Biochemical and Histopathological Effects of Exposure to Diesel Fumes on Vital Organs of Wistar Rats (*Rattus Norvegicus*)

*[1]Hilary I. Okoh, [2]Ikenna M. Odoh, [3]Jamila Ahmed, [4]Tolulope Adewemimo, [1]Aaron O. Nwana and [1]Olalekan A. Akano
[1]Department of Animal and Environmental Biology, Federal University, Oye - Ekiti, Nigeria
[2]University Medical Centre, Federal University, Oye - Ekiti, Nigeria
[3]Department of Zoology, Ahmadu Bello University, Zaria, Nigeria
[4]Department of Public Health, Ekiti State Ministry of Health and Human Services, Ado-Ekiti, Nigeria
iwegbunem.okoh@fuoye.edu.ng

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ORIGINAL RESEARCH

Abstract- Diesel is a volatile petrochemical product and exposure to diesel fumes may be harmful to normal body physiology by increasing serum lipid peroxidation, creatine, and PCV levels. In this study, the effect of diesel fumes inhalation on Wistar rats was assessed. Twenty Wistar rats were clustered into 4 groups (A, B, C, and D) each containing 5 rats after acclimatisation for 2 weeks. Groups B, C, and D were exposed to diesel fumes for 1, 3, and 6 hours per day, five times a week for 8 weeks respectively, while rats in group A served as unexposed control. At the end of the experiment, blood was collected from rats in each group and haematological and biochemical parameters were analysed using automated haematological and chemical analysers respectively. Histopathological analyses of the kidney, liver, and lungs were done following tissue sectioning and staining. There was a significant increase ($P< 0.05$) in the packed cell volume and leucocytes of rats exposed to diesel fumes as compared to the control. Exposure to diesel fumes also caused elevated levels of the liver and kidney biomarkers. Histopathological studies showed diffuse tubular degeneration and congestion of the renal cortex; mild portal congestion and periportal connective tissues in the liver and mild congestion at the pulmonary interstitium in the lungs. The results from this study highlights the negative effects of exposure to diesel fumes and the need for safer alternatives like solar energy for power generation.

Keywords- Diesel fumes, Wistar rats, kidneys, liver, lungs

1 INTRODUCTION

Petrochemical products such as diesel and gasoline contain compounds such as benzene, xylene, and toluene among others which are harmful to health (Moro *et al*., 2017). The volatile nature of these products makes the aforementioned compounds readily available in the air, and exposure to them is by direct inhalation (Cecil *et al*., 1997). Benzene in particular has been reported to be genotoxic, causing cancer in humans (Carrieri *et al*., 2018), and its toxicity is further compounded by the presence of xylene and toluene (Keenan *et al*., 2010). Both short and long-time exposure to benzene has been reported to cause haematological disorders such as leukaemia and myelodysplastic syndrome (Uzma *et al*., 2010; De Palma *et al*., 2012; Moro *et al*., 2019). In an enclosed area, volatile petrochemical products are likely to reach very high concentrations which can lead to loss of consciousness or even death as a result of respiratory failure (Chilcott, 2007). Exposure to petrochemical fumes can affect vital organs like kidneys, liver, and lungs resulting in elevated levels of their biomarkers in the blood which may lead to various related diseases and several forms of genotoxic, carcinogenic, and neurotoxic effects (Owagboriaye *et al*., 2016; Moro *et al*., 2019). Although the mechanism of benzene action is not fully known, it has been established that the reactive oxygen species resulting from the breakdown of benzene damages biomolecules, and induces oxidative stress causing elevated levels of biomarkers in the body system (Uzma et al., 2010; Moro *et al*., 2019).

In Nigeria, due to inadequate supply of electricity, diesel is mainly used to power electrical generating sets in homes, workplaces, and industries. This has led to an increase in the demand and use of diesel, thereby increasing the frequency of exposure to diesel fumes and those who are exposed occupationally have been reported to be more affected (Moro *et al*., 2013; 2019). Some of the occupational hazards associated with exposure to diesel fumes include hepatic and renal function impairment, increased cancer risk, anaemia, and immunotoxicity among others. Most research on exposure to diesel especially in Nigeria have focused on diesel exhaust with little or no attention paid to diesel fume inhalation (Jiang *et al*., 2014; Long and Carlsten, 2022). Hence, this research was designed to assess the effect of diesel fume inhalation on vital organ functions using Wistar rats as a mammalian model.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMAL

Ethical approval for this study was obtained from the Ethics Committee, Faculty of Science, Federal University, Oye – Ekiti. Twenty healthy adult male and female Wistar rats, weighing 160g - 220g were obtained from the breeding section of the animal house of Ekiti State University (EKSU), Ekiti State. The animals were kept in a well-ventilated cage in the departmental animal house

*Corresponding Author

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at regular room temperature (25°C-30°C) and under a controlled light cycle for two weeks for acclimatisation, prior to the experiment. The animals were then clustered into four groups A, B, C, and D with each group containing 5 rats (3 males, 2 females). Group A served as the control group which was not exposed to diesel fumes while groups B, C, and D served as the treatment/exposure groups.

