ORIGINAL ARTICLE

Toxicological impact of gasoline generator emissions using rat models

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ABSTRACT

Nigeria has been identified as one of the largest active users of gasoline generators globally. Due to the widespread incessant use of gasoline generators, the present study assessed the exposure to gasoline generator emission (GGE) and some toxicity endpoints. This study employed a laboratory-based experimental design. An emission chamber was designed using clear thermoplastic to house the rats during exposure to 0.5KVA GGEs. Three experimental groups, each with eight rats respectively, were subjected to 5, 10, 15 minutes of of daily GGE exposure for fourteen days for fourteen days, while the fourth was a control group. Selected air quality parameters were measured using the appropriate samplers. Assays like liver function tests, antioxidant enzyme activity, and micronucleus frequency were conducted after the experiment. Micronucleus frequency significantly increased with time of GGE exposure ($p=0.000$), while the liver tissue antioxidant activity of the test groups was significantly higher than that of the control group ($p<0.05$). Moreover, histopathological examinations revealed lesions like sinusoidal congestion and vacuolar degeneration of the hepatocytes in liver tissues of the exposed rats. However, the liver function test showed a non-significant association with their level of GGE exposure. The result of this study suggested that exposure to GGEs at a non-lethal concentration in a sub-acute or chronic manner has deleterious effects on mammalian biochemical systems. Therefore, the use of exhaust filters and improvement in the ventilating systems of houses are encouraged to reduce exposure rates in developing countries like Nigeria, where GGEs are widespread due to erratic electricity supply.

Key words: gasoline generator emissions, toxicity effects, health hazards, air pollutants

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INTRODUCTION

Air pollution is the contamination of indoor and/or outdoor environments by any chemical, physical, or biological agent that modifies the natural characteristics of the atmosphere. Common sources of air pollution are combustion devices, industrial facilities, motor vehicles, forest fires, and other forms of fire outbreaks. Air pollutants of major public health concerns are particulate matter, CO, oxides of sulphur and nitrogen, and ozone.[1]

It has become very essential for almost every Nigerian home and business that demands electric power to own at least one generator because of the epileptic form of power supply in the country and this has sporadically increased the importation of generators. According to the Centre of Management development, there were about 60 million generators in Nigeria at the ratio of one per household of 2.5 people in 2013.[2] As reported between 2009 and 2012, Nigeria spent approximately N1.3 trillion on the importation of generators, which is the largest among nations of the world.[3]

The estimated carbon dioxide (CO_2) emitted from the world's electrical power industry is 10 billion tonnes yearly.[4] With continuous growth in industrialization and urbanization, one can only expect a commensurate increase in the rate of generator importation and usage in Nigeria, thereby leading to a simultaneous increase in the country's contribution to the global CO_2 burden. Besides its CO_2 release, these generators have also been reported to generate a broad range of gaseous and particulate environmental pollutants, of which CO is a notable constituent.[5-7] CO arises from the incomplete combustion of fuel and has been reported to be directly hazardous to human health. It is particularly lethal because it is odourless and colourless, thereby leaving those exposed unaware of the exposure. Depending on the proximity of those affected, there are different detrimental consequences of exposure to CO from gasoline or diesel engines. The effects of exposure to high concentrations of CO has resulted in several deaths in Nigeria.

The impacts of health and environmental hazards associated with constituents of emissions from gasoline generators have been recorded over the years. Environmental pollutants and petrol fumes have been identified as factors that can enhance peroxidative processes and oxidative stress within cells.[8] A study showed that generator fumes contained levels of poly-aromatic hydrocarbons (PAHs) capable of increasing the risk of cancer in an environment where generator use is common.[9] Other reported adverse effects of exhaust pollutants included increased infant mortality, acute heart attacks, chronic deficits in lung development of children aged 10–18 years, and ovarian cancer.[10-13] Numerous epidemiological studies have also shown that exposure to a large amount of petroleum-related particles causes an increase in morbidity and mortality, which often arises from respiratory diseases and their negative impact on human health.[14-16]

However, the scientific literature on the toxicological risks of exposure to generator smoke remains incomplete. There are numerous biological indices yet to be characterized in humans after exposure to gasoline generator emission (GGE) at varying time periods and concentrations. Research in these areas will add to the knowledge base on the level of risk posed by exposure to generator emissions on the liver and the antioxidant status in the body. Pulmonary data from exposure to GGE are available, but little is known on the hepatotoxic effect of GGEs in animals.[17] Thus, the study was designed to determine the effects of GGE on some biomarkers of liver dysfunction, antioxidant status, and histology. In this study, we attempt to record the biotoxicity and genotoxicity effects of GGEs on albino Wistar rats by measuring some of these parameters.

Histopathological changes in the liver tissues of both the control and experimental animals were examined to support the biochemical findings.

MATERIALS AND METHODS

Experimental Design

The emission chamber was designed using the dynamic (airflow) system. The material used was transparent thermoplastic, 3 mm thick. The dimension of the chamber was 70 cm by 60 cm by 60 cm (27 by 24 by 24 inches). The top of the chamber was removable to aid cleaning of the interior chamber space; when fixed, however, it was air-tight. To allow movement of air, ventilation orifices were made at 30 cm and 50 cm from the base of the chamber; each hole was 5 cm x 5 cm wide, with two rows of six holes each and on two sides of the chamber only. At 28 cm (11 inches) from the base of the chamber, four suspended platforms – each in one corner of the chamber were created. The platform served to carry the gas and particulate matter (PM) monitors, namely CO and PM monitors.

