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HISTOLOGICAL CHANGES IN THE BRONCHI OF THE ADULT WISTAR RAT FOLLOWING EXPOSURE TO CEMENT DUST

Ehi-Omosun, M.B., Iyawe, V.I.and Sakpa, C.L.
Departments of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria.
Corresponding Author Email address: mabel.ehi-omosun@uniben.edu; Tel: +2347049948914.
Co-Authors' Email: vincent.iyawe@uniben.edu ; christopher.sakpa@uniben.edu

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ABSTRACT

Cement dust is a harmful air pollutant. Previous research has linked cement dust to respiratory disease, but its effects on bronchi are poorly understood. The present study examined the impact of cement dust on body weight and bronchial function in Wistar rats. Twenty four Wistar rats between250g and 280g were divided into 4 groups of 6 rats each. Group A rats were placed in a cement dust free chamber while Group B - D animals were exposed to cement dust dispersed from 5g, 10g and 20g of cement, respectively via dust distributor glass-chamber of dimensions 32.5 cm^3 × 32.5 cm^3 × 16.5 cm^3 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. After 28 days, animals were euthanized and bronchiwere collected for histology. Body weight significantly increased ($P<0.05$) in all groups. The control group had the highest weight gain compared to the exposed groups. The histological sections of the bronchi of rats in Group A revealed normal bronchi. There were observable histological variations in the bronchial architecture of the exposed rats (Group B-D) which include mucosal ulceration, mucosal infiltrates of inflammatory cells, activated lymphoid follicles and luminal haemorrhage. Histological findings are consistent with usual histological features in bronchiectasis. Cement dust had histomorphological effects on the bronchiwhich are capable of compromising the health of the research animals.

Keywords: Cement particles; bronchi; histoarchitecture; bronchiectasis; weight changes

INTRODUCTION

Cement dust is an atmospheric pollutant that poses a significant threat to the environment and humans. It is generated during the entire value, from cement production and processing to transportation, storage, concrete cutting and workers' activities like bag dumping (Alakijaet. al.,2017; Nwaforet. 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 bronchi and may lead to pathogenesis of various respiratory diseases including bronchitis(Akinola et. al., 2019).

The bronchi, also known as windpipe orbronkhia in Greek, are a vital part of the respiratory tract, situated within the lung (Mark et al.,2016). They function primarily as

passageways for air, bringing oxygen into the lungs and expelling carbon dioxide (Drake et al., 2010; Harrison, 2018). Any injury to the bronchi causes distortion of the smooth functioning of the respiratory system.

Bronchiectasis is a disease of the bronchi that commonly involve the bronchioles, characterized by destruction of the bronchi/bronchioles, causing them to widen (Mark et al., 2016). Damaged bronchi cannot clear mucus like they are supposed to (Harrison, 2018). Bacteria then grow in the mucus, causing bronchiolar mucosal ulceration, vascular ulceration, luminal haemorrhage and inflammation which generally involve the muscularis and mucous membrane of the bronchi. Signs and symptoms of cement dust-related bronchiectasis include fever, wheezing, dyspnoea, haemoptysis, cough and phlegm production (Moore and Dalley, 2014; Ross and Wilson, 2018).

The most reported occupational hazards for cement workers are allergies and other respiratory illnesses (Omigie et. al., 2016). Several authors have reported that exposure to cement dust may result in some metabolic disorders, respiratory diseases and cardiovascular disorders Iyawe and Ebomoyi (2015); Iyaweet.al (2016). There is a significant knowledge gap regarding the health effects of cement dust on the bronchi. This study aimed to investigate the histological effects of cement dust exposure on the bronchi of Wistar rats.

MATERIALS AND METHODS

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.*One thousand and fifty gram (1050g) of Dangote cement was purchased from Phalbon Group Cement Depot in Iguosa Community, Ovia North East Local Government Area of Edo State, Nigeria and was kept in a clean, dry storeroom.

The transparent dust distributor glass chamber (DDGC) of dimensions 32.5cm^3 in length, 32.5cm^3 in width and 16.5cm^3 in height, 2010 model, that was used in this research for the dispersion of cement dust particles was manufactured by Hoddlier and Stoughton Group of Company, USA. The 24 animals were divided into 4 groups comprising 6 rats each. 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 28 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 28 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 28 days. The weights of the animals in each group were taken and recorded weekly and the difference between them and previous weights were noted. The weight measurement data was handed over to the statistician for statistical analysis (Table 1).

