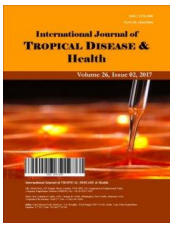

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Physiological, Oxidative Stress and Histopathological Effects of Exposure of White Mice, *Mus musculus* to Petrol Generator Exhaust

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the potential damage *in vivo* due to exposure to petrol generator exhaust fumes using White mice, *Mus musculus*. Four mice per group (experimental and control groups) were exposed to the exhaust fumes for 30 days. Weight changes, activity quotient and oxidative stress indicators (superoxide dismutase-SOD, catalase-CAT, reduced glutathione-GSH, glutathione-S-transferase-GST and malondialdehyde -MDA) were assessed for both groups. The results showed that there was no significant difference in activity quotient ($P > 0.05$) between both groups after the 30d study. The activities of the enzymes, GSH and GST, were inhibited ($P < 0.05$) and the level of MDA in the liver of the exposed mice increased significantly ($P < 0.05$) while those of SOD and CAT were not significantly different ($P > 0.05$) from the control. Various degrees of thickening of the alveolar septa and mononuclear inflammatory cells which were largely perivascular, peribronchiolar, and subpleural were the histological changes observed in the lungs of exposed mice. Hepatic tissue sections of the exposed mice also revealed some degree of pan lobular hepatocyte hyperplasia while the kidney sections showed a ghost outline of the tubule and glomeruli-acute cortical necrosis. The results indicated that the generator exhaust fumes had

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detrimental effects on the exposed mice, raising concerns regarding daily human exposures in cities like Lagos with a huge reliance on petrol generators. The need for improved provision of public electricity and adoption of cleaner household energy sources is advocated.

Keywords: Fossil fuel; carboxyl-haemoglobin; air pollution; environmental health; green energy.

1. INTRODUCTION

The erratic power supply in Nigeria has created a culture of the use of petrol generators to provide household energy across the country. The conditions worsen every day as most Nigerians go without power supply for weeks and sometimes months. This has hindered the nation's economic development as industries, companies, corporate organisations, and private establishments have to rely on generators [1] which implies increased cost and the attendant air pollution consequences.

In densely populated areas in Nigeria, generators are placed on balconies, doorsteps, chained beside windows and on top of houses to prevent theft. The generators are often run daily from nightfall to morning in households and during business hours for most establishments. The exhaust fumes produced by these generators contain some hydrocarbons and other toxic substances like nitrogen oxides, of carbon, sulphur, lead and carcinogenic benzo(a)pyrene which pose serious health challenges [2]. Cases of death sequel to the use of generators at home are daily reoccurrences because of exhaust fumes inhalation [3].

On the basis of the above, the aim of this study is to evaluate the potential health implications in households which are daily exposed to exhaust fumes using the white mice as a model.

2. MATERIALS AND METHODS

2.1 Test Organism

Healthy mature female white mice (*Mus musculus*), weighing 9.3 ± 2.8 g were used for the experiment. The same sex was used to reduce physiological differences in experimental animals. The mice were obtained from the Zoological garden of the University of Lagos where they had been bred and acclimatised already. They were divided into two groups (experimental and control), each comprising 4 animals. Mice were housed in separate wooden cages labelled 'exposed' and 'control'. Section 2.2 further describes the experimental design.

2.2 Bioassay Experiment

A brand new blue Two-Stroke Gasoline internal combustion generator (Tiger TG 950) with a maximum output power of 800 W was employed in this study to generate the exhaust fumes. This is the commonly used model in low-income household and small-scale businesses across Nigeria due to its low procurement and running cost.

In the experiment, mice were exposed to the exhaust fumes for a duration of 30 d. Eight (8) mice were used in two (2) cages (one experimental and one control), measuring about 56 cm x 30 cm x 30 cm each containing 4 mice. The control cage was kept at a different location (more than 500m) away from the effect of the exhaust fumes and the experimental ones were exposed to exhaust fumes 1 m from electric generator for 6 hours daily fed both group mice equally on daily bases throughout the duration of the experiment. The mice were sacrificed after the 30 d period using sterilised dissecting instruments.

2.3 Weight Change Studies

The mice were weighed before and during acclimatisation using an OHAUS Triple beam balance (700/800 Series). During experiments, they were also weighed daily and their body weight recorded.

