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Histomorphological Assessment of Lungs Architecture in Rat Model Exposed to Petroleum Product Fumes

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ABSTRACT

Petroleum products are used for various purposes owing to their importance; however, the need for their constant application has led to persistent exposure to humans and the environment. This study seeks to investigate the effect of long-term exposure to these products on the histoarchitecture of the lungs in the rat model. A total of forty adult Wistar rats with weights ranging from 158 g to 230 g were procured. The rats were randomly divided into four groups of ten animals each. Group C_T animals (Control) were exposed to fresh air only for twenty weeks. The Test Groups, Group P animals were exposed to 100 ml of petrol fumes between four and twenty weeks, Group K animals were exposed to 100 ml of kerosene fumes between four and twenty weeks, while Group D animals were exposed to 100 ml of diesel fumes between four and twenty weeks. The normal histomorphology of the lungs as seen in the control group showed clearly the bronchioles, pulmonary alveoli, and blood vessels. Generally, prolonged exposure to petroleum products results in a number of respiratory complications. There were different histoarchitectural changes seen in the test groups. Such changes include congested blood vessels with distorted pulmonary alveoli, distorted, dilated alveoli, hemorrhagic and inflammatory cells. The damage seen on the histoarchitecture of the lungs is characterized by the breakdown of fats and proteins, which weakens the structure of the lung lining and may eventually lead to the development of chronic diseases like chronic obstructive pulmonary disease (COPD).

Keywords: petroleum products, histoarchitecture, lung disease, prolonged exposure, fumes

INTRODUCTION

Petroleum products (diesel, kerosene, petrol (gasoline)) are derivatives of the fractional distillation of crude oil (petroleum). The fractions of petroleum are made up of both aromatic and aliphatic varieties of saturated and unsaturated hydrocarbons: Toluene, Benzene, and Xylene (TBX) [1]. Petroleum products are used for various purposes owing to their importance; however, the need for their constant usage has led to persistent exposure to humans and the environment [2]. This exposure to humans is increasingly becoming a public health concern because of its effects on human health [3]. Exposure to these products can be grouped into two sources: domestic and occupational. However, those exposed due to the nature of their job may be mostly impacted [1].

Occupational exposure can be seen as any contact between humans and a potentially harmful agent or environment in the workplace [4]. Occupationally exposed individuals in this case include petrol tanker drivers, petrol attendants, petroleum refinery workers, automobile mechanics, cobblers who use petrol in producing local gum for shoe repairs, and roadside petrol dispensers (Black market traders). However, domestic exposure involves individuals and households that use petroleum products to power heavy-duty machines, power-generating sets, and other household appliances. The various routes that these products can be absorbed include inhalation, ingestion, dermal, and ocular routes [5]. Owing to the lipophilic nature of petroleum products, biological membranes are targeted, leading to structural and functional discrepancies upon exposure [6].

In humans, some snails, and a number of fish, the primary organ of the respiratory system is the lungs. In most vertebrates and mammals, the lungs are located on either side of the heart near the backbone. They function in the transportation of oxygen from the atmosphere into the bloodstream and subsequent

release of carbon dioxide (CO_2) from the blood to the atmosphere through a process called gaseous exchange [7]. Airflow provided by the lungs makes human speech and vocal sounds possible.

The tissue of the lungs can be affected by a number of respiratory diseases, including pneumonia and lung cancer [8]. Chronic obstructive pulmonary diseases include chronic bronchitis, emphysema, and several occupational lung diseases that can be related to smoking or long-term exposure to harmful substances, which may include tetraethyl lead in petroleum products, coal, dusts, asbestos fibers, and crystalline silica dust [9].

A well-refined petroleum product obtainable in developed countries is devoid of poisonous chemicals. Nevertheless, in developing countries like Nigeria, many petroleum marketers cut corners to maximize profits [10], hence resulting in distributing poorly refined petroleum products containing poisonous chemicals such as tetraethyl lead and other contaminants to the public. It is against this background that this study seeks to investigate the effect of long-term exposure to these products on the histoarchitecture of the lungs in the rat model.

