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Histological Changes in the Larynx of Adult Wistar Rats Following Exposure to Cement Dust

EHI-OMOSUN, M.B.*, IYAWE, V.I., SAKPA, C.L.
Departments of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria.

Author for Correspondence*:mabel.ehi-omosun@uniben.edu/+234-704-994-8914.
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Abstract
Cement dust is a fine powder that is produced during the manufacture, handing and use of cement. 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 larynx. Therefore, this present study was carried out to investigate histological changes in the larynx of Wistar rats following exposure to cement dust. 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 (DDGC) of dimensions 32.5 cm^3 in length, 32.5 cm^3 in width and 16.5 cm^3 in height for 1 hour daily for 30 days. The weights of the rats were taken weekly, and the difference noted. At the end of the 30^{th} day of exposure, the animals were euthanized under chloroform anaesthesia and the larynges were harvested and processed for histological examination. The histological sections of the larynx of rats in group A revealed normal mucous membrane, muscular layer, cartilage, subepithelial areolar connective tissue and lumen. There were observable histological variations in the laryngeal architecture of the exposed rats (Groups B-D) which include severe mucosal ulceration, sub-epithelial oedema and heavy mucosal infiltrates of inflammatory cells. These injuries are consistent with usual histological findings in laryngitis. It was concluded that cement dust had histomorphological effects on the mucosa, submucosa and muscuaris of the larynx which are capable of compromising the health of the research animals.

Keywords: Cement dust; larynx; histoarchitecture; laryngitis

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; Akinola *et al.,* 2019; Nwafor *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 larynx and may lead to pathogenesis of various respiratory diseases including laryngitis (Akinola *et al.,* 2019).

The larynx, commonly called the voice box, is a hollow muscular organ located above the trachea and in front of the esophagus (Ross and Wilson, 2018). It is a key part of the respiratory tract (Mark *et al.,* 2014). The larynx contains the vocal cords that function as a voice box for phonation (Moore and Dalley, 2014; Harrison, 2018). It also protects the lower respiratory tract from aspirating food into the trachea while breathing (Drake *et al.,* 2010; Harrison, 2018). Any injury to the larynx causes distortion of the smooth functioning of the respiratory system. Due to the fact that cement dust contains many hazardous chemicals, prolonged or repeated exposure to it may cause dysphonia and if not corrected may lead to laryngitis (Mark *et al.,* 2014; Alakija *et al.,* 2017). About 1% laryngitis occurs every year in United States due to dysphonia (Harrison, 2018)**.**

Laryngitis is a disease that causes inflammation of the larynx (Mark *et al*., 2014). The inflammation generally involves the mucous membrane, submucosa and muscularis of the larynx and lowers its ability to protect the lower respiratory tract and vocal cords which function as a voice box for producing sounds (Iyawe and Ebomyi, 2005; Omigie *et al*., 2016). A severely inflamed larynx can block the respiratory tract, causing dyspnoea and discomfort. Laryngtis may occur when environmental irritants such as pollution, dust, or chemicals or smoking causes destruction and inflammation of the laryngeal mucosa, submucosa or muscularis which then makes passage of inspired and expired air into and out of the lungs difficult (Iyawe *et al.,* 2012; Iyawe and Ebomoyi, 2005). Signs and symptoms of cement dust related laryngeal disorders include fever, general malaise, dry cough, nasal congestion, phlegm, sore throat, hoarseness and loss of voice (Mark *et al.,* 2014).

The most reported occupational hazards for cement workers are allergy and other respiratory illnesses (Omigie *et al.,*2016) 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). . There is a significant knowledge gap regarding the health effects of cement dust on the larynx. Therefore, this present study was carried out to investigate histological changes in the larynx of Wistar rats following exposure to cement dust.

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.*

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 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 30th 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 larynx. 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 x40 and x100.

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).

***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. $P \leq 0.05$ was considered significant. Post hoc analysis was done with Scheffe's test to compare means between groups.