The generator used as the source of emission for the experiment was the Sunshine SS1200 portable generator which had been in use for 8-12 months.

Procurement of rats and acclimatization

Forty male albino rats of Wistar strain at seven weeks of age with their weights between 90-105g were procured from the Veterinary Physiology Department, University of Ibadan. The animals were fed with rat chow feed and water *ad libitum* for three weeks, at the end of which they attained weights between 143g and 211g.

Exposure Design

The generator exhaust was placed at 5 cm from the chamber. The fumes could infiltrate the chamber space where the rats were placed. For every exposure session, the generator was always allowed to run for five minutes before being placed against the chamber to allow the stabilization of the emissions from the generator. The generator was placed on a rubber tyre to attenuate the noise from the generator. The control group was subjected to every other treatment apart from being placed in the exposure chamber.

To determine the length of time that the rats can survive inhalation of fumes from the generator, a pilot test was conducted. Eight experimental rats ≥ 250 g were placed in the emission chamber and whole-body inhalation of fumes was adopted. The rats were closely monitored for behavioral and morphological responses and the mortality rate was set at 50%.

After the exposure limit test, the test animals were then divided into four groups of eight rats each. Three of the groups were experimental groups exposed at 15, 10, and 5 minutes, respectively, while the fourth group was the control group. The type of inhalation employed for this study was whole-body inhalation. The exposure was done daily for fourteen days and at regular intervals between the hours of 11 am and 12 pm. During exposure periods, the rats were monitored for morphological and behavioural changes.

Some components of the generator emissions were monitored during the periods of exposure; CO was monitored using EXTECH CO monitor model CO10 and PM with a diameter below 2.5 microns ($PM_{2.5}$) was monitored using the Thermo Scientific PM monitor (pDR1500).

Sample Collection

Twenty-four hours after the last exposure, the animals were sacrificed by cervical dislocation.

Biological analysis

Micronucleus assay was conducted by assessing the polychromatic erythrocytes of the bone marrow as stipulated by past literature.[18] Serum samples were analysed for estimation of Aspartate Aminotransferase (AST), Alanine aminotransferases (ALT), and Alkaline Phosphatase (ALP) using the standard colorimetric method.[19] The activity of Superoxide Dismutase (SOD) was determined by the method of Misra and Fridovich.[20] Glutathione Peroxidase (GPX) activity was measured according to the procedure of Rotruck et al. with some modifications. Catalase (CAT) activity was determined according to the method of Claiborne.[21,22] The liver specimens were routinely processed and sectioned at 4-5µm thickness. The obtained liver sections were stained with Hematoxylin-Eosin (H&E) dye before mounting in a neutral Dibutylphthalate Polystrene Xylene (DPX) medium. Prepared slides were examined at 100 x and 400 x magnifications.

Data analysis

Data obtained were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20.0. For micronucleus assay of the bone marrow, at least 1000 anucleate Polychromatic Erythrocytes (PCEs) were counted for each animal. Observations included the number of PCEs with Micronuclei (MC). Data were presented as Mean ± Standard deviation. One-way Analysis of Variance (ANOVA) was conducted to determine significant differences across the groups. A P-value less than 0.05 ($P< 0.05$) was considered statistically significant.

Ethical Consideration

The research protocol was reviewed and approved by the University of Ibadan Animal Ethics Review Committee.

RESULTS

Lethal Concentration (LC_{50}) Determination

The rats were exposed to emission for a total of 40 minutes. At about twenty-eight minutes into the exposure, the CO, $PM_{2.5}$ monitors read 1708 ppm and 98 mg/m³, respectively. At this stage, there was reduced movement in the housing chamber and some of the rats were observed to stop breathing as their abdominal movements ceased completely. After forty minutes, the generator was switched off and the chamber was opened. Mortality was recorded in seven out of the eight rats (88% casualty). The only survivor was then kept in a well-aerated cage to allow maximum resuscitation. About one hour later, it died. The LC_{50} value was 1708 ppm/98mg/m³ at 20 minutes. The maximum exposure period for any of the experimental groups in the main study was about half of the time taken to arrive at 50% casualty in the exposure limit test.

Results from Experimental Setup

A similar setup was made for the 4 groups (Group 1- 15 minutes of daily exposure; Group 2-10 minutes of daily exposure; Group 3- 5 minutes of daily exposure; Negative control- No exposure) after Lethal Dose (LD_{50}) determination. The rats were weighed before the exposure to generator emissions commenced and were also weighed on a weekly basis to check for changes as a result of the exposure. After one week of exposure, the percentage of change in weight for the control, group 1, group 2, and group 3 were -0.91, 0.07, and 10.79% respectively. From day 8 to day 14, there was -7.00, -0.50, and 0.45% change in weight respectively. At the end of the experiment, the exposed rats had a significantly higher weight compared to the control group. The rats in Group 3 exposure group showed significantly lower liver weight compared with group 2 ($p<0.05$), while the rats in group 1 showed

significantly higher liver weight compared to all other study groups. Table 1 shows the average weight differences between the groups across different exposure duration, while Figure 1 shows the liver weight differences among the groups.

