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EFFECT OF PETROLEUM PRODUCT INHALATION ON THE HISTOLOGICAL INDICES OF THE LUNGS OF MALE WISTAR RATS

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Abstract

Petroleum product exposure is a known risk factor for respiratory diseases. This study investigated the effect of petroleum product inhalation on oxidative stress parameters and histopathological indices of the lung tissue of male Wistar albino rats. Forty-five rats aged 3 months and weighing 180–220 g were divided into five groups: Group A (Control), Group B (PMS exposure - 500 ml), Group C (Kerosene exposure - 500 ml), Group D (Diesel exposure - 500 ml), and Group E (Mixed petroleum products - 500 ml). The exposure lasted for 28 days, and lung tissue homogenate samples were collected at days 0, 14, and 28 to measure superoxide dismutase (SOD), catalase (CAT) activity and glutathione-s-transferase (GST) activity. The lungs were harvested using dissection to test the histological effects of the exposure. The results showed that SOD and CAT activity were significantly decreased ($p<0.05$) (11.12 ± 0.48 and 1.63 ± 0.23) when compared with the control (12.87 ± 0.93 and 3.29 ± 0.70) while GST activity significantly increased ($p>0.05$) (24.02 ± 0.54) compared with the control (23.20 ± 0.45). Histology of the lungs showing degeneration of the dentate granular layer in male Wistar albino rats exposed to diesel. These results emphasize the harmful effects of inhaling petroleum products on critical organ systems, particularly the lungs.

Introduction

A complex mixture of hydrocarbons, including volatile organic compounds (VOCs) like xylene, toluene, and benzene, is found in petroleum products (Saeedi *et al*., 2024). These substances are known to be harmful to a variety of biological systems and to present major health risks in situations involving occupational and environmental exposure (Ahmed and Fakhruddin, 2018).

Exposure to these toxicants can induce oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system, leading to cellular damage and inflammation

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(Albano *et al*., 2022). ROS are produced during metabolism or when exposed to external contaminants. According to research studies, inhaling petroleum products raises oxidative stress by decreasing the activity of important antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) (Kemabonta *et al*., 2020). Particularly in organs with high metabolic needs like the lungs, caused by a disruption in the redox state of equilibrium, which can harm tissues and impede the functions of cells.

Petrol compounds have a major impact on the lungs, which are the main sites of exposure during inhalation (Zhao *et al*., 2019). The respiratory tract may be irritated by the volatile ingredients in these goods, resulting in fibrosis, inflammation, and alveolar damage. Histopathological alterations in lung tissue, such as thicker alveolar walls, inflammatory cell infiltration, and higher mucus production after exposure to petroleum vapors, have been observed in studies conducted on experimental animals (Owumi *et al*., 2021). These results imply that the harmful effects of petroleum products target the respiratory system. A systemic pattern of toxicity was observed in the lungs through oxidative damage and inflammatory reactions.

Although considerable progress has been made in understanding the toxic effects of petroleum products, several gaps remain in the literature. The interplay between oxidative stress and histopathological changes in the lungs has not been thoroughly explored. A comprehensive understanding of these interactions is essential for assessing the systemic effects of petroleum product exposure and its health implications.

Given the extensive use of petroleum products in underdeveloped nations, where exposure control regulations are less strict, the importance of this research is further emphasized. Employees in the transportation, filling station, and refinery industries are particularly vulnerable to long-term exposure to petroleum vapors (Olabisi *et al*., 2018). Environmental contamination from leaks, spills, and inappropriate disposal methods further increases the risk of unintentional exposure to the public. These cases illustrate just how important it is to learn about the toxicological implications of petroleum inhalation and to propose approaches to lessen these effects (Ivshina *et al.,* 2015).

By evaluating oxidative stress markers, mainly antioxidant enzymes, with histopathological assessments, this study sought to comprehensively assess the effects of petroleum product inhalation on the lungs. The results will enhance existing knowledge on petroleum toxicity and can help shape public health policies and occupational safety measures to minimize exposure risks.

Justification of the study

The increasing use of petroleum products due to industrialization and urbanization has raised concerns about the release of toxic compounds into the environment (Robinson *et al*., 2017). Human and animal populations are at risk of exposure to these volatile compounds, which can lead to health risks (Antonelli *et al*., 2020). The effects of inhaling petroleum product fumes on oxidative stress parameters and histopathological changes are not fully understood. Studies on rats can provide insights into the risks to humans in high-exposure environments. This study aimed to address existing knowledge gaps by evaluating the effects of petroleum fume inhalation on histopathological changes in male Wistar albino rats.

Aim of the study

The aim of this study was to investigate the effect of inhalation of petroleum products on the lungs of male Wistar albino rats.

Objectives of the study

The specific objectives of the study were as follows:

- Determine the effect of petroleum product inhalation on superoxide dismutase (SOD) activity in male Wistar albino rats.

- Determine the effect of petroleum product inhalation on catalase (CAT) activity in male wistar albino rat.
- Determine the effect of petroleum product inhalation on glutathione -s- transferase (GST) activity in male Wistar albino rats.
- Determine the effect of petroleum product inhalation on the histological indices of the lung tissue of the male wistar albino rat.