Results

Changes in body weights of the animals in all the experimental groups are presented in Table 1. It was observed that there was a significant increase in body weights ($P<0.05$) of the rats in Group A (Control Group) while Groups B, C and D (Groups that were exposed to cement dust) showed a significant decrease ($P<0.05$) in body weight. (A positive integer typically connotes an increase. However, in this context of degradation in the body weights of the animals over time, the observed positive integer connotes a decrease in a particular direction (Kenneeth and Rosen, 2018).

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- 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
3rd week	7.40 ± 1.24*	0.06 ± 0.17*	0.20 ± 0.05*	0.16± 0.07*	0.000
4th 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

LEGENDS FOR PHOTOMICROGRAPHS

Figures 1 and 2 are micrographs of a section of rat's larynx in the control group (Group A), (H&E at x 40 and x 100) magnifications respectively showing normal muscular layer (M), cartilage (C), subepithelial areolar connective tissue (SA), mucous membrane (MM) and normal tracheal lumen (L).

Figures 3 and 4 are photomicrographs of a section of rat's larynx exposed to cement dust dispersed from 5 g of cement (Group B), H&E at x 40 and x 100 magnifications respectively showing patchy mucosal ulceration (PM), subepithelial infiltrates of inflammatory cells (SI) and subepithelial oedema (SO).

Figures 5 and 6 are photomicrographs of a section of rat's larynx exposed to cement dust dispersed from 10 g of cement (Group C), H&E at x 40 and x 100 magnifications respectively showing severe mucosal ulceration (MU), subepithelial infiltrates of inflammatory cells (SI) heavy mucosal infiltrates of inflammatory cells (MI) and mucosal congestion (MC).

Figures 7 and 8 are photomicrographs of a section of rat's larynx exposed to cement dust dispersed from 20 g of cement (Group D), H&E at x 40 and x 100 magnifications respectively showing severe mucosal ulceration (MU) and heavy mucosal infiltrates of inflammatory cells (MI).

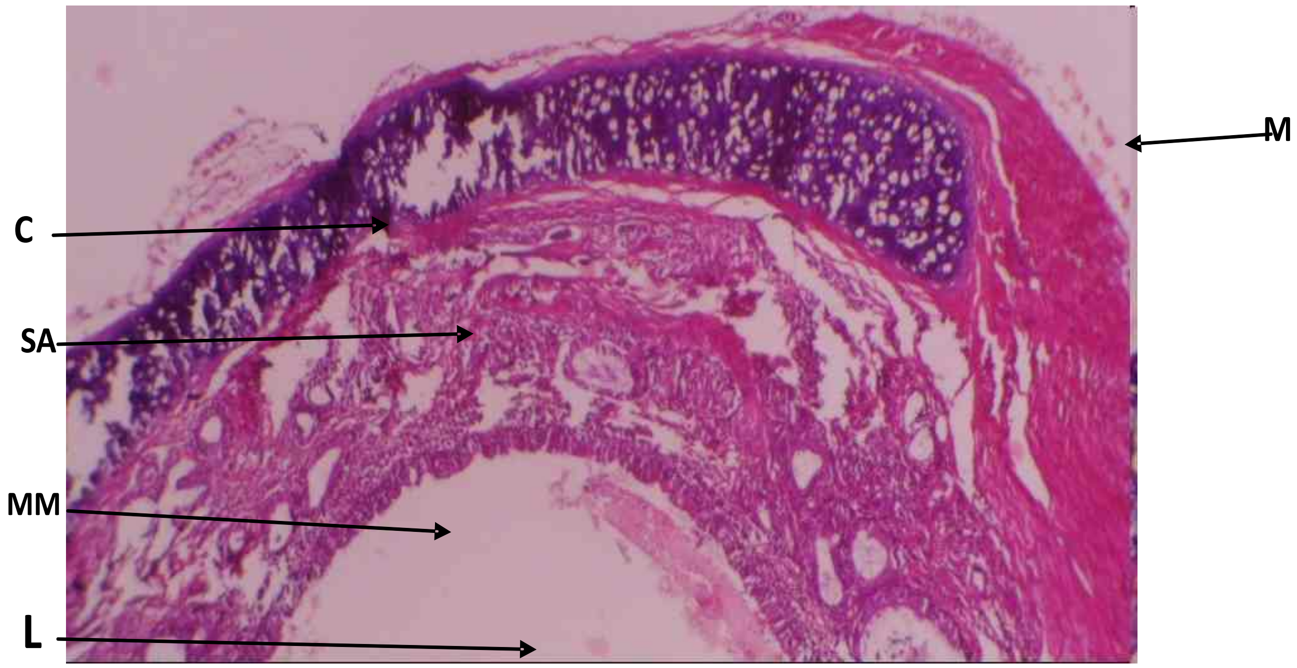


Figure 1: Rat larynx (control). Composed of: M muscular layer, C, cartilage, SA, sub epithelial areolar connective tissue, MM, mucous membrane and L, lumen (H&E x 40)

