



# Histological Changes in the Trachea of the Adult Wistar Rat Following Exposure to Cement Dust

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Website:  
jecajournal.com

Doi:  
[doi.org/10.4314/jeca.v21i1.7](https://doi.org/10.4314/jeca.v21i1.7)

## Abstract

**BACKGROUND AND AIM:** Cement dust is an atmospheric pollutant which poses a significant threat to the environment and humans. Previous studies have shown that cement dust causes bronchitis, chronic obstructive pulmonary disease, lung cancer and other lung diseases. Not much has been reported on cement dust exposure on trachea. Therefore, this present study was carried out to investigate histological changes in the trachea of Wistar rats following exposure to cement dust.

**METHODOLOGY:** Twenty-four (24) Wistar rats weighing between 250g and 280g were divided into 4 groups of 6 rats per group. Group A rats were placed in a cement dust free chamber while Group B - D animals were exposed to cement dust dispersed from 5g (low dose), 10g (medium dose) and 20g (high dose) of cement, respectively via dust distributor glass-chamber of dimensions 32.5 cm<sup>3</sup> in length, 32.5 cm<sup>3</sup> in width and 16.5 cm<sup>3</sup> in height for 1 hour daily for 30 days. The weights of the rats were taken weekly and the difference between them and previous weights were noted. At the end of the 30<sup>th</sup> day of exposure, the animals were euthanized under chloroform anaesthesia and the tracheae were harvested and processed for histological examination.

**RESULTS:** The histological sections of the trachea of rats in Group A revealed normal lumen, mucous membrane, sub-epithelial areolar connective tissue, cartilage, muscularis layer and glands. There were observable histological variations in the tracheal architecture of the exposed rats (Groups B-D) which include mural oedema, sub-epithelial infiltrates of inflammatory cells, mucosal ulceration, and luminal haemorrhage. These injuries are consistent with usual histological findings in tracheitis.

**CONCLUSION:** It was concluded that cement dust had histomorphological effects on the mucous membrane, lumen, muscularis layer and sub-epithelial areolar connective tissue of the trachea which are capable of compromising the health of the research animals.

## Keywords:

Cement dust; trachea; histoarchitecture; tracheitis

Submitted: 24<sup>th</sup> April, 2024

Revised: 4<sup>th</sup> June, 2024

Accepted: 5<sup>th</sup> June, 2024

Published: 30<sup>th</sup> June, 2024

## INTRODUCTION

Cement dust is an atmospheric pollutant which poses a significant threat to the environment and humans. It is emitted during the manufacturing and processing of cement, transportation, bag dumping and emptying, storage, usage and concrete cutting (Alakija *et al.*, 2017; Nwafor *et al.*, 2019; Akinola *et al.*, 2019). Cement dust contains substances such as aluminium, silicon, calcium, manganese, iron and zinc which at high levels of exposure can trigger inflammatory changes in the trachea and may lead to pathogenesis of various respiratory diseases including tracheitis (Akinola *et al.*, 2019).

The trachea is a key part of the respiratory

system that leads from the larynx to the bronchi (Drake *et al.*, 2010). It is located anteriorly in the neck and dives posteriorly in the mediastinum as it travels towards the carina (Harrison, 2018). The trachea is a long, U-shaped structure that is composed of hyaline cartilage on the anterior and lateral walls, with the trachealis smooth muscle forming the posterior border of the trachea (Ross and Wilson 2018). It is made up of 16-20 tracheal cartilages anterolaterally and a fibromuscular wall posteriorly, which help to keep it open and prevent it from collapsing (Mark *et al.*, 2016). The entire tracheal lumen is lined with a mucous membrane, which produces mucus to help trap dirt and dust particles (Moore and Dalley, 2014). The main

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**How to cite this article:** Ehi-Omosun, M.B., Iyawe, V.I. and Sakpa, C.L. Histological Changes in the Trachea of the Adult Wistar Rat Following Exposure to Cement Dust. *J Exp Clin Anat* 2024; 21(1):45-49.

<https://dx.doi.org/10.4314/jeca.v21i1.7>

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function of the trachea is to allow passage of inspired and expired air into and out of the lung (Mark *et al.*, 2016). Any injury to the trachea causes distortion of the smooth functioning of the respiratory system.