Method

Experimental design

Forty-five (45) adult male Wistar rats aged 3 months, weighing between 180 and 220 g were procured from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. They were housed in wire-gauze ventilated cages at Power-Tech Analytical and Scientific Research Laboratory, Independence Layout, Enugu, Enugu State. The rats were fed standard rat chow and clean water *ad-libitum* and were kept under a normal room temperature of 25 ± 2^0C with humidity of $45 \pm 5\%$. The rats were allowed to acclimatize for 2 weeks before the start of the experiment. Procedures involving animals and their care were performed in accordance with the National Institutes of Health (NIH) guidelines for animal care and use. The rats were grouped into five (5) cages labeled A-E, which consisted of three (3) rats each, each replicated three times. The groups were grouped as follows: Group A (Control Group) comprised nine rats that were fed with normal rats' chow and clean water without exposure to any petroleum products; Group B comprised nine rats that were exposed to 500 ml of Premium Motor Spirit (PMS); Group C comprised nine rats that were exposed to 500 ml of dual purpose kerosene (DPK); Group D comprised nine rats that were exposed to 500 ml of diesel; and Group E, comprised nine rats that were exposed to 500 ml of a combined mixture of PMS, DPK, and diesel. The experiment lasted for 28 days after exposure to the petroleum products. Lungs tissue homogenate was taken from three rats each from the experimental and control groups for oxidative stress parameters. The lungs were harvested from the three rats for histological indices on weeks 0, 2, and 4 post-exposure. The samples were taken to the Power–Tech Analytical and Scientific Research Laboratory for oxidative stress and histological analysis.

Animal Model Selection (male Wistar rat)

Forty-five (45) adult male Wistar rats aged 3 months, weighing between 180 and 220 g were obtained from the Department of Human Physiology, University of Nigeria, Enugu campus, Enugu State. They were housed in well-ventilated cages. The rats were fed standard rat chow and tap water *ad-libitum*.

Petroleum-Product Inhalation Protocol

The method of exposure employed in this study was inhalation. The animal was placed in an exposure chamber that was sealed and ventilated. Petroleum products (PMS, DPK, diesel) was gotten from Nigerian National Petroleum Corporation (NNPC) Mega Filling Station Enugu, Nigeria. Four highly perforated 1000 ml cans containing 500 ml of diesel, dual purpose kerosene (DPK), premium motor spirit (PMS), and mixed doses of the three petroleum products were placed in the exposure chamber, and the animals were allowed to inhale the fumes evaporating from the cans. The exposure period lasted for 5 h. The experiment lasted for 28 days. The exposure time was between 9.00 am and 2.00 pm, after which the animals were transferred to the fume-free section of the experimental animal house.

Collection and preparation of respiratory tissue homogenates

The lungs of the sacrificed rat were harvested using the dissection method, weighed, and homogenized with potter-Elvenhjem tissue homogenizer in a potassium phosphate buffer 10MmPH (7.4). The respiratory tissue homogenate was centrifuged at 10,000 revolutions per minute for 15 min in a cold centrifuge, and the resultant supernatant was used for different estimations of oxidative stress markers.

Oxidative Stress Analysis

Superoxide dismutase (SOD) activity

The method described by McCord and Fridovich (1969) was applied to the determination of superoxide dismutase. We estimated this in the erythrocyte lysate prepared from the 5% RBC suspension. 50µL of the lysate, 7 Mm of Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM of pyrogallol. The increase in absorbance was

recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity represents 50% inhibition of the rate of autoxidation of pyrogallol as determined by the change in absorbance per min at 402ηm. The activity of SOD is expressed as units/mg protein.

Catalase (CAT) activity

Catalase activity was determined in erythrocyte lysate using Aebi's method (Aebi, 1984). Fifty microliters of the lysate were added to a cuvette containing 2.0 mL of phosphate buffer (pH 7.0) and 1.0 mL of 30 Mm H_20_2. Catalase activity was measured at 240ηm for 1 min using a spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity. One unit of activity was equal to 1.0 mmol of H_2O_2 degraded per minute and expressed as units per milligram of protein.

Glutathione -s-transferase (GST) activity

The method described by Jocelyn (1972) was used. The reaction mixture (1.0mL) consisted of 0.1 N potassium phosphate (pH 6.5), 1.0 nM/L glutathione, 1.0 M/L 1-chloro-2, 4-dinitrobenzene as a substrate, and a suitable amount of cytosol (6 mg protein/mL). The reaction mixture was incubated at 37^0C for 5 min, and the reaction was initiated by the addition of substrate. The increase in absorbance at 340ηm was measured spectrophotometrically.

Histological Examination

Tissue preparation

The surviving experimental animal was sacrificed. Gross lesions were recorded during the postmortem examination. Sections of the lungs were collected using the dissection method after the rat was euthanized through cervical dislocation, and the rat carcass was secured to a dissecting board using tacks (Morton and Snider, 2017). The rat was harvested, prepared, and examined for histopathological changes. After excision, the samples were fixed in 10% phosphate-buffered formalin for 72 h. The tissues were subsequently trimmed using a microtome, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene, and embedded in molten wax. On solidifying, the blocks were cut into 5 µm thick tissue sections using a rotary microtome, floated in a water bath, and then incubated at 60^0C for 30 min. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90 %, 80 %, and 70%). The sections were stained with hematoxylin for 15 minutes. Bluing was performed using ammonium water. Differentiation was performed with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a Dibutylphthalate Polystyrene Xylene (DPX) mountant.

Slide examination and photomicrographs

The prepared slides were examined with a Motic™ compound light microscope using x4, x10, and x30 objective lenses. The photomicrographs were taken using a Motic™ 2.0 megapixel microscope camera at ×120 and ×300 magnifications. The images were stored diligently.

Statistical Analysis

All the statistical analysis were processed by using the Statistical Package of Social Science (SPSS) for the window (version 16). The values of the measured parameters are expressed as mean $\pm$ SEM. Two-way analysis of variance (2-way ANOVA) was used to determine the effect of different inhalants and number of days after exposure on the parameters that were studied, and the differences between means were separated using Duncan's multiple range test. Test for significance was considered at 0.05 probability level.