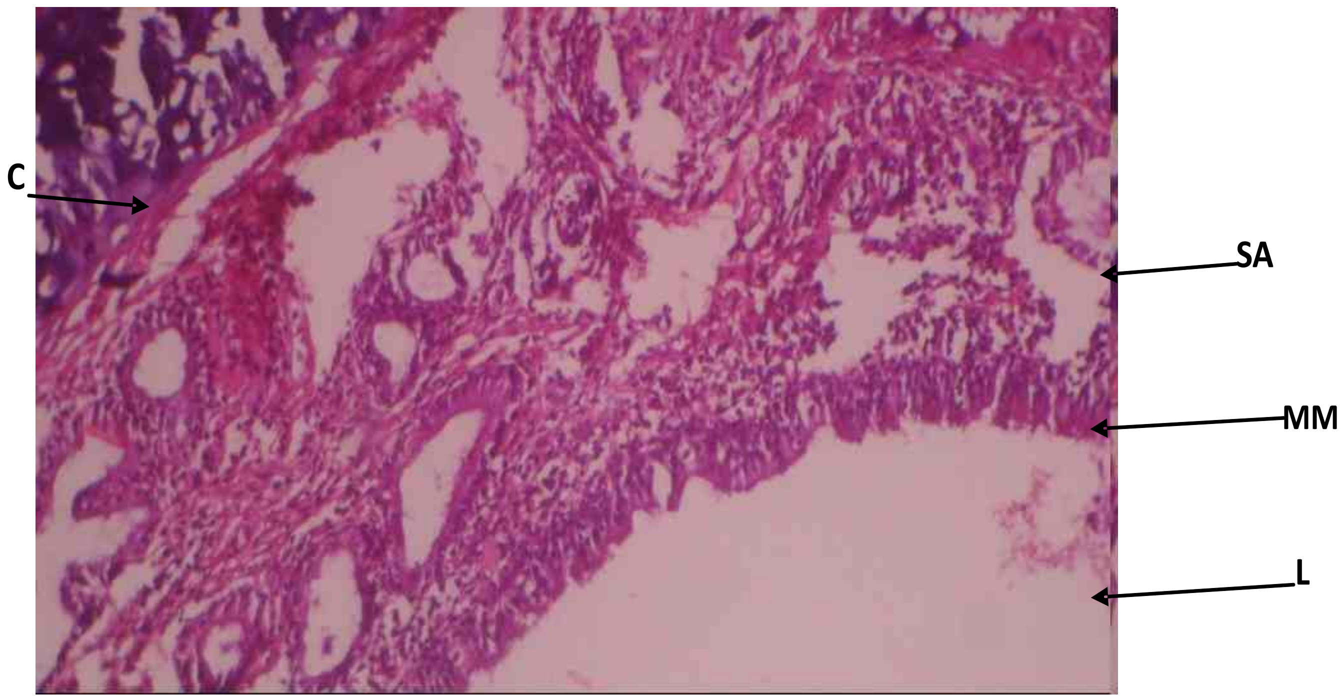
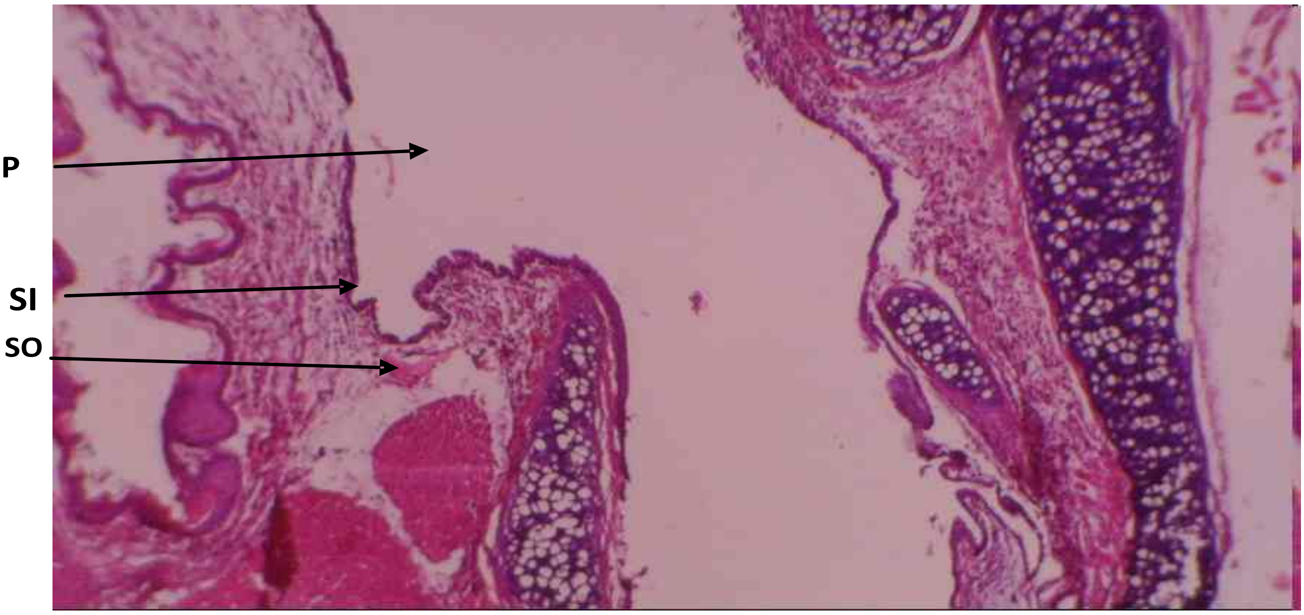