Table 1 Mean weight and standard deviation (in grams) of rats across the groups during the exposure period

	Before exposure	At the end of one week	At the end of two weeks
Control	162.75±10.96	171.38±7.61	177.50±8.37
Group 1 (15 mins)	178.75±15.55	176.75±16.49	164.38±10.77
Group 2 (10 mins)	175.63±19.76	175.75±15.98	174.88±14.89
Group 3 (5 mins)	173.43±13.78	192.14±14.78	193.00±14.63

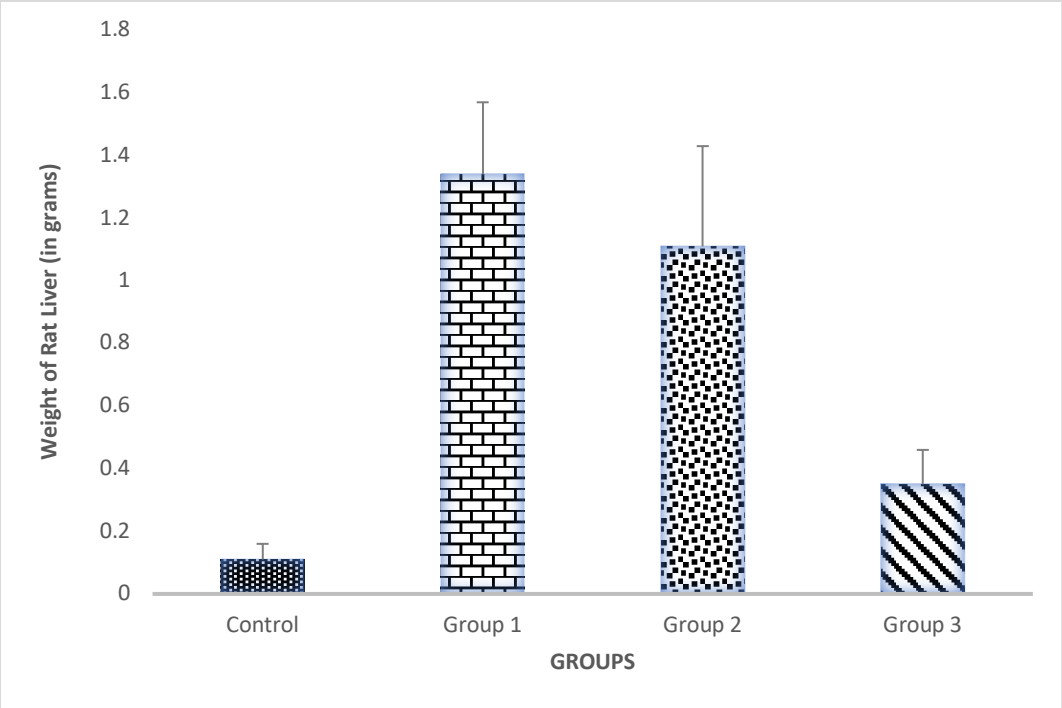

Figure 1 Effect of GGE on the weight of rat liver across the groups

Furthermore, the AST, ALT, and ALP levels of rats in group 1 (15 minutes exposure), group 2 (10 minutes exposure), group 3 (5 minutes exposure) were not significantly higher compared to the control group, while the SOD and GPx activity of rats in group 1 (15 minutes exposure) decreased significantly compared to rats in group 2, group 1, and control group, respectively. An increase in the duration of exposure reduced the activities of antioxidant enzymes in the rats. Table 2 shows the activity of AST, ALT, and ALP among the groups, while Figure 2 shows the activity of SOD, GPx, and CAT in the liver of rats exposed to the varying durations of gasoline generator emission exposure.

Table 2 Effect of GGE on the parameters of liver function in rats

Parameters	Control	Group 1 (15 Mins)	Group 2 (10 Mins)	Group 3 (5 Mins)
Aspartate Aminotransferase (I.U/L)	110.99 ± 26.09	126.75 ± 24.78	126.04 ±30.74	143.34± 48.72
Alanine Aminotransferase (I.U/L)	32.29 ±8.37	26.60 ±7.13	38.30 ±11.75	38.87 ±11.75
Alkaline Phosphatase (I.U/L)	214.51 ±62.97	194.33 ±62.32	106.61 ±54.55	154.19 ±68.37