2.2 Exposure to Diesel Fumes

The exposure methods of Uboh *et al*., (2008) and Owagboriaye *et al*., (2016) were adopted with modifications. Rats in group A (unexposed control) were kept in the Animal house of the Department of Animal and Environmental Biology which is about 150 meters from the diesel tank while the treatment groups (B, C and D) were exposed to diesel fumes by placing them under a 5,000 litres capacity diesel storage tank located within the Faculty of Science, Federal University, Oye – Ekiti, Ekiti State, Nigeria for different durations. Following pre – determined daily exposure periods for each group (1, 3, and 6 hours per day respectively), rats were withdrawn from exposure and taken to the animal house till the next exposure period.

2.3 Sample Collection

At the end of the exposure period, the rats were sacrificed by cervical dislocation after 24 h of the last exposure. Blood was collected into heparin, EDTA, and plain bottles. Vital organs such as kidneys, lungs, and liver were harvested for histopathological studies.

2.4 Haematological Examination

Packed cell volume (PCV), white blood cell (WBC), neutrophils, lymphocytes, monocytes, and eosinophils assessment were done using a haematological analyser. The kidney and liver biomarkers such as Na, K, Cl, urea, creatinine, alanine transferase (ALT), aspartate transferase (AST), alanine phosphate (ALP), albumin (ALB), and total protein were assessed spectrophotometrically using automated chemistry analyser following the method of Elekwa *et al*., (2021).

2.5 Histopathological Examination

The kidneys, lungs, and liver were fixed in 10% formalin which was followed by enclosure in paraffin wax. The tissues were sectioned and stained using hematoxylin and eosin. The prepared slides were then observed with the aid of a light microscope at a magnification of x40 (Abubakar *et al*., 2015).

2.6 Data Analyses

Data were expressed in means including their standard error. Means were compared using a one-way analysis of variance (ANOVA) with the level of significance set as $P<0.05$. The Tukey posthoc test was used to separate the means that were significantly different. All data were analyzed using Microsoft Excel 2019 and SPSS statistics.

3 Result: Effect of Diesel Fumes on Biomarkers

There was a significant difference in all the haematological parameters assessed except for monocytes and eosinophils (Table 1). Table 2 presents the effect of diesel fume exposure on kidney function biomarkers. There was a significant difference in serum electrolytes of the rats exposed to diesel fumes which increased with an increase in exposure hours. Electrolytes of rats exposed to diesel fumes at different hours was observed to be elevated when compared to the control. Creatinine level was also significantly elevated compared to the control while though, the urea level was elevated in the treatment groups, the difference was not significant ($P>0.05$). All the liver function biomarkers increased significantly with increase in exposure hours, compared to the control (Table 3). Plates 1, 2 and 3 presents the photomicrograph of the kidney, liver and lung sections of exposed and unexposed rats.

Table 1. Effect of diesel fumes exposure on the haematological parameters of Wister rat

Exposure	PCV%	WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils
0	45.00±0.71^{a}	5.60±1.00^{a}	55.00±0.71^{a}	25.00±0.71^{a}	2.18±0.60^{a}	1.54±0.42^{a}
1	46.00±0.71^{a}	7.20±1.01^{b}	56.00±0.73^{a}	30.00±0.74^{b}	3.00±0.71^{a}	1.54±0.42^{a}
3	52.00±0.74^{b}	8.00±0.71^{b}	60.00±0.74^{b}	34.00±0.76^{c}	4.00±0.75^{a}	2.18±0.60^{a}
6	54.00±0.77^{b}	11.30±1.0^{c}	63.00±0.77^{b}	35.00±0.79^{c}	4.00±0.78^{a}	2.40±0.71^{a}

^{abc}Mean values in the same column having the same superscript are not significantly different ($p < 0.05$), PCV- packed cell volume, WBC-white blood cell