MATERIALS AND METHODS

Experimental site

This study was carried out in the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. The town of Abakaliki is the capital city of Ebonyi State in southeastern Nigeria. The state is located geographically as seen in fig. 1, between Latitude 6^0 15'N and Longitude 8^0 05'E in the South East derived savannah zone of Nigeria. The climate of Ebonyi State is found within the humid tropical climate regions. It experiences one rainy season and one dry season (8 months of rainfall and 4 months of dryness). Harmattan is felt between December and January. The mean yearly temperature remains at 28^0C. The temperature in the dry season ranges from 20^0C to 38^0C and from 16^0C to 28^0C during the blustery season. The mapped territory has a mean yearly precipitation of 2500 mm. Mugginess here is around 50-60% per annum.

Experimental animals

A total number of forty (40) adult Wistar rats with weights ranging from 158 g to 230 g were procured from the animal house of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. All animals used for this study were treated in accordance with the ethics and guidelines of the Institutional Animal Care and Use Committee (IACUC). An ethical code EB/ANT/004 was issued by the Ethics Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. The animals were kept in well-ventilated polyethylene cages and given normal rat feed and water *ad libitum*. They were left to acclimatize for 2 weeks. The animal house was kept properly ventilated, cleaned, and disinfected at intervals of 3 days to ensure a healthy environment.

Procurement of petroleum products

The three (3) petroleum products (diesel, kerosene, and petrol or premium motor spirit (PMS)) were purchased from a local filling station, Murphy Oil Ltd, in Abakaliki Metropolis, Ebonyi State, Nigeria. The products were tested and confirmed in the Industrial Chemistry Lab of Ebonyi State University.

Animal grouping

The rats were randomly divided into four (4) groups of ten (10) animals each, as seen in Table 1. Group C_T animals (Control) were exposed to fresh air only for twenty (20) weeks. The Test Groups, Group P animals were exposed to 100 ml of petrol (P) fumes between four (4) to twenty (20) weeks, Group K animals were exposed to 100 ml of kerosine (K) fumes between four (4) to twenty (20) weeks while Group D animals were exposed to 100 ml of diesel (D) fumes between four (4) to twenty (20) weeks.

Method of exposure and sample collection

The petroleum products were placed in an improvised wooden fume chamber of diameter 75 cm X 50 cm X 30 cm, a hole was bored in the cover of the chamber to allow little ventilation inside the chamber. The animals were exposed to petroleum product fumes in the chamber for 9 hours daily (to match the average period spent by a worker daily in the workplace) during the experimental period. After exposure, the rats were returned to their cages.

On the last day of exposure to the fumes in each case, all rats were anaesthetized in a jar containing cotton wool soaked in diethyl ether; after that, their lungs were harvested and fixed in formalin-saline.

Histological processing

Formol-saline-fixed lungs were dehydrated and embedded in paraffin wax. Eight micrometer-thick sections were cut on a rotary microtome, and sections were stained with Hematoxylin and Eosin (H&E) as described by Bancroft and Stevens [11]. Images were captured with an MW1-HD2 digital microscope at a magnification of x40.