2.3.1 Activity quotient studies: Body movement per minute and feed consumption pattern

This was measured for control and exposed mice and then the average calculated weekly [4].

$$Activity\ Quotient = \frac{Body\ movement\ per\ minute}{Quantity\ of\ food\ consumed}$$

100 g of feed were fed to the mice every day. Feed consumption pattern was measured and recorded throughout the experimental period. The average was calculated weekly.

2.4 Antioxidant Enzymes Studies

2.4.1 Method for homogenising sample

Three mice were randomly selected from each group and sacrificed by cervical dislocation after anesthetization. Their liver samples were excised and stored in plain bottles after draining in potassium chloride. Specimens were stored in ice packs prior to transportation to the Biochemistry Laboratory for analysis within 2 hrs.

The post mitochondria fraction of the liver samples were prepared as follows;

The samples were washed in an ice cold 1.15% KCL solution, blotted and weighed. They were then homogenised with 0.1 M phosphate buffer (pH 7.2), putting the organs each into the mortar; laboratory sand was added to it (acid washed sand) and it was blended in the mortar with pestle together. The resulting homogenate was centrifuged at 900 rmp speed for 15 mins and the supernatant was decanted and stored -20°C until analysis.

2.4.2 Measurement of anti-oxidative stress enzymes and lipid peroxidation

The Superoxide Dismutase (SOD) activity was determined using the methods of Sun and Zigma [5]. This was based on the ability to inhibit auto-oxidation of epinephrine, determined by an increase in absorbance at 480 nm. The Catalase (CAT) activity was determined according to the method of Sinha [6]. The reduced glutathione (GSH) content of liver tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay [7]. Glutathione-S-transferase (GST) activity was determined by the method according to Habig et al. [8] while the Malondialdehyde (MDA) level which is an index of lipid peroxidation was determined using the method of Buege and Aust [9] using thiobarbituric acid reactions (TBARS) assay.

2.5 Histological Studies (Lungs, Kidney and Liver)

Two mice were randomly selected from the experimental and control group. After dissection, the respective tissues were fixed in 10% formal saline. Before sectioning, the tissues were first dehydrated in graded alcohol concentrations, cleared in xylene and embedded in paraffin wax. Serial sections of 5-6 µm thickness were cut using rotary microtome, and then passed through xylene followed by absolute alcohol and water. The sections were stained with haematoxylin and eosin, dehydrated in graded alcohol, cleared in more xylene and mounted in Canada balsam. The slides were left to dry on the hot plate for 2 hrs before observation under the Olympus binocular light microscope. The histologic sections were translated by a histopathologist at the Anatomy Laboratory of the College of Medicine, University of Lagos.

2.6 Statistical Analysis

The results obtained were subjected to statistical analysis using T-test (unpaired t-test), SPSS Version 20 and Graph-pad prism 6 -statistical software. Significant differences in treatment means were determined at $P<0.05$.

3. RESULTS AND DISCUSSION

Among the main sources of atmospheric carbon monoxide in developing countries like Nigeria is vehicular exhaust and generator fumes particularly because of the common use of less fuel-efficient vehicles which fail modern emission standards and incessant power failures which necessitates the use of electricity generating sets. More so, Two-Stroke generators produce more fumes because the combustion of carbon is poor and they appear to be more commonly used in many low-income countries due to their lower cost of procurement. Thus, this study assessed health effects of exposure to fumes from such generators on white mice as a model for vertebrate (human) exposure.

3.1 Weight Changes

There was an initial increase in the mean weight of the White mice exposed to generator fumes from 27.54g to 28.49 g between the first and second week of the experiment. The weight steadily decreased, however, to 26.94 g in the third week following exposures and further decrease to 9.92 g in the fourth week. There was no detectable change in the mean weight of the control mice (Fig. 1).

The results of the exposure experiment indicated that there was a reduction of weight in the exposed white mice (*Mus musculus*) compared to control after three weeks and this may indicate stress which resulted in a gradual decline in body movement per minute and food consumption from week two to week four. Jeong et al. [10]

reported that retrain stress was associated with weight loss and reduction in food intake. Also, chronic physiological stress has been attributed to weight loss in rodent models in a study conducted by Harris [11] which sought to determine appropriate animal models for investigation of effects chronic and acute stress on energy balance.

3.2 Body Movement per Minute

The body movement per minute of the exposed mice was lower than that of the control in the first week. It reduced continuously throughout the exposure period while that of the control showed slight changes throughout the 30 days period. Overall, mean body movements per minute were not significant (P >0.05) (Fig. 2).