Results

Oxidative Stress Parameters

Superoxide dismutase (SOD) activity of male Wistar albino rats exposed to petroleum products

The effects of petroleum product inhalation on superoxide dismutase activity in male Wistar albino rats are presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test groups and the control. There was also no significant ($p>0.05$) difference in the mean value of superoxide dismutase activity (µ/dl) among the other test groups. SOD activity was reduced on day 14^{th} day post-exposure in the test groups (11.57 ± 0.28, 11.13 ± 0.74, 11.41 ± 0.45, and 11.75 ± 1.39 µ/dl) when compared to the control (12.87 ± 0.93 µ/dl). The superoxide dismutase activity among test groups showed no significant difference ($p>0.05$) among groups B, D and E. On day 28 of exposure, the mean value of superoxide dismutase activity maintained the same trend as that on day 14 when compared with the control; however, the mean value of superoxide dismutase activity of

kerosene was significantly lower ($p<0.05$) when compared with the other test groups. There were no significant difference ($p>0.05$) among groups B, D, and E (table 1).

Table 1: Effect of petroleum product inhalation on superoxide dismutase (µ/dl) activity in male Wistar albino rats.

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	11.44 ± 0.82^{a1}	12.87 ± 0.93^{b1}	12.87 ± 0.93^{c1}
B (PMS Exposure)	10.56 ± 0.45^{a1}	11.57 ± 0.28^{a1}	10.21 ± 0.83^{b1}
C (Kerosene Exposure)	10.22 ± 0.76^{a2}	11.13 ± 0.74^{a2}	8.96 ± 0.93^{a1}
D (Diesel Exposure)	9.89 ± 1.26^{a1}	11.41 ± 0.45^{a1}	10.23 ± 0.30^{b1}
E (Mixed PP)	9.53 ± 0.33^{a1}	11.75 ± 1.39^{a1}	11.12 ± 0.48^{b1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the letter down the column are significantly different ($p<0.05$) while mean values with same figures or number as superscript are not significantly different ($p>0.05$).

Catalase activity of male Wistar albino rats exposed to petroleum products

The effects of petroleum product inhalation on catalase activity in male Wistar albino rats are presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test group and the control. There was also no difference in the mean value of CAT activity (µ/dl) among the test groups. CAT activity on day 14 post-exposure was significantly reduced between the test groups (1.65 ± 0.25, 1.87 ± 0.08, 1.73 ± 0.20, and 1.67 ± 0.63µ/dl) when compared to control (3.29 ± 0.70 µ/dl). The mean value of CAT activity among test groups showed no significant difference ($p>0.05$). On the 28th day of exposure, the mean value of CAT activity maintained the same trend as that on day 14 compared with the control (table 2).

Table 2: Effect of petroleum product inhalation on catalase (µ/dl) activity of male Wistar albino rats.

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	2.74 ± 0.44^{a1}	3.29 ± 0.70^{b1}	3.29 ± 0.70^{b1}
B (PMS Exposure)	1.39 ± 0.24^{a1}	1.65 ± 0.25^{a1}	1.58 ± 0.21^{a1}
C (Kerosene Exposure)	1.34 ± 0.33^{a1}	1.87 ± 0.08^{a1}	1.21 ± 0.18^{a1}
D (Diesel Exposure)	1.28 ± 0.13^{a1}	1.73 ± 0.20^{a1}	1.28 ± 0.05^{a1}
E (Mixed PP)	1.34 ± 0.28^{a1}	1.67 ± 0.63^{a1}	1.63 ± 0.23^{a1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the letter down the column are significantly different ($p<0.05$) while mean values with same figures or number as superscript are not significantly different ($p>0.05$).

Glutathione-s-transferase (GST) activity in male Wistar albino rats exposed to petroleum products

The effects of petroleum product inhalation on glutathione S-transferase activity in male Wistar albino rats are presented. The baseline result (Day 0) showed no significant difference ($p>0.05$) between the test group and the control. There was also no difference in the mean value of the GST value (µg/l) among the other test groups. On the 14th day post-exposure, there were no significant different ($p<0.05$) between the test groups (24.82 ± 1.58, 22.86 ± 1.90, and 23.78 ± 2.60 µg/l) compared with the control (23.20 ± 0.45 µg/l), except for the mixed petroleum product (26.22 ± 1.69 µg/l). On day 28 of exposure, the mean value of GST activity maintained the same trend

as on day 14. However, groups D and E showed no significant difference ($p>0.05$) when compared with the control (table 3).

Table 3: Effects of petroleum product inhalation on glutathione-s-transferase (µg/l) activity Of male wistar albino rats

GROUPS	DAY 0	DAY 14	DAY 28
A (Control)	24.40 ± 0.84^{a1}	23.20 ± 0.45^{a1}	23.20 ± 0.45^{b1}
B (PMS Exposure)	23.42 ± 3.85^{a1}	24.82 ± 1.58^{a1}	19.24 ± 1.00^{a1}
C (Kerosene Exposure)	21.70 ± 0.82^{a1}	22.86 ± 1.90^{a1}	19.54 ± 0.71^{a1}
D (Diesel Exposure)	21.38 ± 1.31^{a1}	23.78 ± 2.60^{a1}	23.20 ± 1.15^{b1}
E (Mixed PP)	23.40 ± 2.39^{a1}	26.22 ± 1.69^{b2}	24.02 ± 0.54^{b1}

The values are expressed as (mean ± SEM)

Mean values with different letters of the letter down the column are significantly different ($p<0.05$) while mean values with same figures or number as superscript are not significantly different ($p>0.05$).