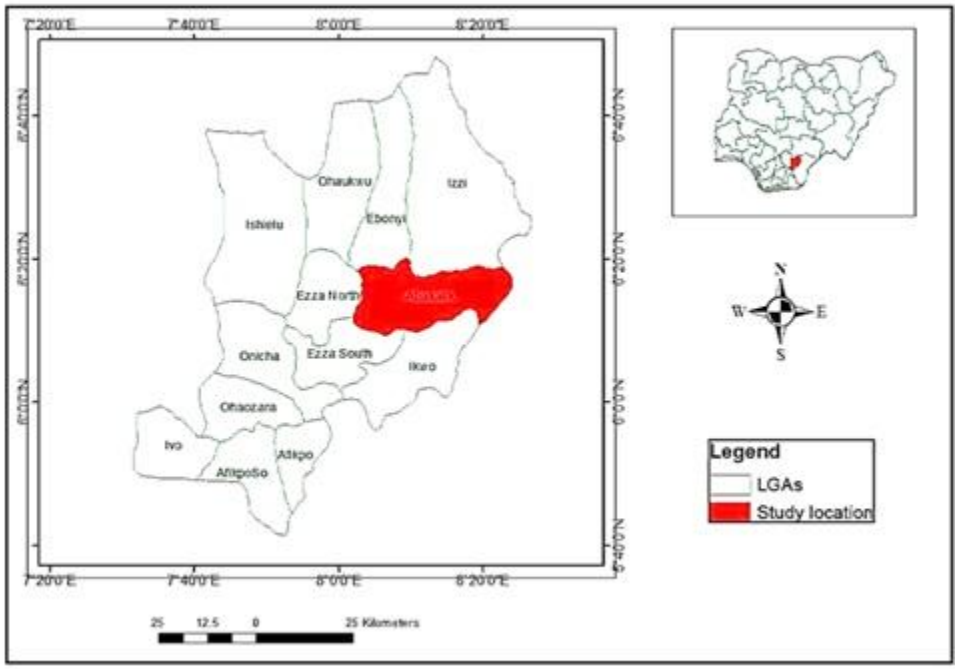

Fig 1: Map of Ebonyi State showing the Study Area, Abakaliki. **Source:** Cartography/GIS Laboratory, Department of Geography and Environmental Management, University of Port Harcourt, Nigeria.

Table 1: Showing the grouping of animals and the period of Exposure

Group	Volume of Petroleum products (ml)	Duration of daily exposure (hrs.)	Duration of exposure (weeks)
C_T (Control)	Fresh air	24:00	20
P (petrol)	100.00	09:00	4-20
K (kerosine)	100.00	09:00	4-20
D (diesel)	100.00	09:00	4-20

RESULTS

The Histomorphology of the lungs

The normal histomorphology of the lungs as seen in the control group showed clearly the bronchioles, pulmonary alveoli, and blood vessels. At the first four (4) weeks of exposure to petroleum products, all the sample groups (P_4, K_4, D_4) showed traces of congested blood vessels with distorted pulmonary alveoli in the histoarchitecture of the lungs. There were noticeable intravascular inflammatory cells with dilated alveoli, which were more prominent in the kerosene-exposed group (K_4), while there were congested blood vessels with dilated alveoli in both petrol and diesel-exposed groups. For eight (8) weeks of exposure, all the sample groups (P_8, K_8, D_8) at X40 showed traces of distorted, dilated alveoli; there were also noticeable scattered dilated alveoli and more visible columnar epithelial cells with the presence of congested blood vessels in all the sample groups. In twelve (12) weeks of exposure, all the sample groups showed the presence of more visible congested blood vessels and blurred, distorted pulmonary alveoli. There was a presence of dilated pulmonary alveoli, inflammatory cells, columnar epithelial cells of the bronchioles, and traces of congested blood vessels in all the sample groups. Exposure for sixteen (16) weeks showed noticeable hemorrhagic cells and dilated pulmonary alveoli in all the sample groups. There was also a noticeable severe hemorrhage. At twenty (20) weeks of exposure, the histoarchitecture of the lungs showed hemorrhagic cells, distorted and dilated alveoli with inflammatory cells across the sample groups.