3.3 Feed Consumption Pattern

The generator fume exposed mice group fed more per day than the control on the first week but their feeding continued to decrease throughout the exposure period (Fig. 3). The feeding pattern of the control mice showed improved feeding throughout the 30 day period. However, there was a slight decrease from 6.4 g per day in the second week to 6.2g per day in the third week. Overall there was no significant difference (P > 0.05) between the daily feeding activities of the control and exposed mice.

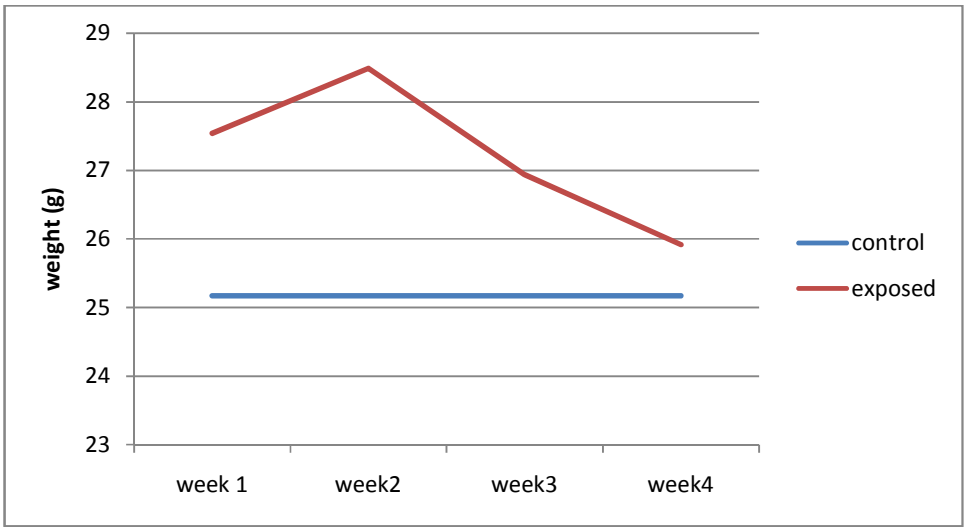

Fig. 1. Weight changes pattern in control and exposed mice over a period of 30 days

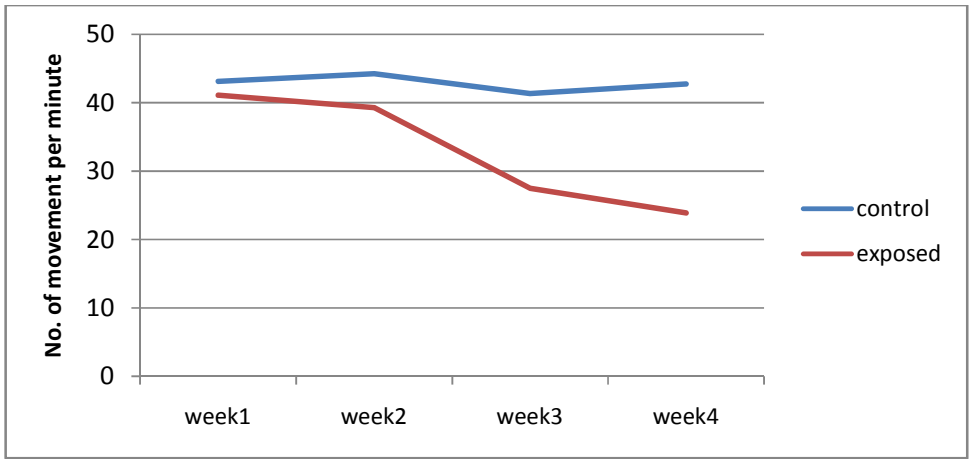

Fig. 2. Body movement per minute pattern in control and exposed mice over a period of 30 days