Figure 2: Rat larynx (control). Composed of: C, cartilage, SA, subepithelial areolar connective tissue, MM, mucous membrane and L, lumen (H&E x 100)



A

Figure 3: Larynx of rat exposed to 5 g of cement dust showing: PM, patchy mucosal ulceration, SI, heavy subepithelial infiltrates of inflammatory cells and SO subepithelial oedema (H&E x 40)

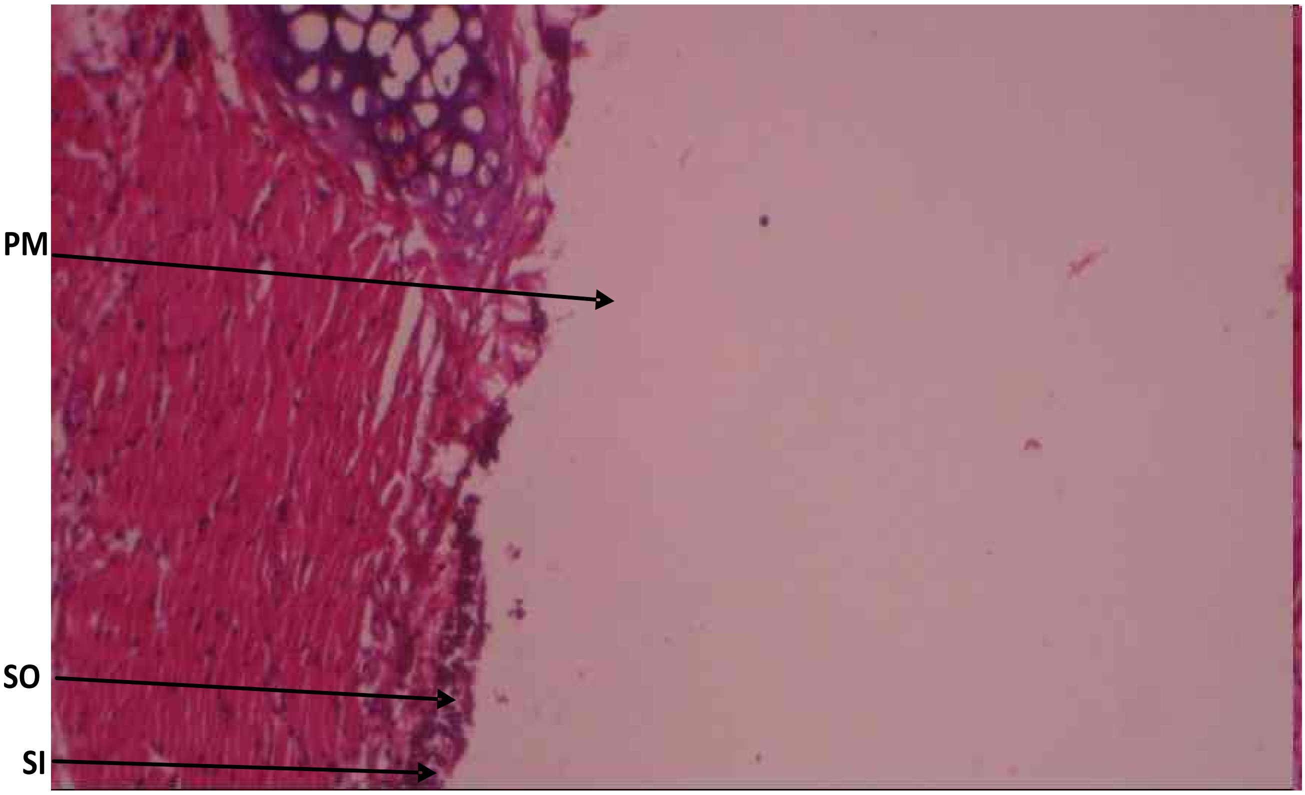


Figure 4: Larynx of rat exposed to 5 g of cement dust showing: PM, patchy mucosal ulceration, SI, heavy subepithelial infiltrates of inflammatory cells and SO, subepithelial oedema (H&E x 100)

Figure 5: Larynx of rat exposed to 10 g of cement dust showing: MU, severe mucosal ulceration; SI, subepithelial infiltrates of inflammatory cells and MC, mucosal congestion (H&E x 40)