Following the end of the28th day of exposure, the animals were weighed and euthanized under chloroform anaesthesiaand a midline incision was made through the ventral wall of thethorax of the rats to access the bronchi. 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 sectionedinto 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 a magnification of x100.

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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 researchBuzek and Chastel (2012).

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. 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. $P \leq 0.05$ was considered significant.

RESULTS

Body Weight Findings

Changes in body weights of the animals in all the experimental groups are presented in Table 1. The results showed that there was a significant increase ($P<0.05$) in body weight of the animals in all experimental groups (Group A-D). The control group (A) showed higher weight gain compared to the exposed groups (B-D).

Histolgical Findings

The photomicrograph of the control group (group A), showed normal features of the bronchi such as mucosal membrane, bronchial lumen and muscularis(figure 1A). In the rats exposed to cement dust dispersed from 5 g of cement (group B), there were activated lymphoid follicles, mucosal infiltrates of inflammatory cells and mild mucosal ulcerations (figure 1B). The group exposed to cement dust dispersed from 10g of cement (Group C), presented heavy mucosal infiltrates of inflammatory cells, severe mucosal ulceration, and luminal haemorrhage (figure 1C). In the rats exposed to cement dust dispersed from 20 g of cement (group D), there weresevere mucosal ulceration and mucosal infiltrates of inflammatory cells (figure 1D).

Table1: Changes in Body Weights of the Rats in all the Experimental Groups:

Periodof Exposure	**Group A**	**Group B**	**Group C**	**Group D**	**P Values**
Ist 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
3rdweek	7.40 ± 1.24*	0.06 ± 0.17*	0.20 ± 0.05*	0.16± 0.07*	0.000
4thweek	7.74 + 0.60*	0.18 ± 0.09*	0.36 ± 0.10*	0.04± 0.08*	0.000