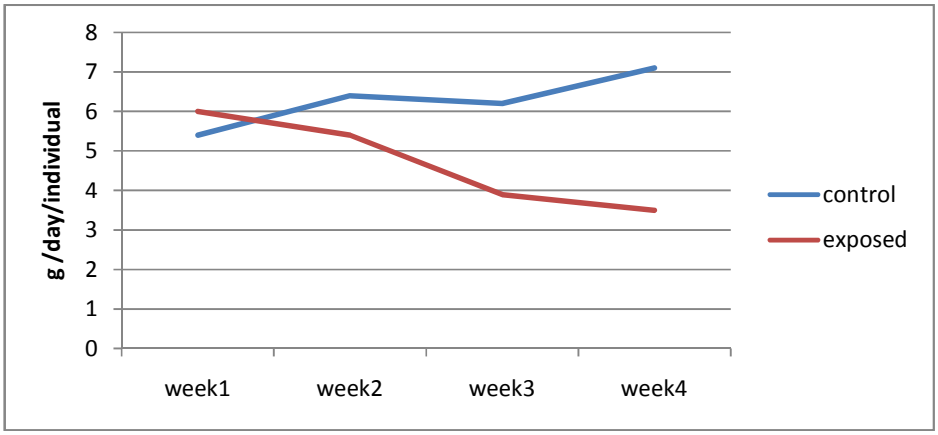

Fig. 3. Feed consumption pattern in control and exposed mice over a period of 30 days

3.4 Activity Quotient

Activity quotient (ratio of body movement to the quantity of feed consumed) increased in the exposed mice and reduced in the control (Fig. 4). Specifically, in the exposed group, there was an increase from 6.85 in the first week to 7.28 in the second week and then a reduction to 7.05 where it remained constant till the fourth week in the exposed mice. In the control mice, it decreased mono-phasically throughout the experimental period. T-test analysis showed that there was no significant difference (*P* >0.05) between the activity quotient of the exposed and control mice group.

3.5 Anti-Oxidative Stress Enzyme Activities and Lipid Peroxidation Levels

The result showed that the level of Glutathione (GSH) in White mice exposed to the generator exhaust fumes was lower than that of the control (P < 0.05) (Fig. 5a). The mean values were 0.63 ± 1.7 µmol/ml/min/mgpro and 1.88 ± 0.01 µmol/ml/min/mgpro for the exposed and control group respectively. The activities of Superoxide Dismutase (SOD) were also lower than those of the control (P >0.05) (Fig. 5b) with values of 1.8 ± 0.3 µmol/ml/min/mgpro and 2.0 ± 0.04 µmol/ml/min/mgpro respectively. However, the activities of Catalase (CAT) in White mice exposed to the generator exhaust fumes were higher than those of the control (Fig. 5c). The values were 13.1 ± 2.6 µmol/ml/min/mgpro and 12.1 ± 0.6 µmol/ml/min/mgpro respectively but they were not significantly different (P >0.05). The activities of Glutathione-S-Transferase (GST) in White mice exposed to the generator exhaust fumes were lower than those of the control(P< 0.05) (Fig. 5d) with the mean values of 0.29 ± 0.02 µmol/ml/mgpro and 0.61 ± 0.01 µmol/ml/mgpro respectively.

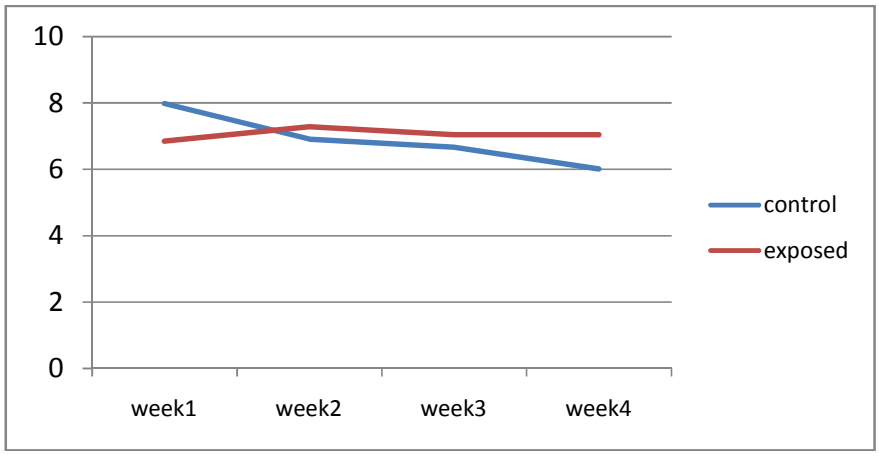

Fig. 4. Activity quotient pattern of control and exposed mice over a period of 30 days

The lipid peroxidation levels showed that the level of Malondialdehyde (MDA) in White mice exposed to the generator exhaust fumes was significantly higher ($P < 0.05$) than that of the control (Fig. 5e). The values were 0.09 ± 0.002 µmol/ml/mg pro and 0.04 ± 0.004 µmol/ml/mg pro respectively in the exposed mice and control group.