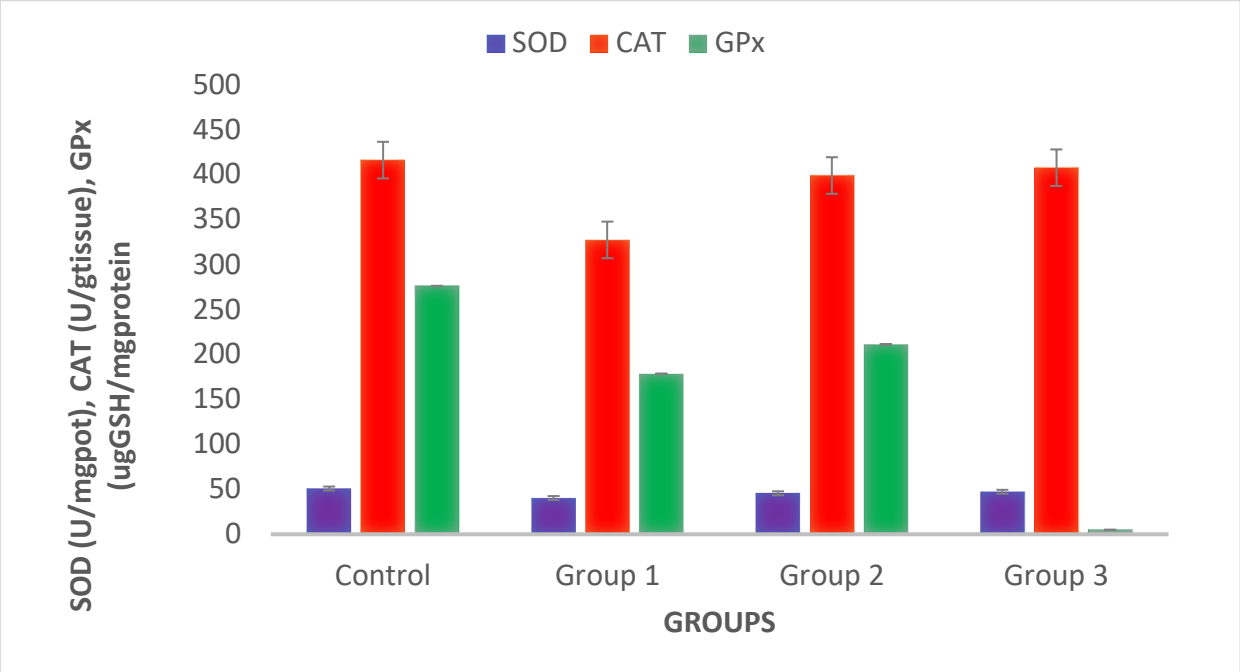

Figure 2 Effect of GGE on antioxidant enzymes

Histopathological Conditions

Histologic examination of the effect of gasoline generator fumes on the livers of exposed rats showed minimal liver damage. There was however sinusoidal congestion, mild vacuolar degeneration of hepatocytes, and moderate hepatic necrosis. The severity of these lesions was in line with the period of exposure. Representative sections of the liver from each group are displayed below. Plate 1 is a representation of the control group where the liver had no visible lesion. Plate 2 is a representation of the fifteen minutes exposure group (group 1), which revealed very mild portal and central venous congestion. Plate 3 is a representation of the ten minutes exposure group (group 2) with moderate portal and sinusoidal congestion and with mild diffuse vacuolar degeneration of hepatocytes. Plate 4 is a representation of the five minutes exposure group (group 1) where severe portal and sinusoidal congestion was observed. Some foci of haemorrhage into the parenchyma and mid-moderate hepatic necrosis were also observed. All the

photomicrographs were taken at 100X magnification.

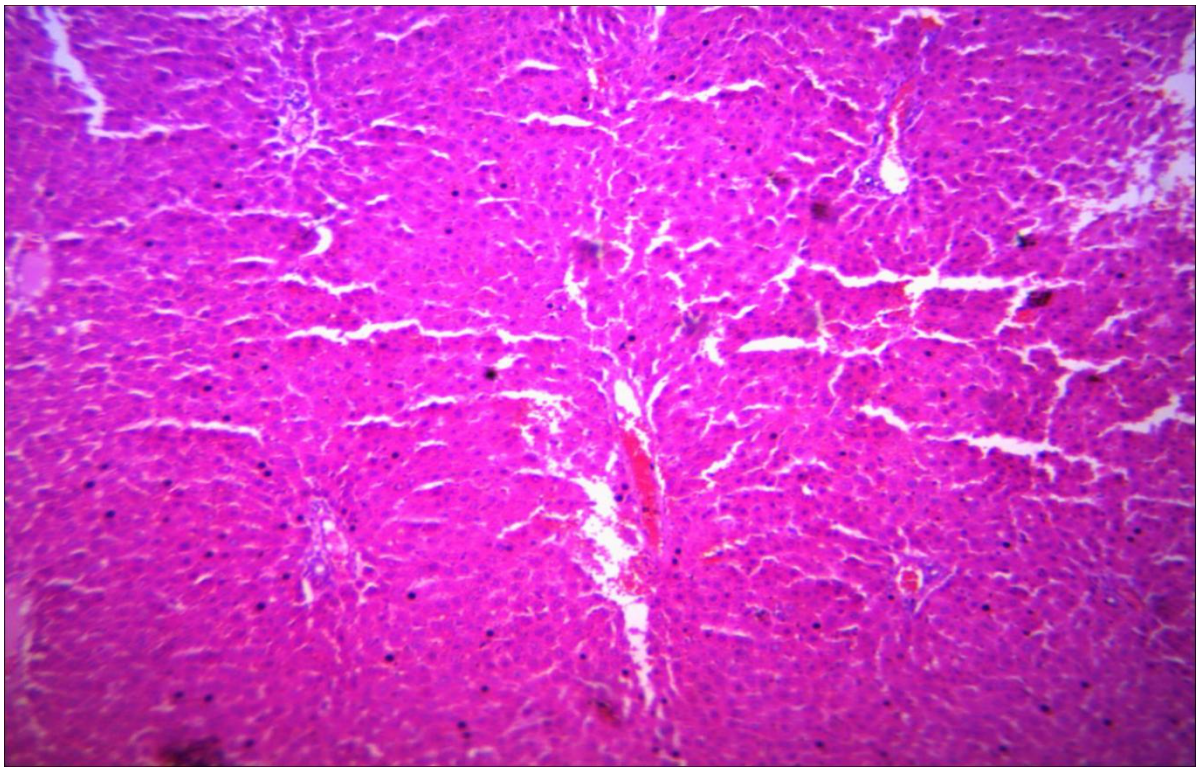

Plate 1 Liver cell condition from the control group (No visible lesion seen)