Histological indices of the lungs

Exposure to premium motor spirits

Sections of the lungs tissue of the experimental rats for control showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 2A). On the first day of exposure after 5 hours of exposure, the lung tissue showed pigmented tissue (Plate 2B). Sections of lung tissue from the experimental rat at week 2 (14 days) showed pigmented tissue (Plate 2C). After 28 days of exposure, the lung tissue showed normal alveolar sacs (S), bronchioles (B), and ducts (D) with intervening connective tissue (T) (Plate 2D).

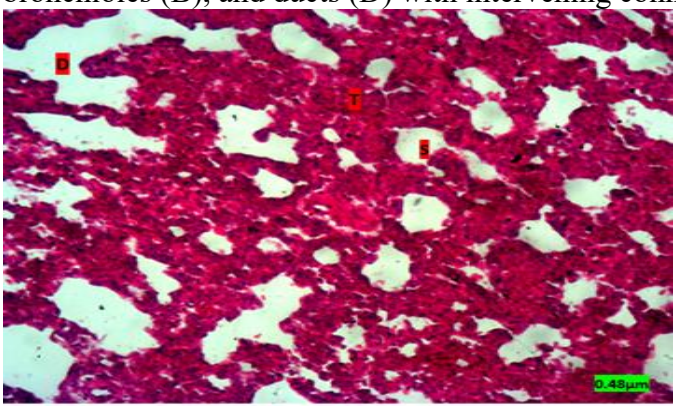


Plate 2A: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H and E. X300).

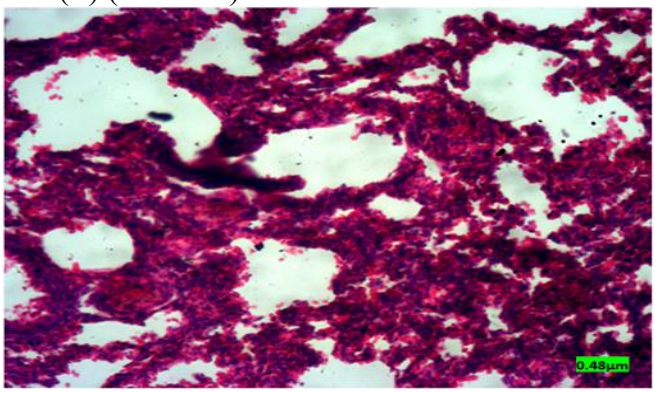
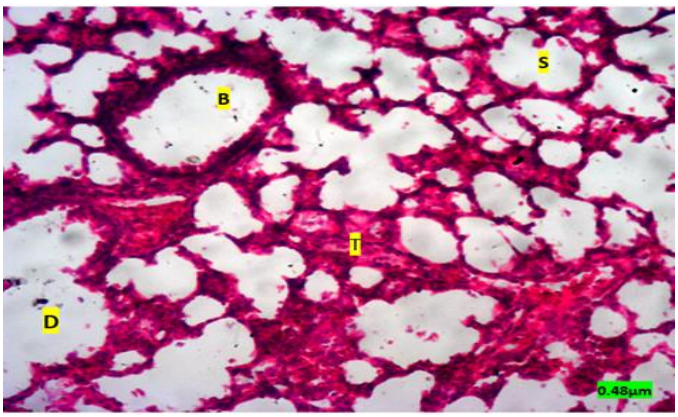


Plate 2B: Photomicrograph of the lung tissue showing pigmented tissue (H & E. X300).



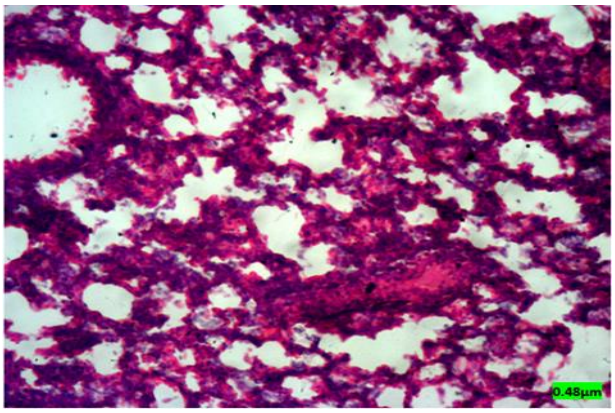


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Plate 2D: Photomicrograph of lung tissue showing alveolar sacs (S), bronchioles (B), and ducts (D) with intervening connective tissue (T) (H and E. X300).

Plate 2C: Photomicrograph of pigmented lung tissue (H and E. X300).

Exposure to dual-purpose kerosene

Sections of the lungs tissue of the experimental rats for control showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 3A). On the first day after exposure, sections of the lungs tissue showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 3B). Sections of lung tissue from the experimental rat at week 2 (14 days) showed general metaplasia (Plate 3C). After 28 days of exposure, the lung tissue showed mild general pigmentation of the alveolar tissue (Plate 3D).

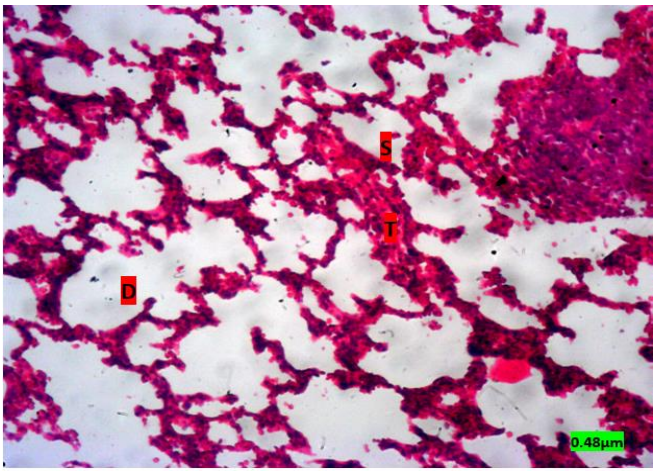

Plate 3A: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H & E. X300).