In this study, the activities of the enzymes GSH and GST were inhibited ($P < 0.05$) in the liver of the white mice exposed to the fumes and this finding is in agreement with those of Otitoloju and Olagoke [12] as well as [13]. Glutathione-S-Transferase (GST) is a cytosolic or microsomal enzyme that catalyses the conjugation of GSH with oxidative products, such as 4-hydroxyalkenals (membrane peroxides) and/or base propenals, resulting from DNA oxidative degradation [14]. Therefore, it plays an important role in protecting tissues from oxidative stress [15]. The decrease in GSH levels indicates that the biomolecule was actively protecting the tissue from oxidative stress induced by the exhaust fumes. Impairment in antioxidant enzymes will produce an imbalance between pro and antioxidant systems causing the formation of toxic hydroxyl radicals, with direct consequences on the cell integrity and cell functions itself [16].

SOD is known to provide cytoprotection against free radicals induced damage by converting superoxide radicals generated in peroxisomes and mitochondria to hydrogen peroxides [4]. The hydrogen peroxide is then removed from the system by the enzyme CAT, which converts it to water and molecular oxygen [4]. The enzyme SOD was inhibited in the liver of the white mice exposed to the test chemicals. However, the reduction in SOD was not significant at $P > 0.05$. The inhibition of the enzyme SOD by the fumes will result in oxidative stress to the liver tissues as a result of the damaging activities of the superoxide radicals. Furthermore, the inhibition of the enzyme SOD is expected to result in a reduction in the activity of CAT, due to a decrease in hydrogen peroxide generation from SOD activities. This was the case as reported by Akpan et al. [4] and [17] but was not reflected in this study. Perhaps exposures to generator exhaust over longer durations as seen in urban populations in cities like Lagos would have far significant stress output.

Exposure of *M. musculus* to generator exhaust was found to cause a significant ($p < 0.05$) increase in the level of malondialdehyde indicative of oxidative damage in the liver of exposed mice compared to control. This result is in agreement with findings of Otitoloju and Olagoke [12] who reported an increase in lipid peroxides (LPO) in tissues of animals exposed to petroleum hydrocarbons. The increase in LPO is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular reactive oxygen species (ROS) [18]. Elevated ROS level in tissues leads to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defence systems.

3.6 Histological Effects in the Lungs, Kidney and Liver

The histological examination of the lungs revealed a normal alveolar epithelium but there was a loss of epithelial cells, and many of the interstitial cells had pyknotic nuclei in control mice (Plate 1). In mice exposed to generator exhaust fumes, there was a thickening of the alveolar septa and mononuclear inflammatory cells were largely perivascular, peribronchiolar, and subpleural (Plate 2) after the 30 days exposure period.

The histological examination of the kidney revealed sections with a normal distribution of the glomeruli and tubule in the control but the renal vessel was congested with some degree of perivascular inflammation (Plate 3). In the mice exposed to generator exhaust fumes, the kidney section showed a ghost outline of the tubule and glomeruli-acute cortical necrosis (Plate 4) after the 30 days exposure period.

The histological examination of the liver showed a normal central vein and portal tract in the control mice group. However, there were a hepatic section with some degree of autolysis and normal outline of hepatocytes (Plate 5). The liver of the mice exposed to the generator exhaust fumes had a normal distribution of hepatocytes, central vein and portal tract with some degree of pan lobular hepatocyte hyperplasia (Plate 6) after the 30 days exposure period.

There was also evidence of various degrees of thickening of the alveolar septa and mononuclear inflammatory cells which were largely perivascular, peribronchiolar, and subpleural. This result agrees with the findings of Zheng et al. [19] where he demonstrated that a “short” exposure (3 weeks) to $PM_{2.5}$ causes a low-grade lung inflammation. This result also agrees with the account of Onarloiglu et al. [20] and El-Nouri

[21] who reported lung histological alterations in mice exposed to lead. Lungs and skin are usually the first contact for environmental exposure to xenobiotics, hence, it is expected that any toxic or sub-lethal effect of the xenobiotic will be observed first in the lungs, more so, in the case of exhaust which is principally inhaled and then directed to the lungs for gaseous exchange.