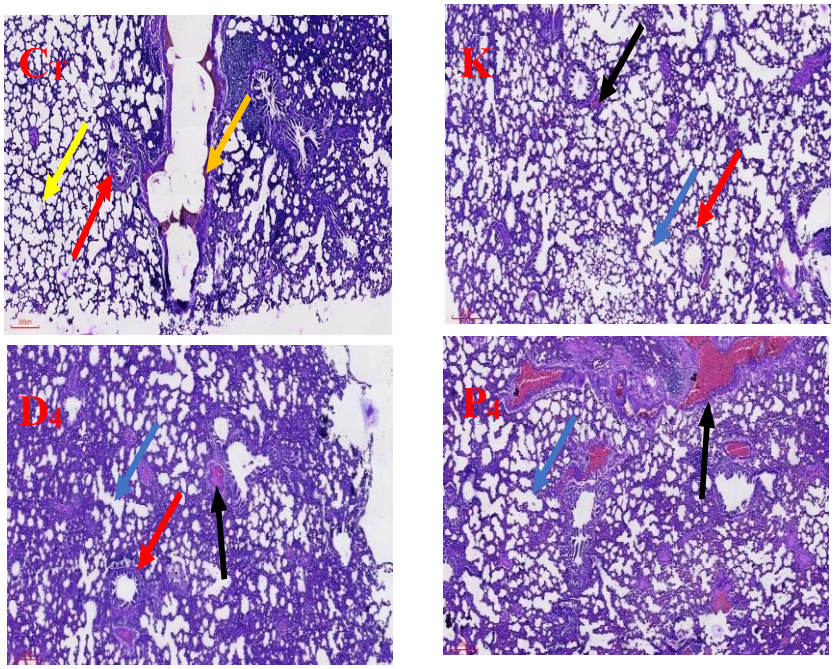

Fig. 2: Histomorphology of Rat Lung Exposed to Kerosene (K_4), Diesel (D_4), and Petroleum (P_4) for four (4) weeks. C_T is the control group. Red arrow = Alveolar sac, Yellow arrow = Pulmonary Alveoli, Blue arrow = Distorted Pulmonary Alveoli, Black arrow = Congested Blood Vessel, and Orange arrow = Bronchiole. Lung: x40 H&E

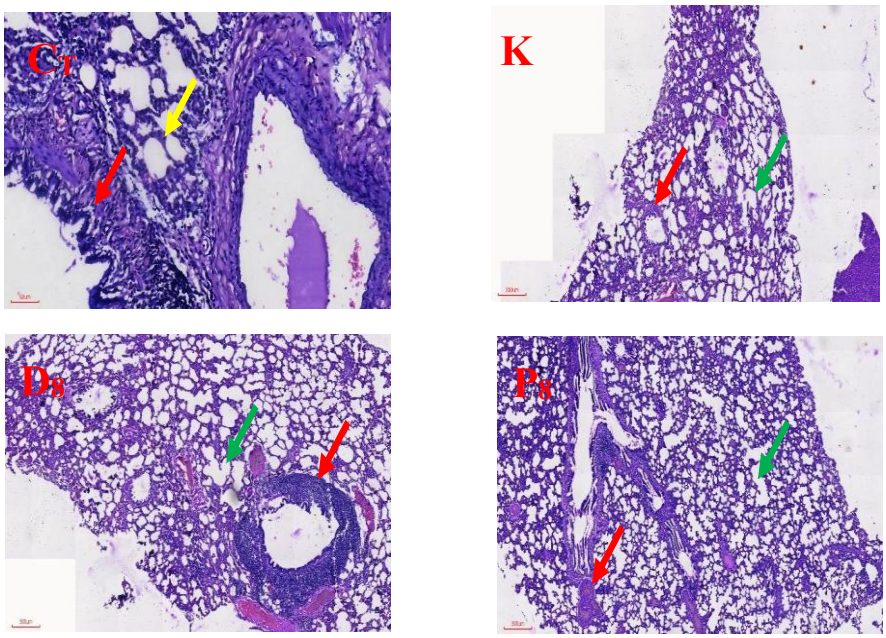

Fig. 3: Histomorphology of the Lung of Rats Exposed to Kerosene (K_8), Diesel (D_8), and Petroleum (P_8) for eight (8) weeks. C_T is the control group. Red arrow = Bronchiole, Yellow arrow = Alveoli, Green arrow = Distorted Alveoli; Lung: x40 H&E