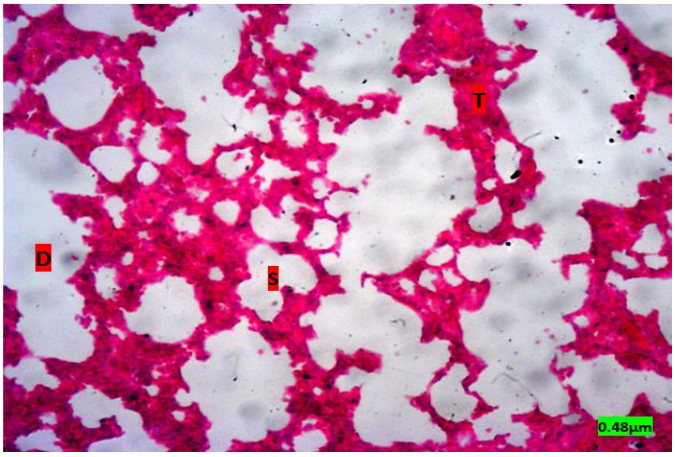

Plate 3B: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H & E. X300).

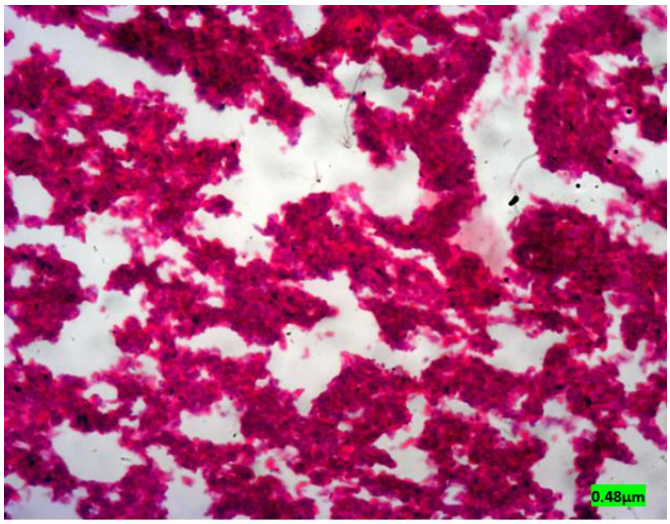

Plate 3C: Photomicrograph of lung tissue showing general metaplasia (H and E. X300).

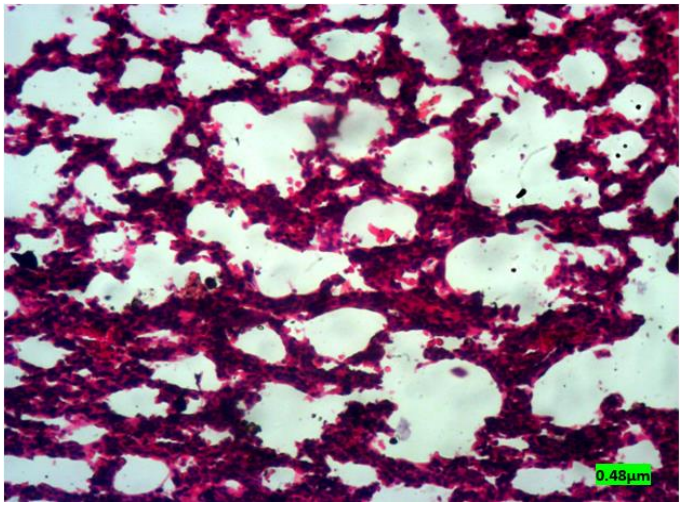

Plate 3D: Photomicrograph of the lung tissue showing mild general pigmentation of the alveolar tissue (H and E. X300).

Exposure to diesel

The lungs tissue of the control group was harvested and showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 4A). On day 0 of exposure, the section of lung tissue harvested showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 4B). Sections of lung tissue from the experimental rat at week 2 (14 days) showed pigmented tissue (Plate 4C). After 28 days of exposure, the lung tissue exhibited severe tissue acute respiratory distress syndrome (Plate 4D).

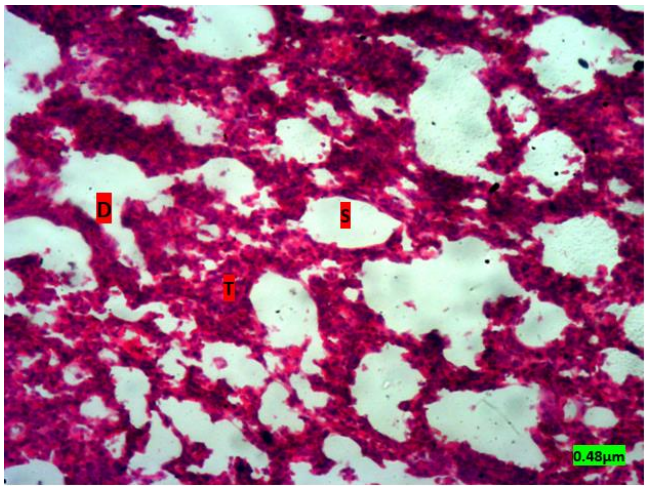

Plate 4A: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H and E. X300).