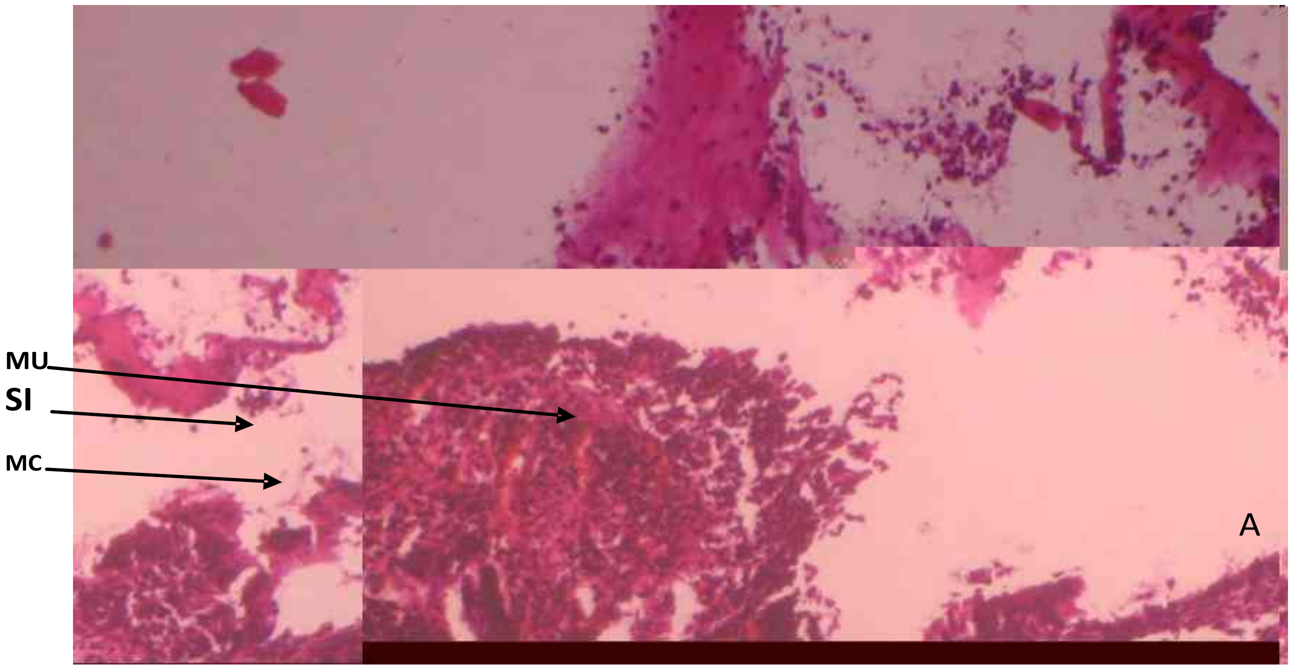


Figure 6: Larynx of rat exposed to 10 g of cement dust showing: MU, severe mucosal ulceration, MI, heavy mucosal infiltrates of inflammatory cells and MC, mucosal congestion (H&E x 100)