Table 2. Effect of diesel fumes exposure on the kidney function biomarkers

Exposure time (h)	K^+ (mmo/l)	Na^+ (mmo/L)	Cl^- (mmo/L)	Urea (mmo/L)	Creatinine (umo/L)
0	7.20±0.13^{a}	142.00±0.10^{a}	108.00±0.10^{a}	4.20±0.10^{a}	60.00±0.10^{a}
1	8.10±0.12^{c}	142.00±0.11^{a}	109.00±0.13^{a}	5.70±0.12^{a}	62.00±0.14^{ab}
3	7.80±0.15^{a}	146.00±0.13^{b}	114.00±0.15^{b}	5.80±0.10^{a}	64.00±0.17^{b}
6	8.40±0.18^{d}	146.00±0.18^{b}	113.00±0.17^{b}	6.10±0.16^{ab}	80.00±0.19^{c}

^{abc}Mean values in the same column having the same superscript are not significantly different ($P < 0.05$), K^+-Potassium ion, Na^+ - Sodium ion, Cl^- - Chloride

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Table 3. Effect of diesel fumes exposure on the liver function biomarkers

Exposure time (h)	AST(u/L)	ALT(u/L)	ALP(u/L)	ALB(g/L)	TP(g/L)
0	277.00±0.10[a]	117.00±0.10[a]	296.00±0.10[a]	27.00±0.11[a]	69.00±0.12[a]
1	293.00±0.13[b]	118.00±0.11[a]	465.00±0.13[b]	30.00±0.13[b]	83.00±0.13[b]
3	324.00±0.15[c]	132.00±0.15[b]	571.00±0.15[c]	32.00±0.14[b]	84.00±0.14[b]
6	371.00±0.17[d]	181.00±0.18[c]	607.00±0.18[d]	32.00±0.15[b]	88.00±0.16[c]

[abc]Mean values in the same column having the same superscript are not significantly different, AST- AspatateTransfarase, ALT- AlaninTransfarase, ALP– Alanine Phospate, ALB- Albumin, TP - Total Protein