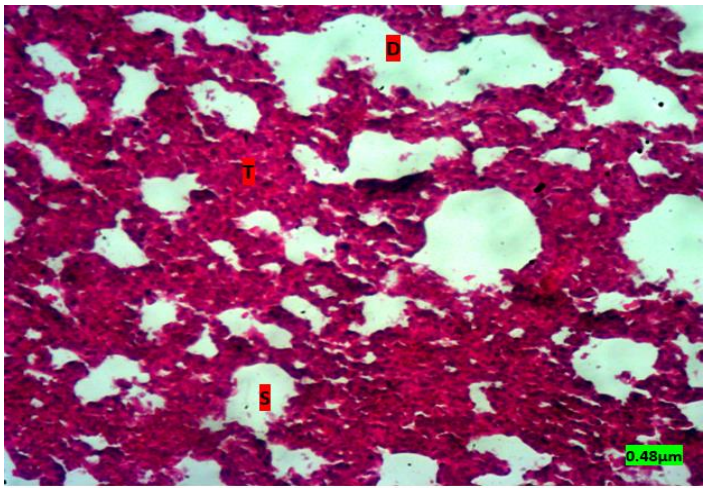

Plate 4B: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H and E. X300).

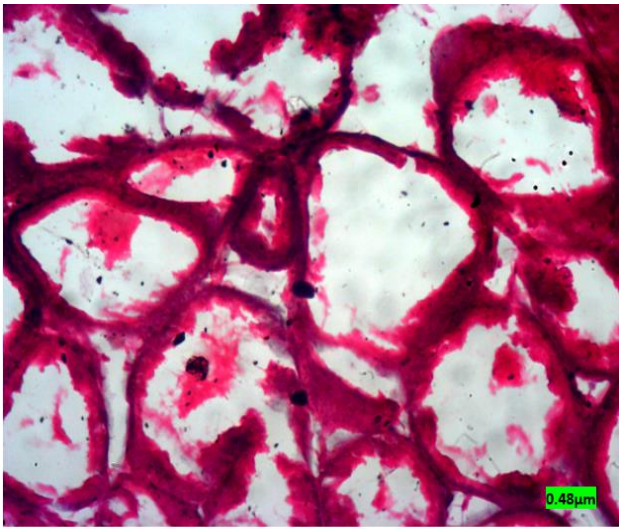

Plate 4D: Photomicrograph of lung tissue showing severe tissue acute respiratory distress syndrome (H and E. X300).

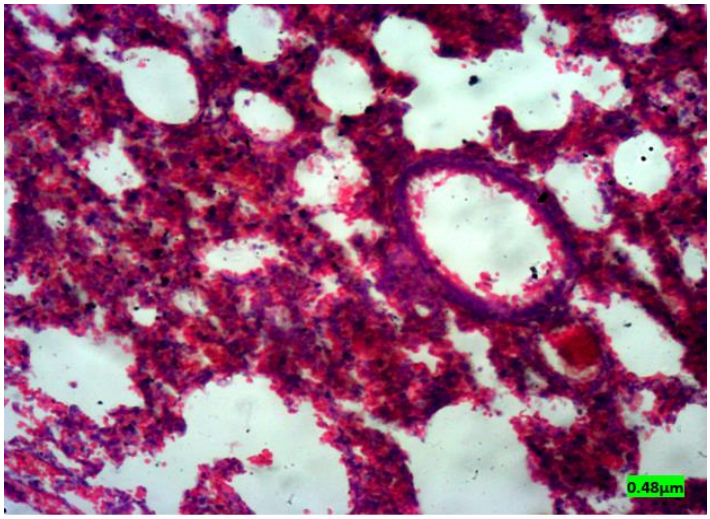

Plate 4C: Photomicrograph of lung tissue showing pigmented tissue (H and E. X300).

Exposure to mixed petroleum products

Sections of the lungs tissue of the control group showed normal alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (Plate 5A). The section of lung tissue on day 0 of exposure showed pigmented tissue (Plate 5B). Sections of lung tissue from the experimental rat at week 2 (14 days) showed metaplastic and pigmented tissue (Plate 5C). After 28 days of exposure, the lung tissue showed normal alveolar sacs (S), bronchioles (B), and ducts (D) with intervening connective tissue (T) (Plate 5D).

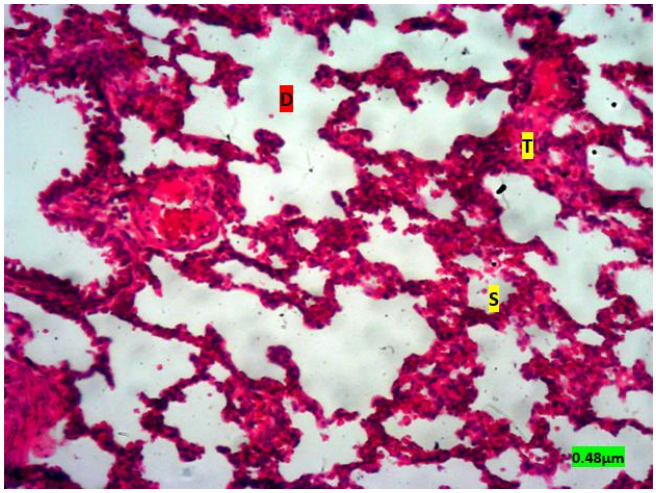

Plate 5A: Photomicrograph of lung tissue showing alveolar sacs (S) and ducts (D) with intervening connective tissue (T) (H and E. X300).

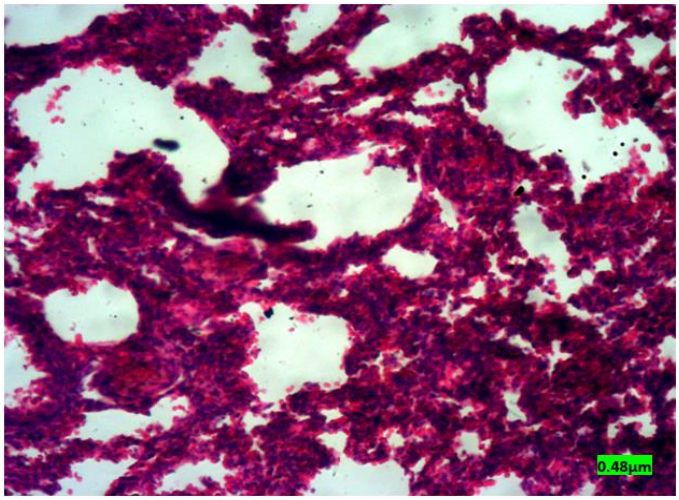

Plate 5B: Photomicrograph of the lung tissue showing pigmented tissue (H & E. X300).