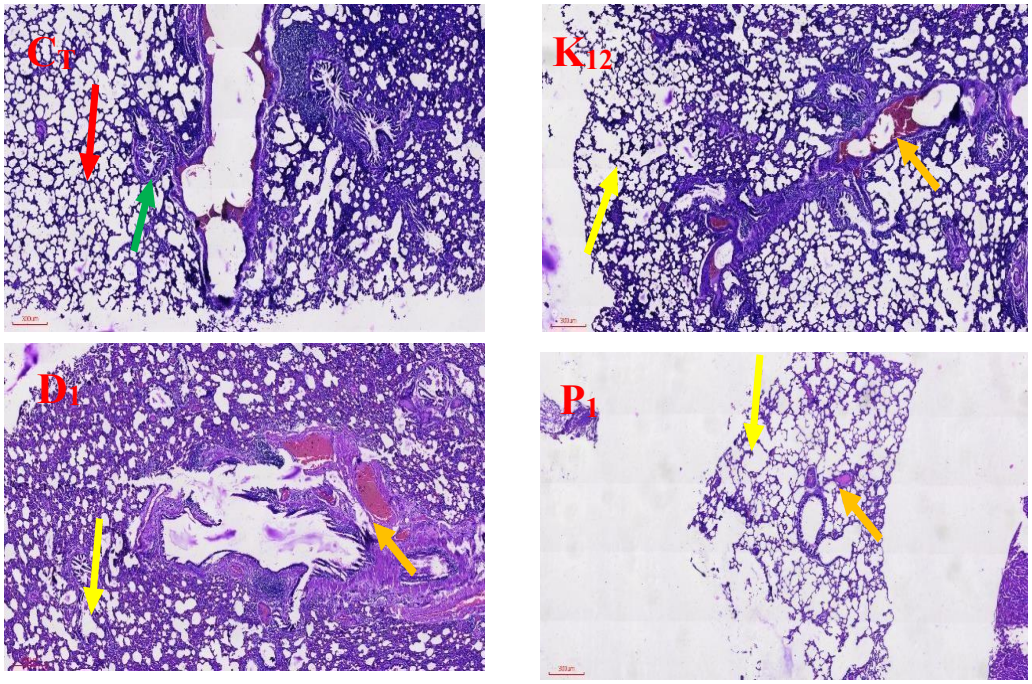

Fig.4: Histomorphology of the Lung of Rats Exposed to Kerosene (K_{12}), Diesel (D_{12}), and Petroleum (P_{12}) for twelve (12) weeks. C_T is the control group. Red arrow = Alveoli, Yellow arrow = Distorted Alveoli, Green arrow = Bronchiole, Orange arrow = Congested Blood Vessel; Lung: x40 H&E

