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Mabel Bilu Ehi-Omosun
Departments of Anatomy and Physiolody, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Victor Oluwadamilola Olotu
Departments of Anatomy and Physiolody, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Correspondence:
Dr. Mabel Bilu Ehi-Omosun
Departments of Anatomy and Physiolody, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria
Email:
mabelehiomosun@gmail.com

Effects of Aqueous leaf Extract of *Cymbopogon citratus* on Cement Dust- Induced Lung Damage in Adult Wistar Rats

Mabel Bilu Ehi-Omosun, Victor Oluwadamilola Olotu

Abstract

This aim of this study was to investigate the effects of aqueous leaf extract of *Cymbopogon citratus* on cement dust-induced lung damage in adult Wistar rats. 30 adult Wistar rats weighing between 240g and 270g were randomly assigned into 5 groups of 6 rats per group. Group A rats were placed on rat food and water only. Group B rats were exposed to 40g of cement dust daily. Group C rats received 500mg/kg body weight/day (BWT/D) of *Cymbopogon citratus*. Group D rats were exposed to 40g of cement dust and received 250mg/kg BWT/D of *Cymbopogon citratus*. Group E rats were exposed to 40g of cement dust and received 500mg/kg BWT/D of *Cymbopogon citratus*. The dosages were given for 30 consecutive days via orogastric method. The haematological outcome showed that cement dust caused some derangements in haematological parameters especially white blood cells, lymphocytes monocytes, mean cell haemoglobin and platelets. Histologically, Group B showed distended alveolar sacs, inflammatory infiltrates, particulate matters in bronchial lumen and florid activation of the lymphoid tissues while Group A, C, D and E revealed normal alveoli, patent bronchiolar lumen and normal arteries. In conclusion, the aqueous leaf extract of *Cymbopogon citratus* has ameliorative effect on cement dust-induced interstitial pneumonitis in Wistar rats.

Keywords: Cymbopogon citrates; cement dust; interstitial pneumonitis.

INTRODUCTION

Cymbopogon citratus is a frost-tender clumping perennial grass that is cultivated in tropical countries especially in Southeast Asia.[1] for its medicinal and culinary uses. *Cymbopogon citratus*, commonly known as lemon grass, barbed wire grass or silky heads is part of the grass family, Poaceae. They contain simple, bluish-green leaves with entire margins and are linear in shape. The blades tend to be 45 - 90 cm long. Like other grasses, the leaves also have parallel venation.[2] Phytochemical constituents of *Cymbopogon citratus* include ketones, terpenes, alcohols, aldehydes, esters, limonene, citral geraniol, neral and nerol. [3] The essential oil of the plant is used in aromatherapy.

The leaves of *Cymbopogon citratus* were well recognized by herbalists in Ovia North-East Local Government Area of Edo State, Nigeria for treating cough in their clients. The leaves are crushed and the resulting liquid can be used to treat cough, difficult breathing, skin rashes, body itching, and other skin diseases. Scientists have opined that the active principles which confer antitussive and soothing activities on the plant are the terpenes, esters and ketones.

Cement dust is a serious atmospheric pollutant. It is emitted during manufacturing and processing of cement, transportation, bag dumping, storage, usage, concrete cutting and when workers empty bags of cement.[4] The basic components of cement dust include: Calcium, Silicon, Aluminium, Manganese, Iron and Zinc. [5,6] Many of the chemical elements of cement dust have been found to be toxic or mutagenic to both animals and humans. [7,8,9,10]

Respiratoy diseases represent a major health challenge among people with airborne-dust related occupations.[11] The most important group of occupational diseases is respiratory disorders related to inhalation of cement dust.[12] Cement dust related diseases that are mainly developed as a result of

occupational exposures include respiratory allergy, laryngitis, tracheitis, bronchitis alveolitis, chronic obstructive pulmonary disease, pneumoconiosis, Interstitial lung disease (ILD) and a lot of other respiratory diseases.[13,14] ILD is a group of disorders that cause progressive scarring of lung tissue. It may be caused by long-term exposure to hazardous materials such as asbestos or cement dust. ILD occurs when an irritating substance causes an inflammation of the alveoli which then makes it difficult for oxygen to pass through the alveoli into the bloodstream.[15,16] Besides cement dust, many other airborne particles e.g., silica dust, coal dust have been linked to ILD.[15] Hence, the present study investigates the effects of aqueous leaf extract of *Cymbopogon citratus* on cement dust-induced injury in the lungs of adult Wistar rats.

MATERIALS AND METHOD

The leaves of *Cymbopogon citratus* that were used in this research work were harvested from a farm in Iguosa Community, Ovia North East Local Government Area Edo State, Nigeria. The harvested plants were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

The fresh matured leaves were oven-dried at 40ºC after air-drying for about a week. The dried leaves were then grounded using a 2018 model mechanical grinder, manufactured by Dozenmann Group of Company, U.S.A. The powdered material was extracted by cold maceration method[17] which was done by soaking 500g of powdered *Cymbopogon citratus* leaf in 1 litre of water for 24 hours at room temperature. Using cotton wool as filter, the soaked *Cymbopogon citratus* was filtered and the filtrate was concentrated over hot water bath using evaporating dishes to obtain a concentrated jellylike extract of *Cymbopogon citratus* leaf which when weighed was found to be 20g and was transferred into a sample bottle for storage inside a refrigerator at 4ºC.

Phytochemical constituents of *Cymbopogon citratus* include: ketones, terpenes, alcohols aldehydes, esters, limonene, citral geraniol, neral and nerol.[3] Acute oral toxicity of the extract was evaluated. Appropriate doses of the extract were made by diluting with distilled water into 150mg/kg body weight and 300mg/kg body weight which were administered to the rats.