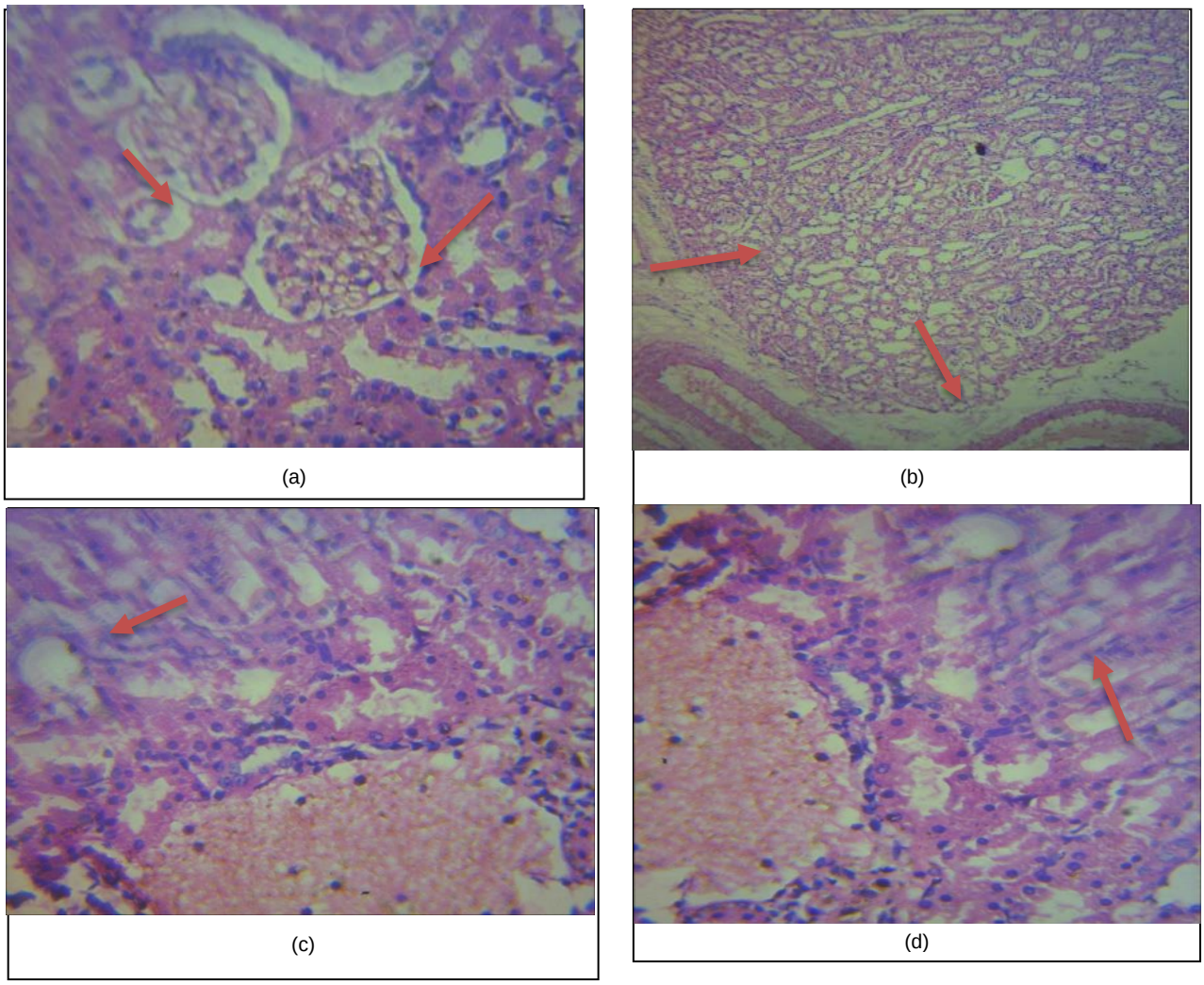
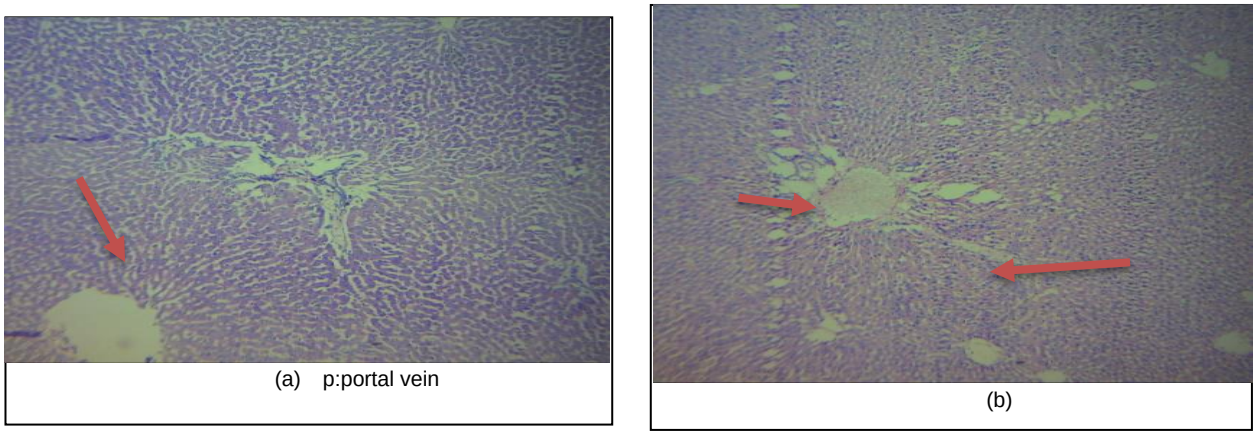


Plate 1: Photomicrograph of wistar rat's kidney sections (a) Control (group A) showing no visible lesions; (b) Group B presenting mild renal cortical congestion; (c) Group C showing mild interstitial congestion of renal cortex;(d) Group D depicting moderate diffuse tubular degeneration