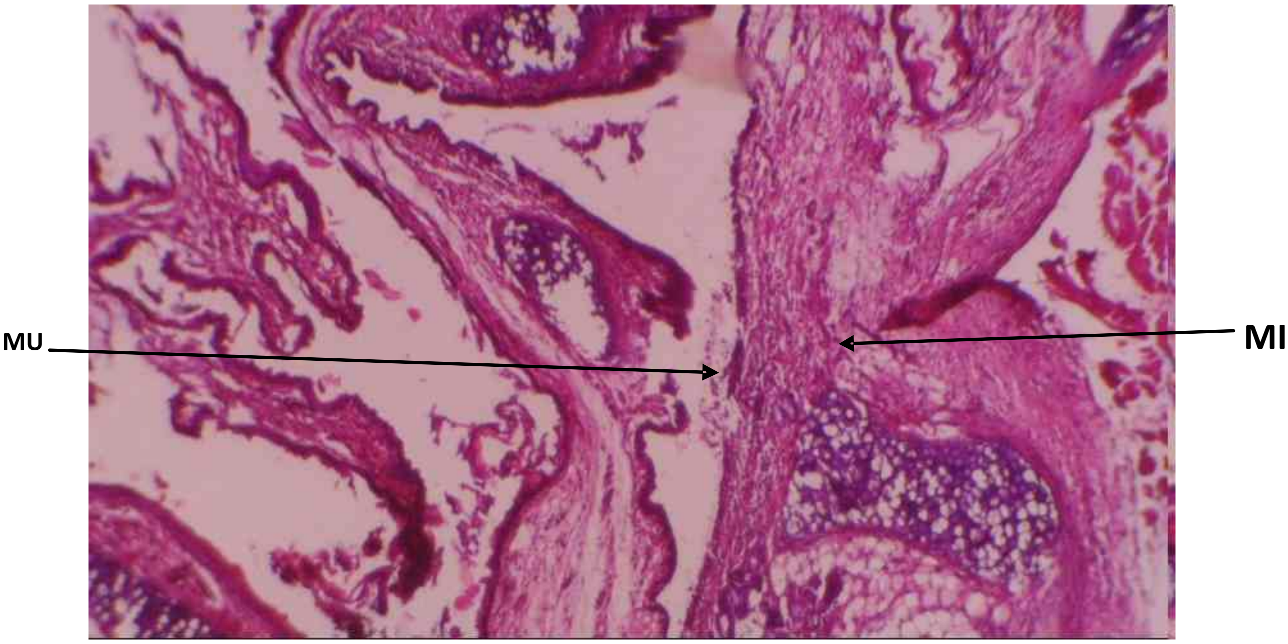


Figure 7: Larynx of rat exposed to 20 g of cement dust showing: MU, severe mucosal Ulceration and MI, heavy mucosal infiltrates of inflammatory cells (H&E x 40)