n=6; Values are Mean ± SEM

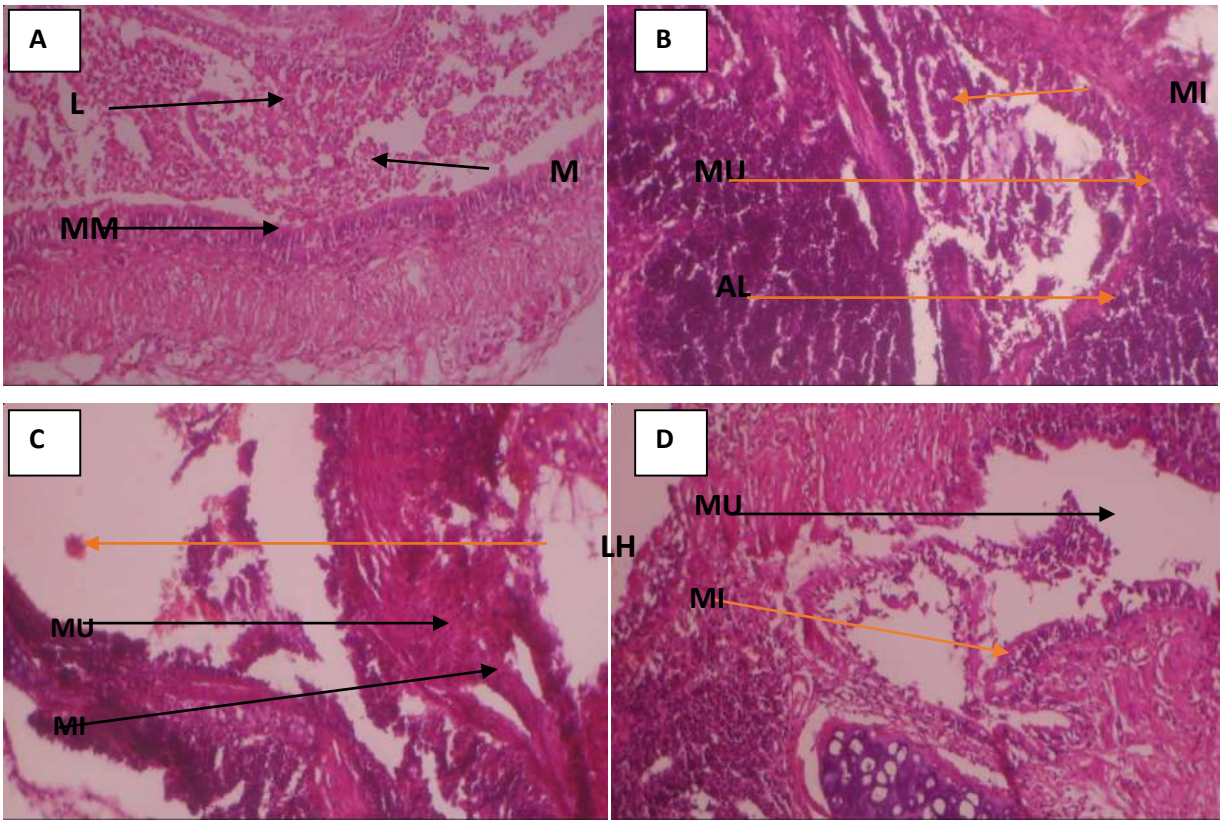

Figure 1: Photomicrographs ofH&E sections of the bronchi of the experimental animals

'A' shows the Group A (Control group) showing normal bronchial lumen**'L'**, mucosal membrane **'MM'**, and normal muscularis**'M'**. 'B' shows Group B exposed to cement dust dispersed from 5 g of cement daily for 28daysshowing mucosal infiltrates of inflammatory cells,**'MI'**, activated lymphoid follicles**'AL'**, and mucosal ulceration**'MU'**. 'C' shows Group Cexposed to cement dust dispersed from 10 g of cement daily for 28 days showing severe mucosal infiltrates of inflammatory cells,**'MI'**, severe mucosal ulceration,**'MU'**andluminal haemorrhage**'LH'.**'D' shows Group D exposed to cement dust dispersed from 20 g of cement daily for 28 days showing severe mucosal infiltrates of inflammatory cells **'MI'** and severe mucosal ulceration**'MU'**.

DISCUSSION

Cement dust inhalation has been implicated in a variety of maladies. Several 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; Nwafor et al., 2019). There is a significant knowledge gap regarding the health effects of cement dust on the bronchi. In light of this, the present study investigated the impact of cement dust on the bronchi of adult Wistar rats.

The findings of the study for body weight showed a significant increase ($P<0.05$) in body weight of the animals in all experimental groups (Group A-D). The control group (A) showed higher weight gain compared to the exposed groups (B-D). Inhalation of cement dust triggered a systemic inflammatory response (Figure 1B-1D) in exposed animals, leading to bronchial toxicity, which likely impacted their overall health and contributed to reduced weight and this concurred with previous studies (Akinola et al., 2019; Alakija et al., 2017). The implications of these results are crucial for the development of innovative weight management strategies in humans.

Histological findings from this study were almost consistentin the exposedgroups (Groups B, C and D) and they includemucosal ulceration, activated lymphoid follicles, mucosal infiltrates of inflammatory cells and

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luminal haemorrhage(Figures1B, 1C and 1D).The activated lymphoid follicle observed in the exposed animals is a sign of an immune response.It implicates inflammation, tissue damage and repair, immune system activation and respiratory problems (Mark et al., 2016).Understanding their implications is essential for developing effective treatments and therapies.The observed mucosal ulceration may cause significant chest pain, recurrent bronchial infections, airway obstruction, impaired lung function, chronic cough, dyspnoeaand haemoptysis. The luminal haemorrhage observed in the exposed animals may cause asphyxiation due to airway obstruction or blood filling the bronchial lumen while the mucosal infiltrate of inflammatory cells observed in the exposed animas may cause disruption of the normal functioning of the muscularislayer and underlying tissues leading to impaired barrier function and increased susceptibility to infections.The implications of the histological findings agree with those of a similar work done by Poinen-Rughooputhetal.,(2016) where they used silica dust to induce bronchitis.

For the rats in Group B (Figure 1B), at low dose, cement dust showed mild histomorphological damagewhile 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 bronchi. The histomorphological changes indicate diseases and pathological symptoms of a variety of maladies including bronchiectasis which are capable of compromising the health of the research animals (Alakija et al., 2017; Harrison, 2018). But early treatment may give the bronchi time to heal.

CONCLUSION

Cement dust was shown to reduce body weight and distorted bronchial histoarchitecture which areconsistent with usual histological findings in bronchiectasisthat may ultimately lead to loss of bronchial 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 tomoderate and high-dose concentrations of cement dust.

Recommendation

The cement dust-related bronchial toxicity observed in this study 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.

Conflict of Interest Declaration: The authors, Prof. Iyawe, VI, Prof. Sakpa, CL and Dr. Ehi-Omosun MB hereby declare that potential conflict of interest does not exist in this manuscript.

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