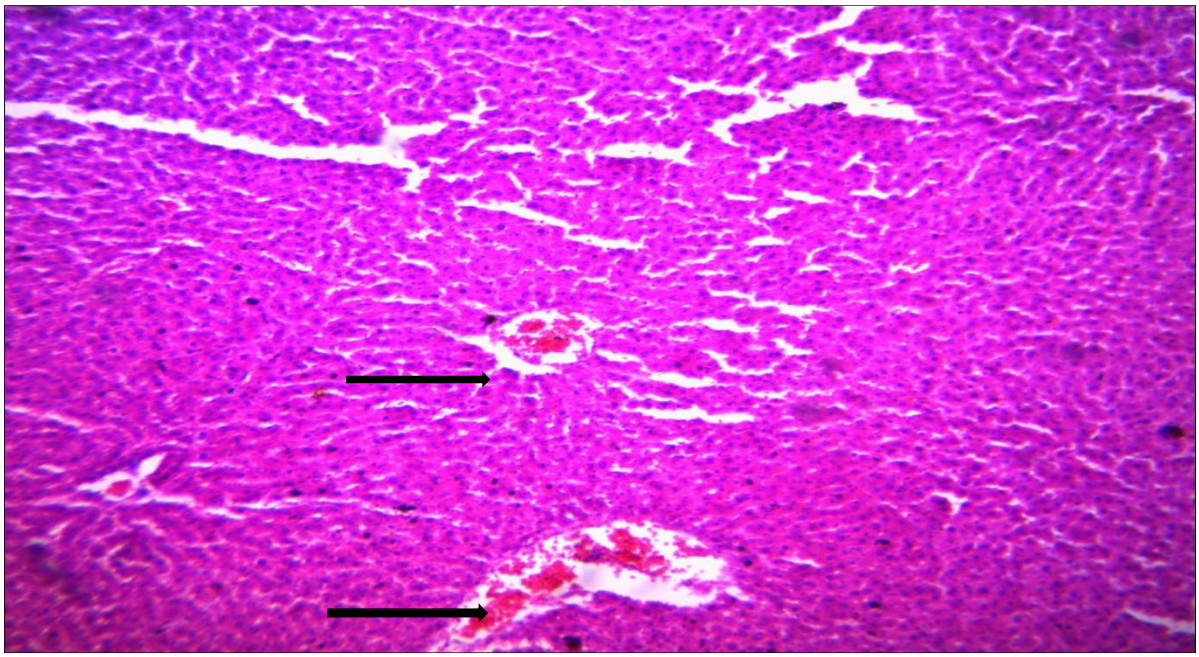

Plate 2 Liver cell condition from fifteen minutes exposure- Group 1 (Very mild portal and central venous congestion {arrows})