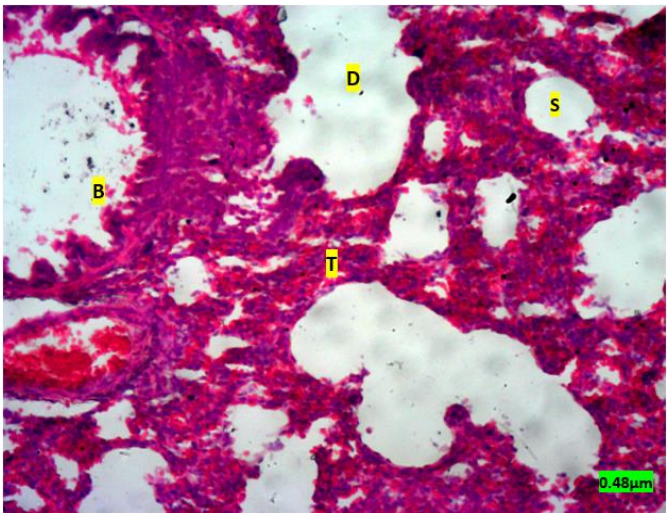

Plate 5D: Photomicrograph of lung tissue showing alveolar sacs (S), bronchioles (B), and ducts (D) with intervening connective tissue (T) (H and E. X300).

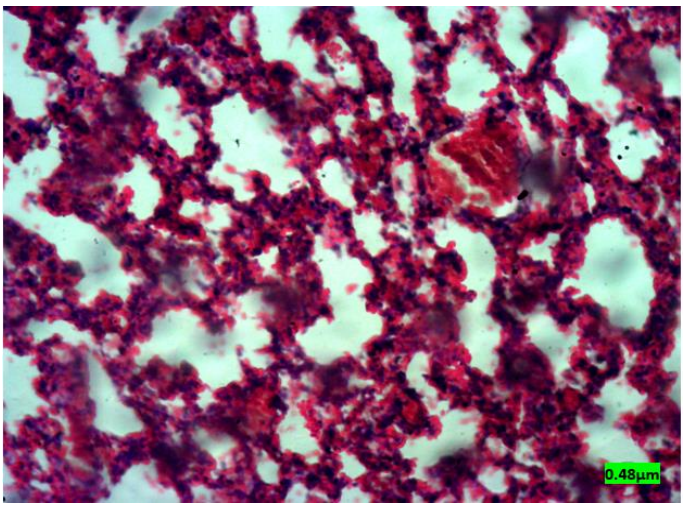

Plate 5C: Photomicrograph of lung tissue showing metaplastic and pigmented tissue (H and E. X300).

Discussion, Conclusion, and Recommendations

Discussion

Oxidative stress parameters of male wistar albino rats exposed to petroleum products

The current study showed that petroleum product inhalation significantly decreases superoxide dismutase activity. The study agreed with Kemabonta *et al*. (2020), who studied SOD activity in rats exposed to petrol, xylene, and thinners, where they found that there is a significant decrease in the value of SOD activity, indicating toxic buildup in the blood, implying that exposures to these (Volcanic Organic Carbon) VOCs inhibit natural processes.

The results of the present study showed that petroleum product inhalation significantly decreased catalase activity. This study agreed with the research conducted by Olufunke *et al*. (2018), who exposed rats to gobbled crude oil painting, leading to a significant decrease in catalase exertion in liver atkins. The study also aligned with the

research conducted by Amara *et al*. (2016), who delved into the impact of crude oil painting exposure on the lungs of rats, resulting in a significant decrease in catalase expression.

The results of the present work indicate that petroleum product inhalation significantly decreases glutathione-s-transferase activity. The results also agreed with the research of Olufunke *et al*. (2018), who exposed rats to gobbled crude oil painting and observed a significant drop in GST exertion in the liver at kins.

The present study showed pigmented tissue on the lung section as well as acute respiratory distress syndrome, which is in agreement with the research conducted by Uboh *et al*. (2010), who discovered that frequent exposure to gasoline vapors in male Wistar albino rats resulted in significant changes in the lungs' tissues. These changes included thickening of the walls separating the air sacs, bleeding, and the infiltration of cells that cause inflammation, indicating acute lung injury. The study showed general metaplasia and mild general pigmentation of the alveolar sac, which is in agreement with the findings of Okoro *et al*. (2006), who studied how gasoline fumes affect the respiratory system of albino rats. Their findings revealed that prolonged exposure reduced protective enzyme levels in the lungs, increasing harmful chemical reactions that damage lung tissue. This damage is characterized by the breakdown of fats and proteins, which weaken the structure of the lung lining and may contribute to the development of chronic diseases like COPD. The findings also agreed with the results of Ita and Udofia (2011), who investigated the impact of inhaling kerosene fumes on the respiratory system of rats. The results showed that exposure to kerosene vapors leads to significant respiratory problems, including coughing, wheezing, and difficulty breathing. The analysis of lung tissue revealed abnormal growth of cells lining the airways, enlargement of mucous-producing glands, and increased scar tissue around air passages, all of which indicated conditions similar to chronic bronchitis and asthma.