Tracheitis is a disease that causes inflammation of the trachea (Mark *et al.*, 2016). This inflammation generally involves the mucous membrane, lumen, muscularis layer and sub-epithelial areolar connective tissue of the trachea and lowers the trachea's ability to provide a safe sturdy passageway for inspired and expired air into and out of the lung (Harrison, 2018). A severely inflamed trachea can block the respiratory tract, causing severe breathing difficulties and discomfort.

Tracheitis may occur when an irritating substance causes destruction and inflammation of the tracheal lumen which then makes passage of inspired and expired air into and out of the lung difficult (Mark *et al.*, 2016; Iyawe, 2005). Signs and symptoms of cement dust related tracheitis include high fever, general malaise, wheezing, dyspnoea, cyanosis, tracheal stenosis, haemoptysis and dry hacking cough.

The most reported occupational hazards for cement workers are allergy and other respiratory illnesses (Omigie *et al.*, 2016). Some authors have reported that exposure to cement dust may result in some metabolic disorders, lung diseases and cardiovascular disorders (Alakija *et al.*, 2017; Akinola *et al.*, 2019). There is a significant knowledge gap regarding the health effects of cement dust on the trachea. Therefore, this present study was carried out to investigate histological changes in the trachea of Wistar rats following exposure to cement dust.

## MATERIALS AND METHODS

**Ethical Considerations:** Ethical approval was obtained from College Ethical Committee of the University of Benin, Benin City, Edo State, Nigeria (Approval number: CMS/REC/2012/302). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

**Experimental Animals:** Twenty-four (24) adult Wistar rats, weighing between 250 g and 280 g purchased from the Animal house, Department of Anatomy, University of Benin, Nigeria, were utilized for this study. The animals were left to acclimatize for two (2) weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed and water *ad libitum*.

The transparent dust distributor glass chamber (DDGC) of dimensions 32.5cm<sup>3</sup> in length, 32.5cm<sup>3</sup> in width and 16.5cm<sup>3</sup> in height, 2010 model, that was used in this research for the dispersion of cement dust particles was manufactured by Hoddler and Stoughton Group of Company, USA. The 24 animals were divided into 4 groups comprising of 6 rats per-

group. Group A rats, which served as control, were placed in a cement dust-free dust distributor glass chamber (DDGC). Group B rats were exposed as a group to cement dust dispersed from 5 g of cement via DDGC at 10 am for 1 hour daily for 30 days. Group C rats were exposed as a group to cement dust dispersed from 10 g of cement via DDGC at 10 am for 1 hour daily for 30 days. Group D rats were exposed as a group to cement dust dispersed from 20 g of cement via DDGC at 10 am for 1 hour daily for 30 days. The weights of the animals in each group were taken and recorded weekly and the difference noted. All the results of the weight measurements were handed over to the statistician who analyzed the data (Table 1).

Following the end of 30<sup>th</sup> day exposure, the animals were weighed, euthanized under chloroform anaesthesia and a midline incision was made through the ventral wall of the lower neck and the superior mediastinum of the thorax of the rats to access the trachea. The harvested organs were immediately fixed in 10% formal saline for 24 hours to prevent tissue degradation and autolysis before the histological procedures. The tissues were sectioned into about 3-5 mm thick sections and processed according to the method of Drury and Wallington, (1980). The thin tissue sections were histologically processed using the methods of fixation, embedding and tissue staining for microscopy. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x100.

**Statistical Analysis:** Statistical analysis was carried out with Statistical Software Package, Microsoft Excel, (2010) and Statistical Package for Social Sciences (S.P.S.S.) version 20. (The two were used together to create a more efficient and comprehensive data analysis workflow). Results were presented as Mean (X) ± Standard error of mean (SEM). The one way Analysis of Variance (ANOVA) was used to determine the significance of the difference in means at 95% confidence interval. P<0.05 was considered significant. *Post hoc* analysis was done with Scheffe's test to compare means between groups.

## RESULTS

**Findings on Body Weight Changes:** Changes in body weights of the animals in all the experimental groups are presented in Table 1. Whereas all the groups gained weight weekly, weight gain was significantly lower (P<0.05) in all the groups exposed to cement dust (Groups B to D) when compared with the control group.