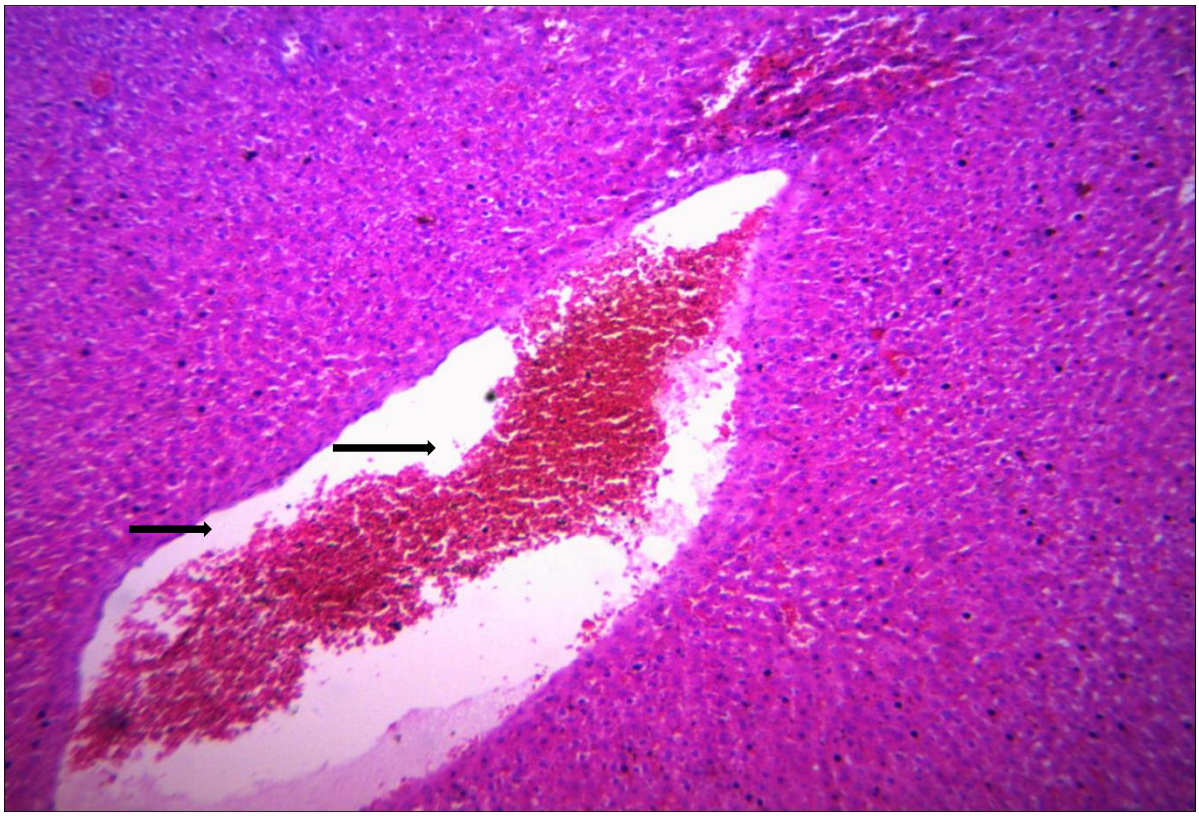

Plate 3 Liver cell condition from ten minutes exposure- Group 2 (Moderate portal {arrows} and sinusoidal congestion, with mild diffuse vacuolar degeneration of hepatocyte)

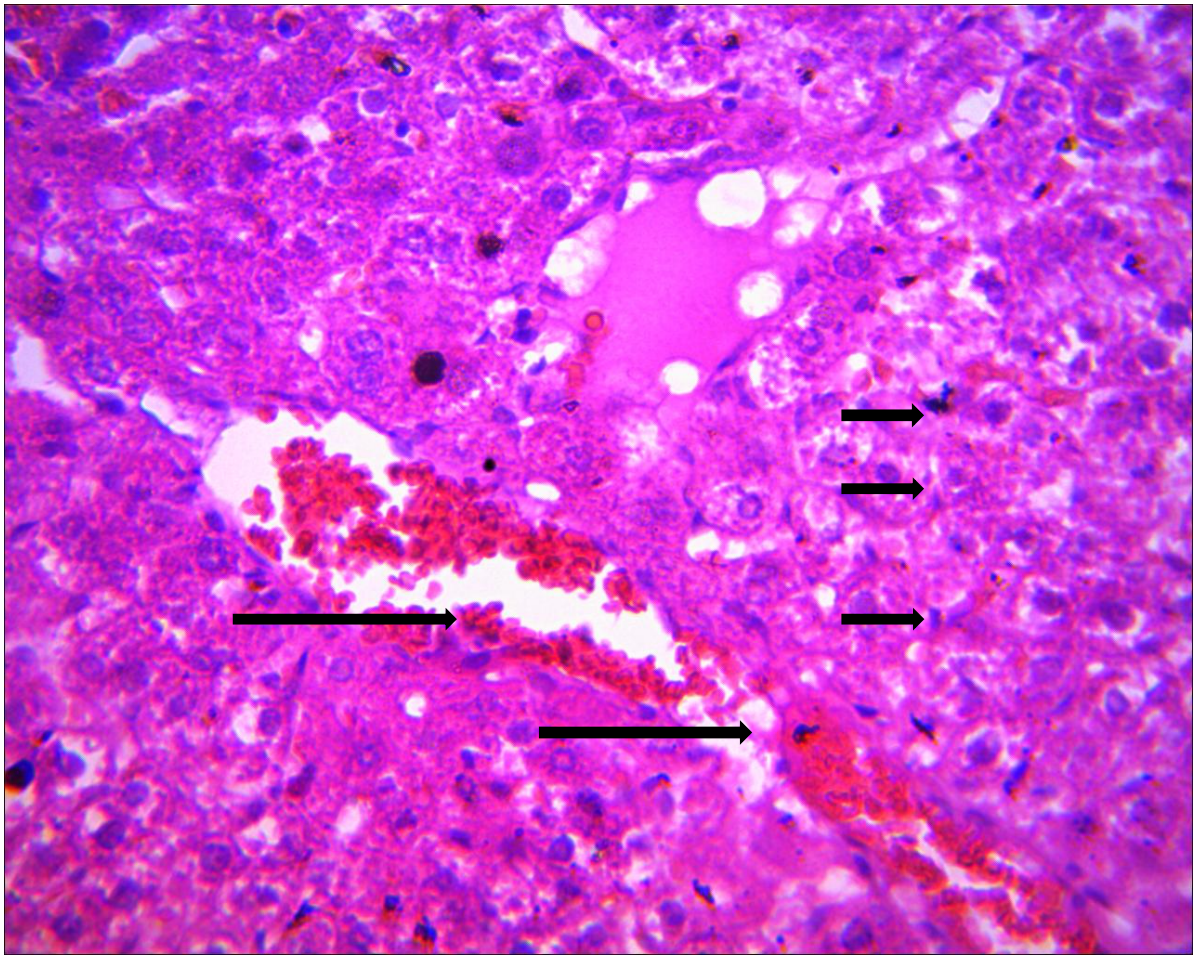

Plate 4 Liver cell condition from five minutes exposure- Group 3 (Severe portal and sinusoidal congestion {long arrows}. There are some foci of haemorrhage into the parenchyma. There is also mid-moderate hepatic necrosis {shorter arrows})

Micronucleus Frequency

The micronucleus frequency (%MNPCE) for the three exposure groups and the control group are presented in Fig. 2. The highest frequency of 1.34±0.23 was recorded in group 1 (15 minutes exposure group) while the lowest frequency (0.11± 0.05) was recorded in the control group. The 10 minutes group showed higher frequency (1.11 ± 0.32) compared to group 3 (5 minutes exposure group) (0.35 ± 0.11). Figure 3 shows the levels of CO/PM2.5 observed in the groups in relation to the micronuclei frequencies in the groups and Figure 4 shows the effect of GGE on %MNPCE in exposed rats via a whisker plot.