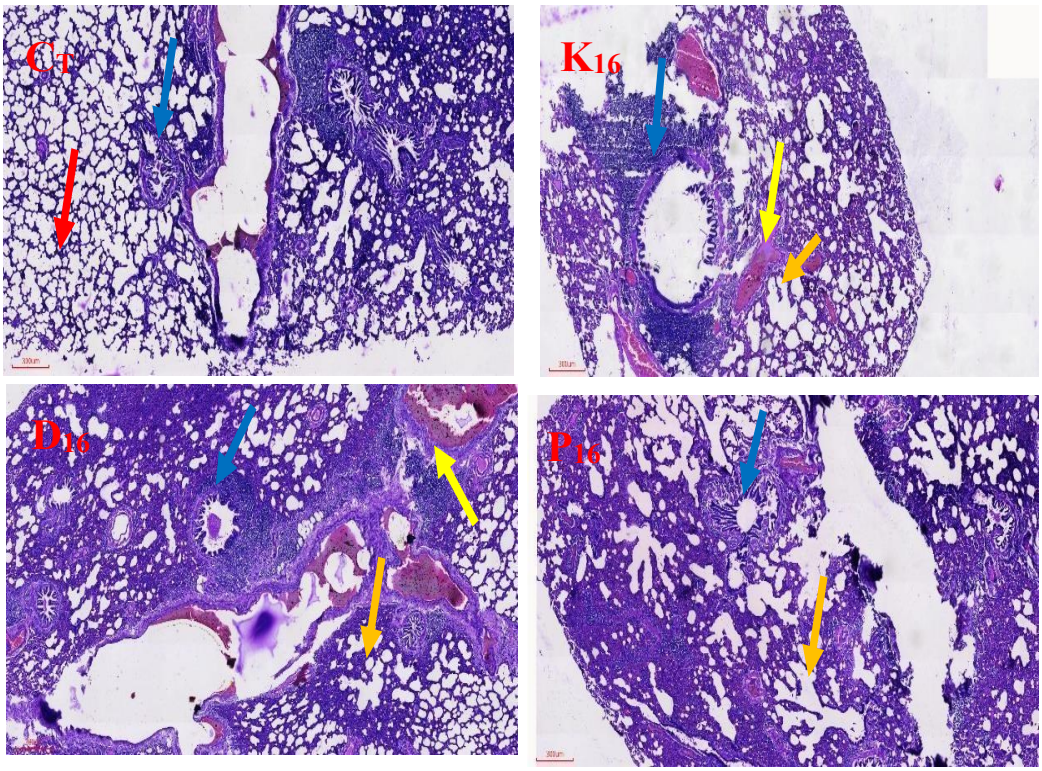

Fig.5: Histomorphology of the Lung of Rats Exposed to Kerosene (K_{16}), Diesel (D_{16}), and Petroleum (P_{16}) for sixteen (16) weeks. C_T is the control group. Red arrow = Alveoli, Orange arrow = Dilated Alveoli, Blue arrow = Bronchioles, Yellow arrow = Hemorrhage; Lung: x40 H&E