It has been reported that exposure to particulate air pollution plays some role in a variety of human diseases involving the cardiovascular, nervous, and urinary systems [22]. The histological section of the liver tissues of the exposed mice revealed some degree of pan lobular hepatocyte hyperplasia. A prolonged exposure as described by Zheng et al. [19] allows particulate matter to hit the liver and when this happens, they cause hepatic kupffer cell activation, inducing an inflammatory response [23]. Also, the kidney sections of the exposed mice showed a ghost outline of the tubule and glomeruli-acute cortical necrosis. This is in support with findings of Massey and Taneja [24] who pointed out that inhaled and ingested nanoparticles can penetrate through the alveolar as well as the digestive walls to enter the blood system and subsequently be transported to any organ in the body. It also supports a reported research where an *in vivo* experimental evidence from single-dose exposure to DEP in the lung aggravated the renal, pulmonary, and systemic effects of cisplatin (CP)-induced acute renal failure in rats [25].

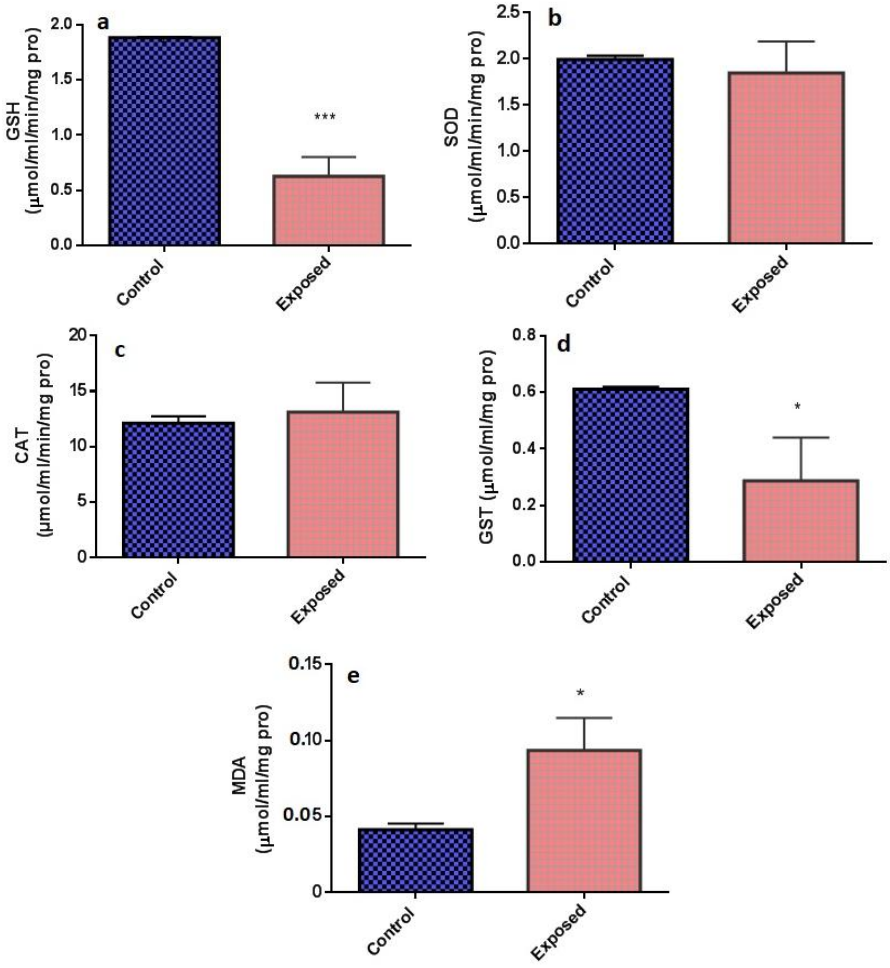

Fig. 5a-e. Mean activities of a- Reduced glutathione (GSH), b- superoxide dismutase (SOD), c- Catalase (CAT), d- glutathione-S- transferases (GST) and e- the levels of lipid peroxidation product (Malondialdehyde-MDA) in White mice *(Mus musculus)* exposed to sub-lethal concentration of generator exhaust fumes and control