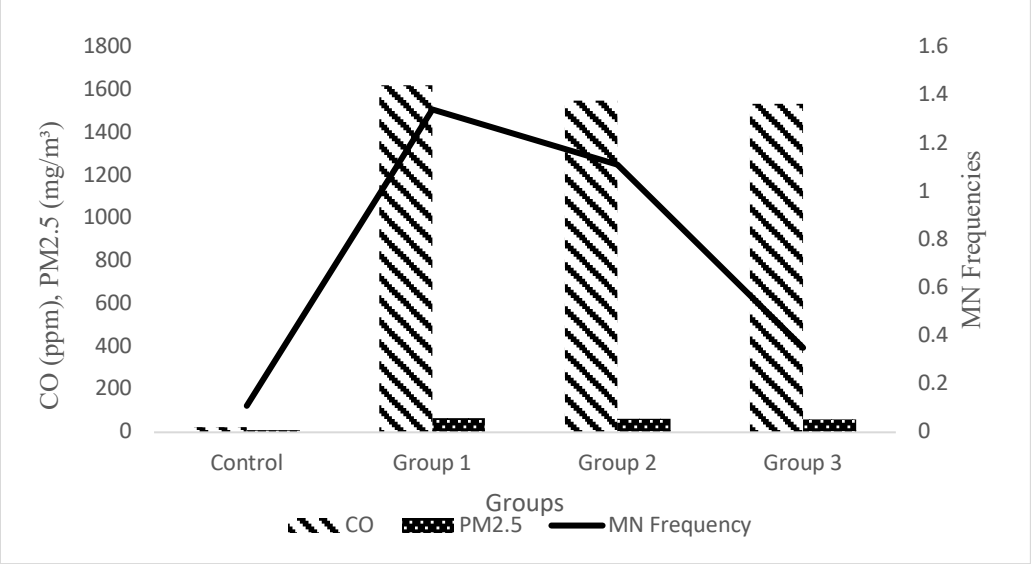

Figure 3 The levels of CO/PM2.5 observed in the groups in relation to the micronuclei frequencies in the groups

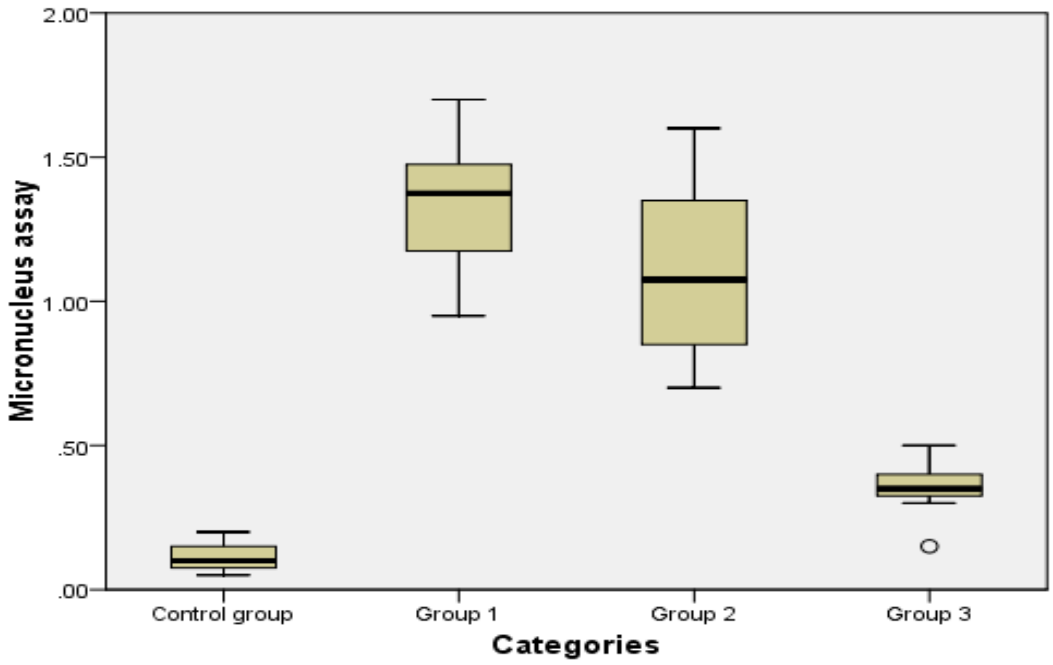

Figure 4 Effect of GGE on MNPCE in exposed rats

Relationship between liver function status, antioxidant enzymes activities, micronucleus frequency, and exposure to air pollutants

Spearman's rank correlation was used to test the strength of the association between the micronuclei frequencies across the groups and the time-dependent levels of gas/ particulate matter recorded during the exposure. Micronuclei frequencies (p-value=0.000) showed a statistically significant, and very strong positive correlation with CO and PM25. The summary of the correlational analysis is presented in Table 3

.