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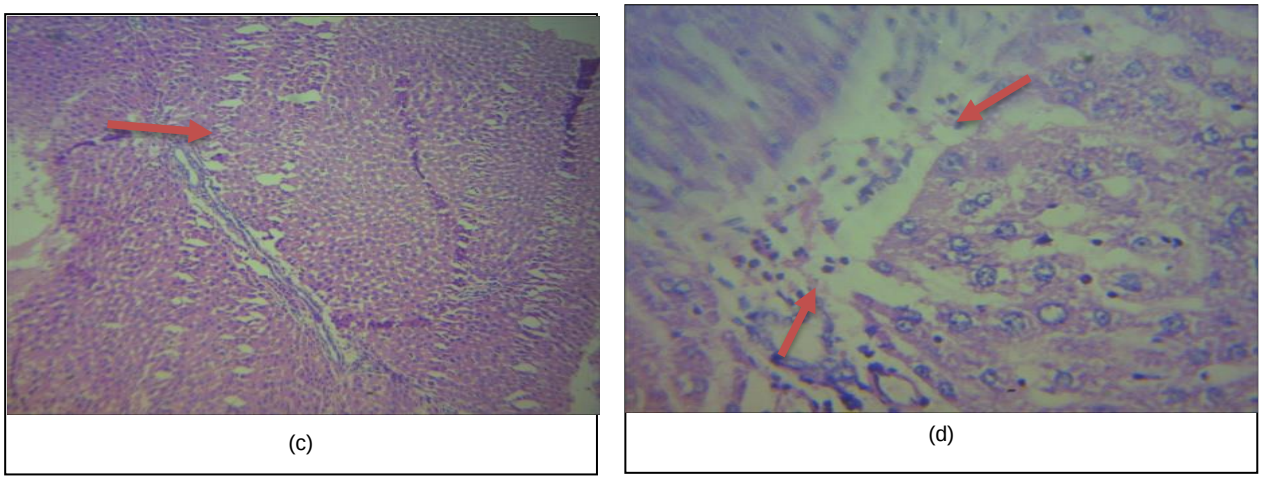
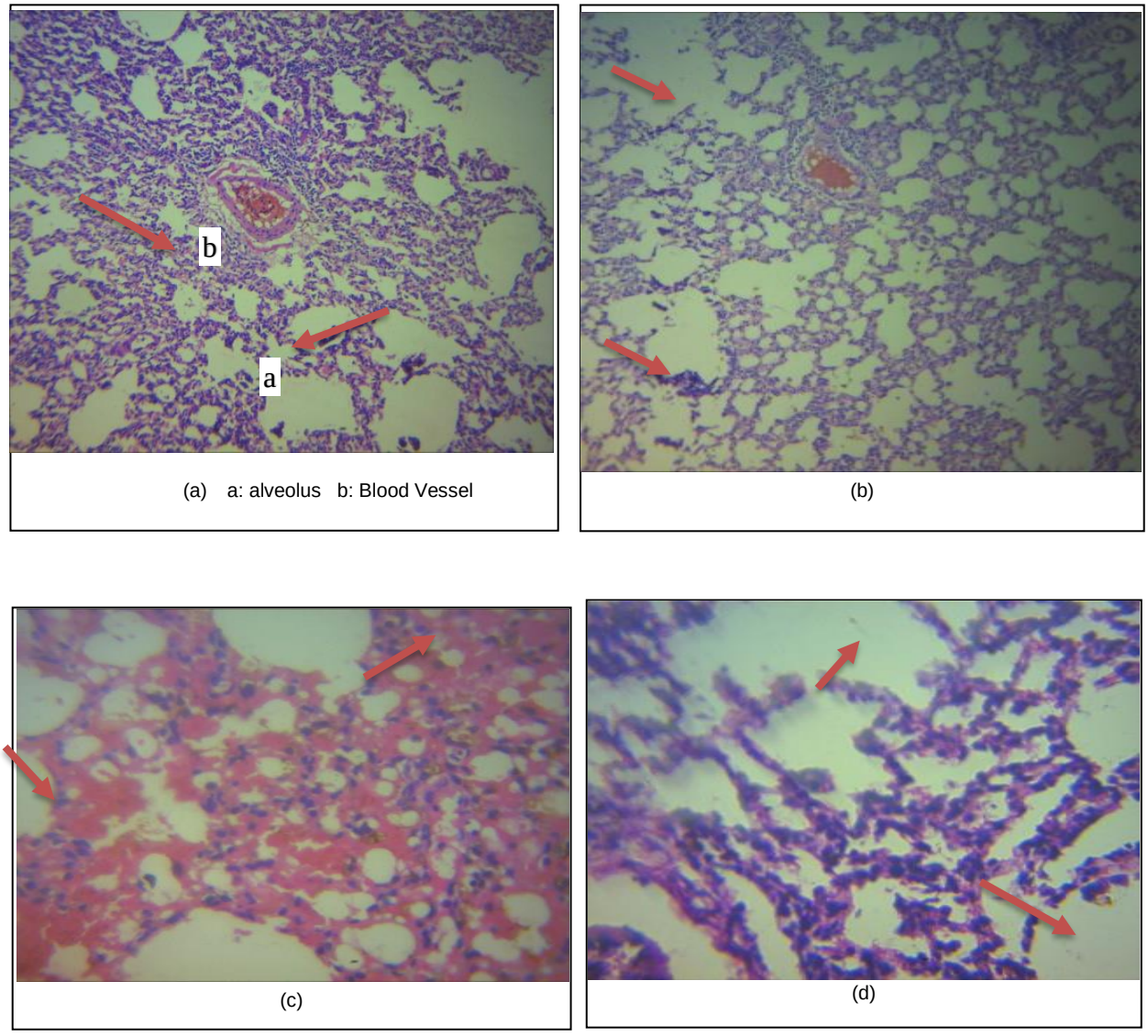