Conclusion

The findings suggest that exposure to petroleum products triggers oxidative stress, as indicated by elevated reactive oxygen species levels and disruption of antioxidant enzyme activity. Histological examination also revealed generalized metaplasia and mild pigmentation in the alveolar sacs of the lung tissue. These results highlight the harmful impact of inhaling petroleum products on vital organ systems, particularly the lungs.

Recommendations

This study emphasizes the necessity of enforcing rigorous environmental regulations and implementing targeted public health interventions to mitigate exposure to petroleum products, thereby safeguarding vulnerable populations. Further studies are therefore recommended to conduct comparative studies with other environmental pollutants to establish synergistic or antagonistic effects on lung pathology. This approach is intended to improve knowledge of the cumulative effect on the lungs.

Declaration

We declare that this manuscript titled "Effect of Petroleum Product Inhalation on Histological Indices of Lung Tissue of Male Wistar Albino Rats" is original and has not been published nor submitted elsewhere for publication. All data were collected and analyzed following ethical guidelines for animal research. There are no conflicts of interest to declare, and all authors have approved the final version of the manuscript for submission.

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Conflict of Interest: This study focuses on eco-toxicological effect of petroleum product on human health. The authors declare no conflicts of interest regarding the manuscript titled *"Effect of Petroleum Product Inhalation on Histological Indices of Lung Tissue in Male Wistar Albino Rats.*

References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, **105**: 121–126.

Ahmed, F., Fakhruddin, A. N. M. (2018). A review on environmental contamination of petroleum hydrocarbons and its biodegradation. *International Journal of Environmental Sciences & Natural Resources*, **11**(3): 1-7.

Albano, G. D., R. P. Gagliardo, A. M. and Profita, M. (2022). Overview of the mechanisms of oxidative stress: impact in inflammation of airway diseases. *Antioxidants*, **11**(11): 2237.

Antonelli, M., Donelli, D., Barbieri, G., Valussi, M., Maggini, V. and Firenzuoli, F. (2020). Forest volatile organic compounds and their effects on human health: A state-of-the-art review. *International Journal of Environmental Research and Public Health*, **17**(18): 6506.

Ita S. O. and Udofia U. A. (2011). Comparative study of hematological parameters in rats following ingestion of crude oil (Nigeria bonny light), petrol, kerosene, and diesel. *Asian J Biol Sci*, **4**: 498-505.

Ivshina, I. B., Kuyukina, M. S., Krivoruchko, A. V., Elkin, A. A., Makarov, S. O., Cunningham, C. J. and Philp, J. C. (2015). Oil spill problems and sustainable response strategies through new technologies. *Environmental Science: Processes & Impacts*, **17**(7): 1201-1219.

Jocelyn, P. C. (1972). Biochemistry of the SH Group. *Academic press, London,* 10.

Kemabonta, K. A., Ikerodah, A. A. and Anumudu, P. C. (2020). Toxicological Effects of Petroleum, Xylene, and Thinner on Mus musculus (Albino Mice).

McCord, J. M. and Fridovich, I. (1969). Superoxide dismutase: an enzymic function of erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, **244**(22): 6049–6055.

Morton, J. and Snider, T. A. (2017). Guidelines for collection and processing of lungs from aged mice for histological studies. *Pathobiology of Aging and Age-Related Diseases* **7**(1): 1313676.

Okoro, A. M., Ani, E. J., Ibu J. O. and Apkogomeh B. A. (2006). Effects of petroleum product inhalation on hematological parameters of fuel attendants in Calabar metropolis, Nigeria. *Niger J Physiol Sci*, 21:71-5.

Olufunke, O. J., Oyewopo, A. O., Bawo, S. D., and Otache, P. O. (2018). Effects of inhaled crude oil on biochemical markers of oxidative stress in rats. *Journal of Environmental Science, Toxicology, and Food Technology*, **12**(9), 12-16.

Owumi, S. E., Elebiyo, T. C. and Oladimeji, B. N. (2021). Oxido-inflammatory responses and histological alterations in rat lungs exposed to petroleum product fumes. *Environmental toxicology*, **36**(1): 132-143.

Robinson, P. R. and Hsu, C. S. (2017). Petroleum and its products. *Handbook of Industrial Chemistry and Biotechnology*, 13-106.

Saeedi, M., Malekmohammadi, B. and Tajalli, S. (2024). Interaction of benzene, toluene, ethylbenzene, and xylene with human body: Insights into characteristics, sources and health risks. *Journal of Hazardous Materials Advances*, **16**: 100459.

Uboh, F., Akpanabiatu, M., Ekaidem, I., Eteng, M., and Eyong, E. (2010). Exposure to gasoline and kerosene vapors: A risk factor for nephrotoxicity in rats. *Internet J Toxicol*, **7**(2): 1-7.

Zhao, Q., Li, Y., Chai, X., Xu, L., Zhang, L., Ning, P. and Tian, S. (2019). Interaction of inhalable volatile organic compounds and pulmonary surfactant: Potential hazards of VOC exposure to the lungs. *Journal of hazardous materials*, **369**: 512-520.

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2. **Dr. Cyril Onyekachi Edoga** was born on 2nd August, 1984 in Mgboko Aku, Igbo Etiti LGA of Enugu State, Nigeria. My academic journey led me to earn a Ph.D. in Animal and Environmental Physiology from the University of Nigeria, Nsukka. Currently a senior academic at Enugu State University of Science and Technology (ESUT). In 2024, I was conferred with Chieftaincy title as Ebekuedike 1 of Ukwuoto Edenu Ede-Oballa Ancient kingdom. As a devout Catholic, I integrate faith into my leadership, striving to uplift my people through education and moral guidance. My work bridges academia, research, community development and spirituality, dedicated to the service of God and humanity.
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