Table 3 The relationship between micronuclei (MN) frequencies and levels of CO/PM2.5 based on spearman's rank correlational analysis

		MN Frequency	CO	PM2.5
MN Frequency	R	1.000	0.903**	0.903**
	P-value		0.000	0.000
CO	R	0.903**	1.000	1.000**
	P-value	0.000		
PM2.5	R	0.903**	1.000**	1.000**
	P-value	0.000		

DISCUSSION

The serum levels of ALT, AST, and ALP of rats exposed to GGE for varying periods in this study were statistically significantly different compared to the control. The finding from this study is in contrast with previous findings that had noted a significant increase in the activities of serum ALT, AST, and ALP in rats exposed to gasoline which is indicative of liver cell damage and liver injury.[23-25] Although this study does not agree with most of the previous studies, the finding aligns with Abualgassim et al. that there was no significant difference in the activity of the enzymes, AST and ALT in fuel station workers when compared with control. However, they stated that some occupational chemical hepatotoxicity due to inducing or inhibiting the liver enzymes that play roles in biotransformation may not change liver enzymes used to evaluate liver damage.[26]

PM2.5 is one of the constituents of GGE known to induce inflammatory liver injury. Lin et al. deduced from their study that inhaled CO has an ameliorative effect on liver injury. From this study, histological analysis of the liver tissues of the exposed rats revealed that frequent exposure to GGE affects the structural integrity and architecture of the liver cells, which is not so in the control group as no visible lesion was seen. This implies that the liver is one of the major target organs of GGE-induced injury.[27,28]

It was noted that most of the sections of the liver viewed show sinusoidal congestion, which is a very critical part of the organ. The hepatic sinusoids comprise a complex of vascular conduits to transport blood from the porta hepatis to the inferior vena cava through the liver. Under normal conditions, portal venous and hepatic artery pressures are equalized within the sinusoids, oxygen and nutrients from the systemic circulation are delivered to the parenchymal cells and differentially distributed throughout the liver acini, and proteins derived from liver are carried into the cardiac/systemic circulation. It has been earlier reported that the cumulative oxidative damage is likely to be one of the underlying mechanisms responsible for the hepatotoxic effects of gasoline fumes, as observed in this study.[24] This finding is also in agreement with the histopathological assessment result of Owagboriaye et al. from the liver sections of rats exposed to gasoline for varying periods, which revealed an increasing level of distorted histoarchitecture, swelling/degenerated hepatocytes, patchy inflammation with remarkable sinusoidal space, and large central vein.[23]

Exposure to GGE significantly reduced the levels of antioxidant enzymes such as SOD, CAT, and GPx in the experimental rats. These three key enzymes top the list of the first-line defense antioxidants, and they help to suppress or prevent the formation of free radicals. Oxidative stress occurs when the presence

of reactive oxidative species is in excess of the available antioxidant buffering capacity.[29] The depletion of antioxidant enzymes predisposes the cell to the toxic actions of xenobiotics, which could lead to cell injury or death. It has been reported that the metabolism of aliphatic and aromatic hydrocarbons, the major constituents of petroleum and petroleum-derivatives, as well as other xenobiotics generates a significant increase in the level of free radical species in various tissues.[30,31] The generated reactive intermediates can interact and disrupt the cell membrane of the affected tissues.

A study by Suthur and Kathiresan on the analysis of oxidative stress in chronic exposure to petroleum hydrocarbons in Karnataka, India showed the association between oxidative stress and exposure to hydrocarbon between control subjects and exposed individuals as not evident.[32] The suggested reason for this was penned at the purity of the fuel. These finding does not align with the findings of this study. This study is in agreement with the study by Adamu et al., where a significantly lower plasma antioxidant status was recorded in a group of roadside petrol dispensers compared to a non-exposed group, which indicated a higher level of oxidative stress among the gasoline exposed group than the control.[33]

An increased frequency of micronuclei may be considered a biomarker of permanent genotoxic damage, reflecting either clastogenic or aneugenic modes of action.[34] In this study, there was a statistically significant increase in the frequency of MNPCE compared to the control, which indicated that GGE has the potential to cause genetic changes. This is in agreement with Ifegwu et al. who in their study of the effects of generator fumes on blood levels of 1-hydroxypyrene in rats concluded that the ability of PAHs to form adducts with DNA is an indication of their carcinogenicity, by checking the levels of 1-hydroxypyrene in blood- a metabolic product of pyrene – which is a prominent marker for PAH exposure.[9] The genotoxicity of some components of generator fumes implies that the adverse effects of inhalation can be passed on to generations yet unborn. Previous studies have shown that polyaromatic hydrocarbons (PAHs) are capable of interfering with DNA repair mechanisms, causing strand breakage, deletion of sister chromatid exchange (SCE), and disaggregation of chromatin.[35]

CONCLUSION

This study has revealed that there are adverse effects of chronic inhalation of subtle (non – lethal) amounts of generator fumes. These effects manifest through non – hypoxic mechanisms of generator fumes; an area that has not received adequate attention from researchers. This study has shown that exposure to GGEs may cause adverse biochemical changes, which can compromise the integrity of the immune system and alter liver function. The results also suggest that frequent exposure to GGE may induce genotoxicity, hence impairing the normal gene structure.

This study assumes significance and public health concern considering the increasing use of gasoline generators and consequent exposure to its emission in Nigeria. Regular servicing of generators by users to reduce the volume and components of the exhaust fumes, improvements on the ventilating systems in households to disperse pollution load from gasoline generators, use of exhaust filters and catalytic converters that can reduce the pollution load from gasoline generators and investment in solar, wind, and hydropower as an alternative means of generating electricity are encouraged.

CONFLICT OF INTEREST

Authors declare no competing interest in this research.

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