**Histological Findings:** The photomicrograph of the control group (group A), showed normal features of the trachea such as lumen, mucous membrane, sub-epithelial areolar connective tissue, cartilage, glands and muscularis layer (figure 1A). In the rats exposed to cement dust dispersed from 5 g of cement (group B), there were mild mural oedema, sub-

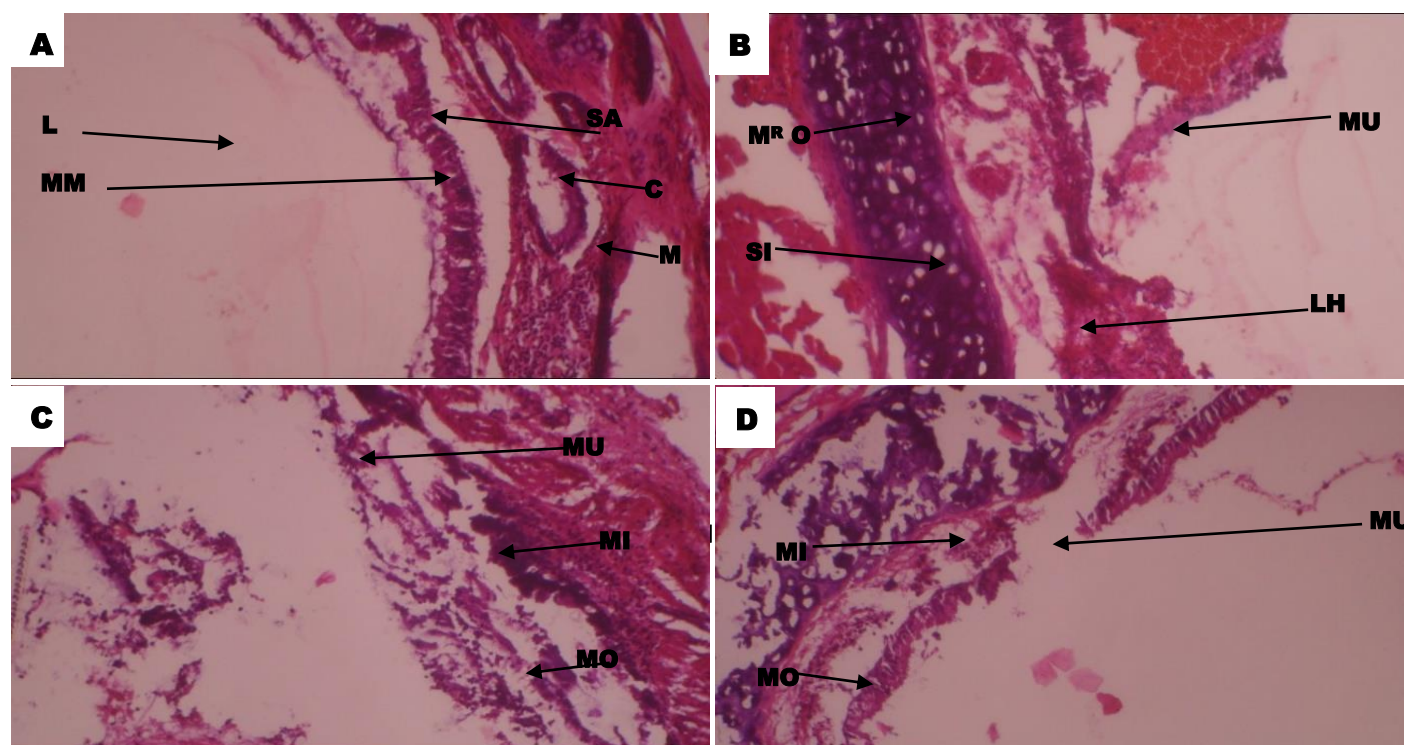
epithelial infiltrates of inflammatory cells, mucosal ulceration and luminal haemorrhage (figure 1B). The group exposed to cement dust dispersed from 10g of cement (Group C), presented severe mucosal ulceration, heavy mucosal infiltrates of inflammatory cells and mucosal oedema (figure

1C). In the rats exposed to cement dust dispersed from 20 g of cement (group D), there were severe mucosal ulceration, severe mucosal infiltrates of inflammatory cells and severe mucosal oedema (figure 1D).