*(*indicates significant difference at P < 0.05)*

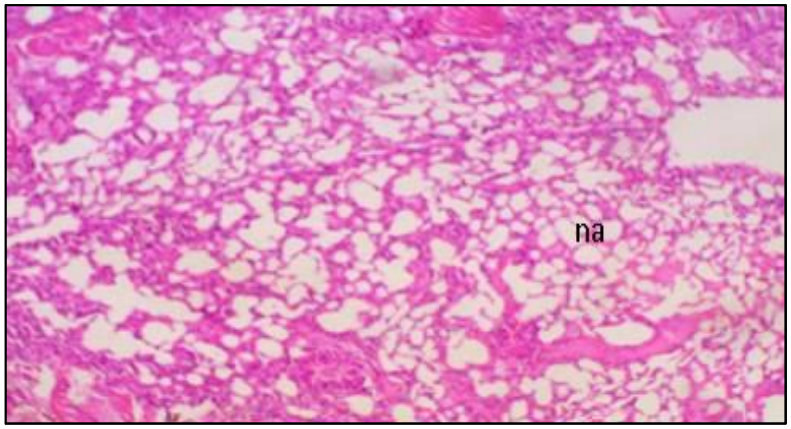

Plate 1. Section of lungs of a control (unexposed) white mice (*Mus musculus*) showing a normal alveolar epithelium (na) but there is a loss of epithelial cells, and many of the interstitial cells have pyknotic nuclei (H&E Stain X40)

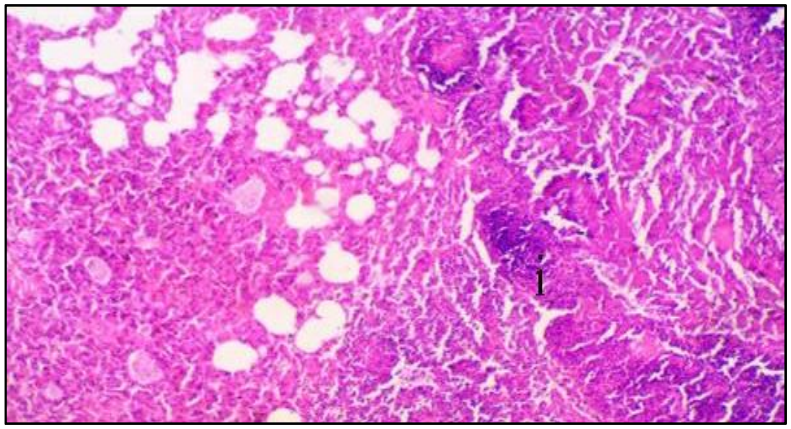

Plate 2. Section of the lungs of a generator fume exposed white mouse (*Mus musculus)* showing thickening of the alveolar septa and mononuclear inflammatory cells (i) are largely perivascular, peribronchiolar, and subpleural. (H&E Stain X40)

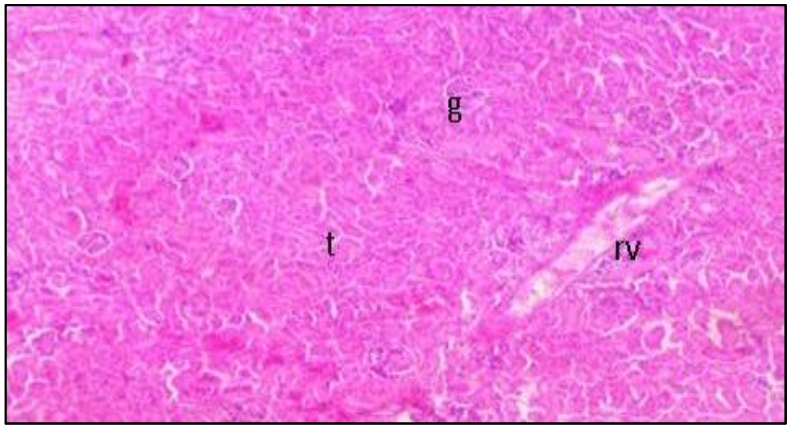

Plate 3. Section of the kidney of control (unexposed) mice (*Mus musculus*) showing a normal distribution of the glomeruli (g) and tubule (t). The renal vessel (rv) is congested with some degree of perivascular inflammation (H&E Stain X40)

