-specific expression patterns (Matouškov, 2018; Bela *et al.,* 2015) and persistent oxidative stress triggers GPX to become an active H2O2 scavenging enzyme after the inhibition of ascorbate peroxidases (Halušková *et al.,* 2009). It detoxifies hydroperoxides by catalysing the reduction of H_2O_2 and hydroperoxides to water and alcohol (Bela *et al.,* 2015). A statistically significant variation was found in the NO values in the rats exposed at a time point of 2 hours at 4 weeks vs 8 weeks and 4 weeks vs 12 weeks, respectively. Furthermore, a significant difference in the 8hr time point of exposure at 4 weeks vs 8 weeks, 4 weeks vs 12 weeks, and 8 weeks vs 12 weeks, was observed. PM has been shown to induce a significant inflammatory response in both in vitro and in vivo models, which is triggered by alveolar macrophages and airway epithelial cells, which then produce pro-inflammatory mediators after phagocytosing the PM, which aids the lung immune response while also causing oxidative stress and systemic inflammation (Yang *et al.,* 2016, Wang *et al.,* 2017). Our findings supported this, as there was a significant increase in NO levels in the exposed rats at various time points, as well as elevated values of proinflammatory mediators such as IL-6 and TNF-α, which can cause cellular and tissue damage by activating TLR4 (Toll-like receptor-4) via DAMPs and PAMPs (Lakey *et al.,* 2016; Münzel *et al.,* 2018).

Conclusion

The consequences of gasoline generator exhaust exposure have been highlighted in this study, further supporting its deleterious effect on mammalian cells. Exposure causes oxidative stress by generating ROS, as evidenced by changes in oxidant-antioxidant homeostasis. This can trigger inflammatory responses that transverse the alveolar-capillary barrier to selectively bioaccumulate the liver and kidneys, emphasizing the cellular toxicity evident by histopathological changes observed in the organs studied.

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