(c) (d)

Plate 2: Photomicrograph of Wistar rat's liver sections (a) Control (group A) showing no visible lesions; (b) Group b depicting mild portal congestion (c) Group C showing prominent periportal connective tissues; (d) Group D also showing prominent periportal connective tissues.



(a) a: alveolus b: Blood Vessel (b)

(c) (d)

Plate 3: Photomicrograph of Wistar rat's lung sections (a) Control (group A); depicting no visible lesion (b) Group B showing mild congestion at the pulmonary interstitium; (c) Group C showing mild interstitial congestion; (d) Group D showing severe congestion at the pulmonary interstitium

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4 DISCUSSION

This study presents the biochemical and histopathological effects of exposure to diesel fumes on some vital organs using Wistar rat as a mammalian model. No mortalities were recorded following exposure for 1-6 h/day, 5 times a week for 8 weeks. Similar findings have been reported by Uboh *et al*., 2008 and Abubakar *et al*., (2015) for gasoline fumes.

In this study, exposure to diesel fumes resulted in a significant change in the haematological indices such as PCV, white blood cell count, neutrophils, and lymphocytes. There was Increased PCV levels in exposed rats compared to the control. This may be due to lowered activity in the lungs as a result of diesel fumes causing mild tissue hypoxia leading to the production of red blood cells. Abubakar *et al*., (2015) reported a similar finding. White blood cells including lymphocytes and neutrophils are the body defense cells that may be produced in large numbers in response to the presence of foreign bodies in the body system. This may explain the increase in WBC in exposed rats compared to the control. The presence of toxicants like benzene and xylene which are volatile components of diesel in the blood as a result of exposure might have been responsible for the elevated levels of these blood parameters. Exposure to volatile petroleum products has also been reported to affect blood indices as observed in this study (Abubakar *et al*., 2015; Zamanian *et al*., 2018; Owumi *et al*., 2021 Teklu *et al*., 2021). However, the findings of this study should be interpreted with care as they may not depict the actual situation in humans as reported by other studies where human subjects were used. The result of their findings depicts reduced rather than elevated blood indices (Okoro *et al*., 2006; Teklu *et al*., 2021). The differences in findings may be attributed to the duration of exposure and the concentration of the components of fumes during the period of exposure. The experimental design may also be a factor as opined by some workers (Uboh *et al*., 2008; Uzma *et al*., 2008).

Volatile petroleum products have been reported to impair renal and hepatic functions (Asefaw *et al*., 2020; Owumi *et al*., 2021). A significant increase in liver and kidney biomarkers in the treatment group is an indication of functional impairment resulting from structural damage as observed in the tissue sections of these organs. Elevated creatinine and urea level may be attributed to the presence of reactive oxygen species resulting from benzene metabolism causing renal impairment. This observation was also reported by Asefaw *et al*., (2020). A significant increase in liver biomarkers such as AST, ALT, and ALP may be attributed to the breakdown of hydrocarbons (the major component of diesel) which might have occurred in the liver resulting in the production of free radicals that could induce lipid peroxidation leading to the release of hepatic enzymes. Similar observations have been reported by Kim *et al*., (2006); Ekpenyong and Asuquo, (2017).
Photomicrograph of lung sections in this study showed severe congestion at the pulmonary interstitium in rats exposed to 6 h/day of diesel fumes. The breakdown of the antioxidant defense system as a result of excessive free radicals resulting from hydrocarbon breakdown can lead to the destruction of the cell and tissue architecture (Otitoju and Onwurah 2007). This might be responsible for the histopathological changes observed in this study as similar findings have been reported by several authors (Adewole and Ojewole, 2009; Ige *et al*., 2011; Azeez *et al*., 2012).

5 CONCLUSION

The result of this study suggests that prolonged exposure to diesel fumes caused elevated levels of the liver and kidney biomarkers. Histopathological studies also showed diffuse tubular degeneration and congestion of the renal cortex; mild portal congestion and periportal connective tissues in the liver and mild congestion at the pulmonary interstitium in the lungs. Safe alternative like solar energy should be considered for electricity power generation.

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