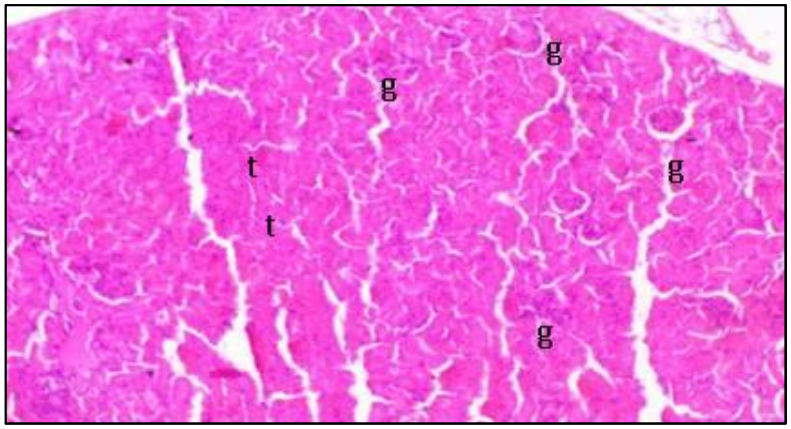

Plate 4. Section of the kidney of a generator fume exposed white mouse (*Mus musculus*) showing normal distribution of the glomeruli (g) and tubule (t). It also shows a ghost outline of the tubule and glomeruli-acute cortical necrosis (H&E Stain X40)

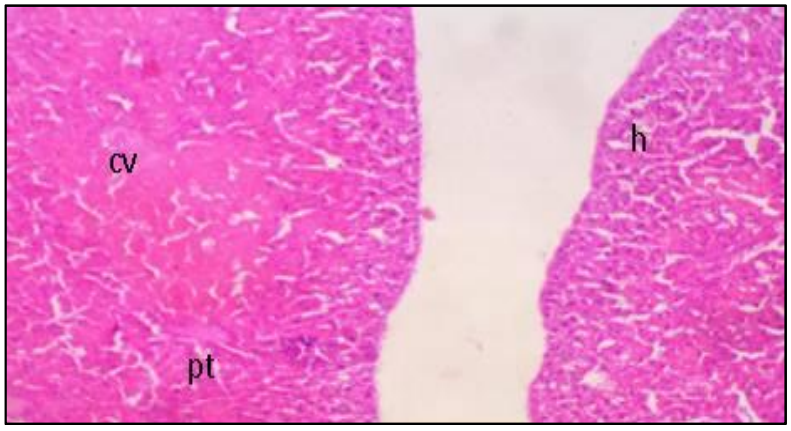

Plate 5. Section of the liver of control (unexposed) white mice (*Mus musculus*) showing some degree of autolysis (on the right) and normal outline of hepatocytes (h) (on the left).The central vein (cv) and portal tract (pt) are normal (H&E Stain X40)

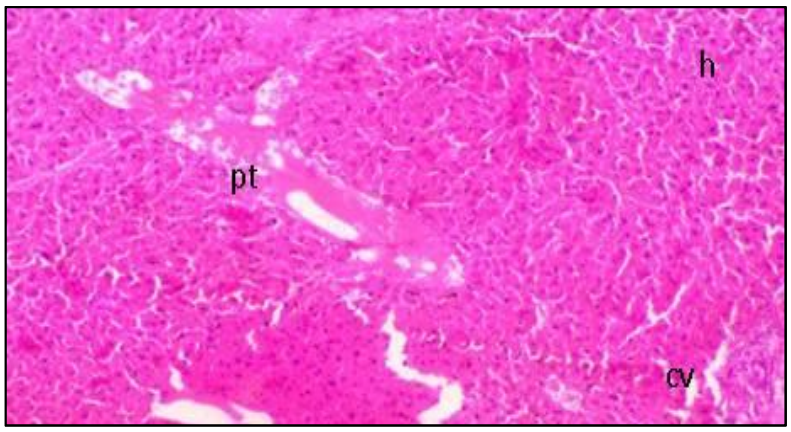

Plate 6. Section of the liver of a generator fume exposed mouse (*Mus musculus*) showing a normal distribution of hepatocytes, central vein (cv) and portal tract (pt) but with some degree of pan lobular hepatocyte hyperplasia (h) (H&E Stain X40)

4. CONCLUSION

The use of biochemical responses and histological changes as biomarkers during environmental monitoring programmes is based on the observations that toxic effects, manifests at the subcellular level before it becomes apparent at higher levels of biological organisation. The observed histological changes and inhibition of the antioxidants defence enzymes; GST and GSH in conjunction with an increase in MDA levels in the liver tissues of test animals exposed to exhaust is a call to city developers to improve electricity infrastructures so as to minimise use of electrical generating sets which leave a trail of pollution and health effects in biological systems.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocol was in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [26] and approved by the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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