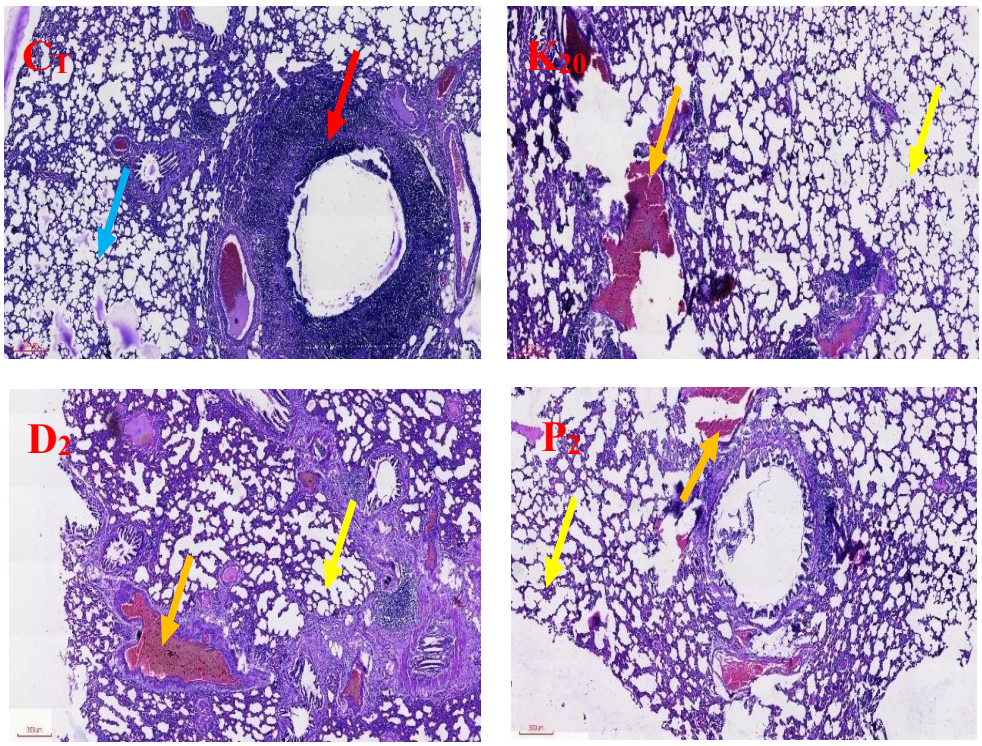

Fig.6: Histomorphology of the Lung of Rats Exposed to Kerosene (K_{20}), Diesel (D_{20}), and Petroleum (P_{20}) for twenty (20) weeks. C_T is the control group. Blue arrow = Alveoli, Red arrow = Bronchiole, Orange arrow = Hemorrhage, Yellow arrow = Distorted Dilated Alveoli. Lung x40 H&E

DISCUSSION

Petroleum products may be laden with harmful contaminants, especially owing to the method in which they were refined. It has been established that most petroleum products refined in developing countries are laden with harmful substances because the majority of the marketers 'cut corners' by resorting to illegal, shabby refining processes to maximize profit [10]. These products have a great impact on humans and the environment because they are often used for both domestic and occupational uses [2]. In this present study, the normal histoarchitecture of the lungs showed pigmented tissues and distorted pulmonary alveoli. This was also observed by Uboh *et al*. [12], where they discovered that frequent exposure to gasoline vapors in male Wistar rats resulted in significant changes in the lungs' tissues. Other noticeable changes include thickening of the walls separating the air sacs, hemorrhagic cells, and infiltration of inflammatory cells in the lung section. This is an indication of injury to lung tissues, which predisposes the lungs to acute respiratory distress syndrome (ARDS). However, there was no significant distinction among noticeable morphological features of the lungs in the three petroleum products that were studied. This research further revealed general metaplasia and mild pigmentation of the alveolar sac, congested blood vessels (CBVs), scattered numerous columnar epithelial cells (CECs) and pulmonary alveoli, which are in agreement with Okoro *et al*. [13], who studied how gasoline fumes affect the respiratory system of Wistar rats. Their findings, however, like this current study, revealed that prolonged exposure can result in damage to the lung tissues. The histoarchitecture of the lungs in the test groups does not present significant progressive worsening conditions with prolonged exposure. This may be as a result of the body system of the animals adjusting itself during the period of withdrawal of the animal from the fume chamber, also as a result of reduced protective enzyme levels in the lungs and increasing harmful chemical reactions. Damages seen in the histoarchitecture of the lung is characterized by the breaking down of fats and proteins, which weakens the structure of the lung lining and may eventually lead to the development of chronic diseases like chronic obstructive pulmonary disease (COPD) which is a progressive, irreversible lung disease primarily caused by smoking or pollutant exposure that restricts airflow, causing chronic cough, wheezing, and severe breathlessness [14]. This COPD may include chronic bronchitis and emphysema, progressing through four stages of mild obstruction, moderate obstruction, increased obstruction, and significantly impaired lung

function requiring administration of oxygen. The findings of this research work are also in tandem with the results obtained by Ita and Udofia [2], where they investigated the impact of inhalation of kerosene fumes on the respiratory system of rats. Their results showed that exposure to kerosene vapor led to significant respiratory complications, including coughing, wheezing, and difficulty in breathing. The lung tissues further revealed abnormal cell growth around the lining of the airways, enlargement of mucous-producing glands, and increased scar tissue around the air passages, all of which indicated conditions similar to chronic bronchitis and asthma.

CONCLUSION

The results obtained from this study are suggestive of the fact that petroleum products are environmental stressors and capable of causing health distress. Prolonged exposure to petroleum products results in a number of respiratory complications.

Conflict of interest

The authors have no conflict of interest to declare.

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Authors' contributions

TIAO: concept and proposal design; benchwork; manuscript writing; EOE: Results processing; final proofreading.

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