**Table 1: Changes in Body Weights of the Rats in all the Experimental Groups**

Period of Exposure	GROUP A	GROUP B	GROUP C	GROUP D	P-value
1st week	5.60 ± 0.68	0.60 ± 0.19*	0.42 ± 0.16*	0.38 ± 0.16*	0.000
2nd week	6.70 ± 0.93	0.30 ± 0.05*	0.30 ± 0.09*	0.20 ± 0.14*	0.000
3 <sup>rd</sup> week	7.40 ± 1.24	0.06 ± 0.17*	0.20 ± 0.05*	0.16 ± 0.07*	0.000
4 <sup>th</sup> week	7.74 ± 0.60	0.18 ± 0.09*	0.36 ± 0.10*	0.04 ± 0.08*	0.000

n=6; Values are Mean ± S.E.M; \* means significantly different ( $p < 0.05$ ) when compared with control



**Figure 1:** Photomicrograph of a section of rat's trachea in the control group (A), group exposed to 5 g of cement (B), group exposed to 10 g of cement (C), group exposed to 20 g of cement (D). Normal tracheal lumen (L), mucous membrane (MM), sub-epithelial areolar connective tissue (SA), cartilage (C), glands (G) and muscularis layer (M), mural oedema (M<sup>R</sup>O), sub-epithelial infiltrates of inflammatory cells (SI), mucosal ulceration (MU) and luminal haemorrhage (LH), heavy mucosal infiltrates of inflammatory cells (MI) and mucosal oedema (MO) (H&E; 100x).

## DISCUSSION

Cement dust inhalation has been implicated in a variety of maladies including tracheitis, lung alveolitis, bronchiectasis, interstitial pneumonitis, pneumoconiosis and other cement factory respiratory diseases both in animals and humans. However, due to the unavailability of appropriate chamber for laboratory use, investigations to determine the impact of cement dust on the organs of respiratory system in Wistar rats and the extent of the associated toxicity have been limited (Nwafor *et al.*, 2019; Omigie *et al.*, 2016; Akinola *et al.*, 2019). Against this background, this study was

conducted to evaluate the effects of cement dust in the trachea of the adult Wistar rats.

At the conclusion of four weeks, the treated groups exhibited a significant weight reduction compared to the control group ( $P < 0.05$ ). The result showed that the cement dust inhaled by the animals caused systemic inflammatory response (Figure 1(B-C) resulting in tracheal toxicity which can affect overall health of the animals and contribute to the observed weight loss (Nwafor *et al.*, 2019; Alakija *et al.*, 2017; Iyawe *et al.*, 2012). These findings have important implications for the development of new obesity treatments in humans.

Histological findings from this study were almost consistent in the various groups (Groups B, C and D) exposed to cement dust, and they include mucosal ulceration, mural oedema, sub-epithelial infiltrates of inflammatory cells and luminal haemorrhages (Figures 1B, 1C and 1D). The observed mucosal ulceration may cause significant pain and discomfort, especially when swallowing or bleeding, causing anaemia or other complications while the observed mural oedema may cause airway obstruction, leading to respiratory distress and potentially life-threatening complications.

The luminal haemorrhage observed in the exposed groups may cause asphyxiation due to airway obstruction or blood filling the lungs while the subepithelial infiltrates of inflammatory cells observed in the exposed animals may cause disruption of the normal functioning of the epithelium and underlying tissues leading to impaired barrier function and increased susceptibility to infections. These implications of the findings agree with those of a similar work done by Poinen-Rughooputh *et al.*, (2016) where they used silica dust to induce pneumonia.

For the rats in Group B (Figure 1B), at low dose, cement dust showed mild histomorphological damage while for the rats in Group C and D (Figures 1C and 1D) at moderate and high doses, cement dust caused severe histomorphological injuries.

The study revealed that cement dust exposure leads to histomorphological changes in the trachea. The histomorphological changes indicate diseases and pathological symptoms of a variety of maladies including tracheitis which are capable of compromising the health of the research animals. But early treatment may give the trachea time to heal.

Cement dust-related tracheal toxicity and its associated complications can be prevented by adherence to proper safety precautions e.g., wearing of personal protective equipment (such as face masks, face shields, goggles, hand gloves, boots and coveralls) in order to minimize the degree of exposure to cement dust; routine medical checkups, especially among cement factory workers and other people with cement dust related occupation should be encouraged so as to avert any occupational health risks and hazards of cement dust; sensitizing the general public regularly by providing them with current information regarding the health risks and hazards of cement dust; and management of cement factories in developing countries adopting the use of modern machines and technologies that can reduce the amount of cement dust released to the environment.

## Conclusion

Cement dust causes body weight loss and distorted tracheal histoarchitecture which are consistent with usual histological findings in tracheitis and may ultimately lead to loss of tracheal function and death of the research animals. The

extent of the histomorphological damage was seen to be directly proportional to the concentration of cement dust as the histomorphological derangements were more severe in the rats exposed to moderate and high-dose concentrations of cement dust.

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