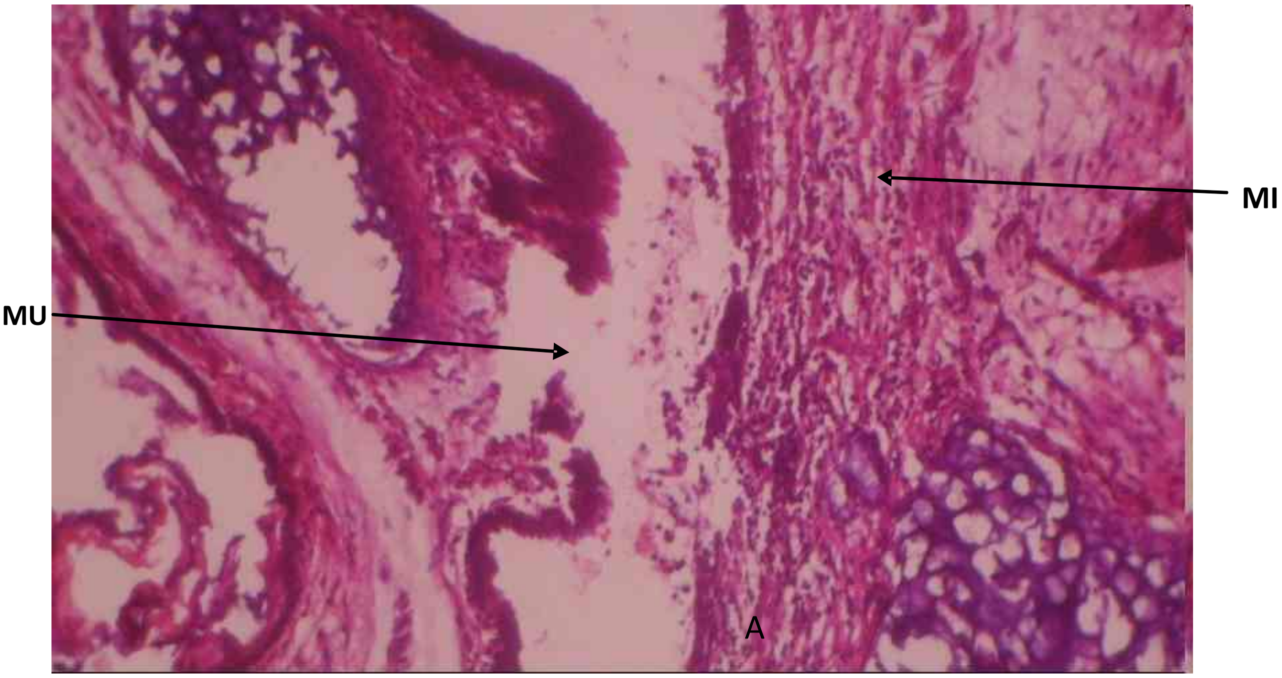


Figure 8: Larynx of rat exposed to 20 g of cement dust showing: MU, severe mucosal ulceration and MI; heavy mucosal infiltrates of inflammatory cells (H&E x 100).

Discussion

Cement dust inhalation has been implicated in a variety of maladies including 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 tract in Wistar rats and the extent of the associated toxicity have been limited (Omigie *et al.,* 2016; Akinola *et al.,* 2019; Nwafor *et al.,* 2019). Against this background, this study was conducted to evaluate the effects of cement dust in the larynx of the adult Wistar rat.

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 (Figures 3 and 4) resulting in laryngeal toxicity which can affect overall health of the animals and contribute to the observed weight loss (Iyawe *et al.,* 2012; Alakija *et al.,* 2017; Nwafor *et al.,* 2019). 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, mucosal congestion and subepithelial oedema (Figures 3-8). The observed mucosal ulceration may cause significant pain and discomfort, especially when swallowing or bleeding, causing anaemia or other complications while the observed subepithelial oedema may cause airway obstruction, leading to respiratory distress and potentially life-threatening complications.

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 3 and 4), at low dose, cement dust showed mild histomorphological damage while for the rats in Group C and D (Figures 5-8) at moderate and high doses, cement dust caused severe histomorphological injuries.

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

Cement dust-related laryngeal 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 laryngeal histoarchitecture which are consistent with usual histological findings in laryngitis and may ultimately lead to loss of laryngeal 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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