Experimental animals: 30 adult Wistar rats weighing between 240g and 270g were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City Edo State, Nigeria and were utilized for this experimental research. The rats were given a period of 2 weeks to adapt to their new environment before commencement of the experiment. During this period, the animals were allowed access to standard animal feed (Vital grower's feed, manufactured by Bendel Flour Mill, Ewu) and clean water *ad libitum*.

Ethical approval was obtained. 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.[18] Interstitial pneumonitis was induced by exposing the test animals to cement dust dispersed from 40g of cement via dust distributor glass-chamber (DDC) 1hour daily for 30 consecutive days.[8] A pilot study was done which confirmed interstitial pneumonitis in the rats.

Experimental design: 30 adult Wistar rats were randomly assigned into 5 groups; (Group A-E) comprising of 6 rats per group. Group A rats which served as control received 1ml of distilled water daily to compensate for stress of administration procured in the test groups. Group B rats were exposed to cement dust dispersed from 40g of cement via dust distributor chamber (DDC) 1hour daily for 30 days. Group C rats received 500mg/kg body weight/day (BWT/D) of *Cymbopogon citratus* aqueous leaf extract. Group D rats were exposed to 40g of cement dust and received 250mg/kg BWT/D of *Cymbopogon citratus* aqueous leaf extract Group E rats were exposed to 40g of cement dust via DDC and received 500mg/kg BWT/D of *Cymbopogon citratus* aqueous leaf extract. The dosages were given via orogastric method for 30 consecutive days.

The weight of the animal in each group was checked weekly so as to get the cumulative weight required for experimental use.

Method of Sacrifice and Sample Collection: At the end of the 30th day exposure, the animals were weighed and then euthanized under chloroform anaesthesia and the lung of each rat was harvested and immediately fixed in 10% formal saline for 24 hours before the histological analysis.

The tissues were trimmed to about 3-5mm thick sections and processed according to the method of Drury and Wallington (1980)[19] And then histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. Histological sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at various magnifications i.e. x40 and 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. 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 AND DISCUSSION

As shown in Table1 below, there was significant difference in body weight of the rats in the various groups exposed to cement dust. The results of the findings showed that *Cymbopogon citratus* aqueous leaf extract caused significant increase in body weight of the rats that were treated with only extract (Group C) while significant decrease was observed in the body weight of rats in Group B, D and E that were exposed to cement dust which concurs with previous work.[20] The weight loss could be attributable to dysguesia[21] anorexia[7] or toxicity of the basic componens of cement dust which include calcium silicon aluminium manganese iron and zinc.[6]

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

Groups	Initial Body Weight	Final Body Weight	p-Value
Group A (Control)	168.00±19.90	195.00±19.22*	0.018
Group B Cement dust exposure only)	214.67±13.30	110.67±17.46*	0.000
Group C (Extract only)	184.00±18.04	232.00±16.70*	0.012
Group D (Cement dust + Low dose Extract)	186.00±18.77	224.33±17.61*	0.000
Group E (Cement dust + High dose ext.	176.67 ±11.86	218.67±16.75*	0.005

Values are Mean ± S.E.M

*****Significantly different from the control group.

As shown below in Figure 1, the histological sections of the lung of control **(**Group A) showed normal histoarchitecture of the lung, viz., normal alveolar sacs, terminal bronchioles, interstitial space, bronchial artery and lymphoid aggregates

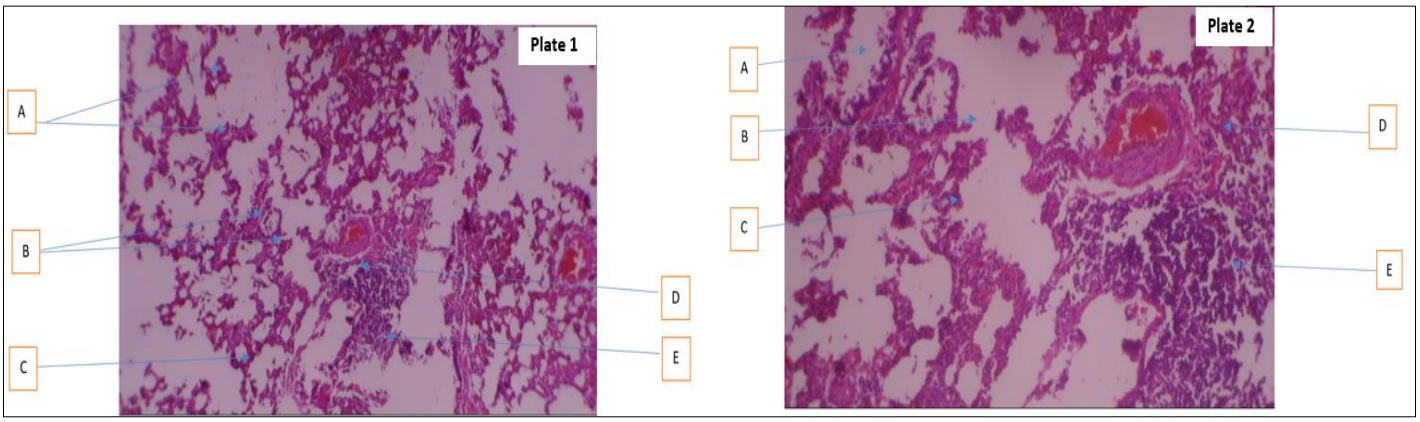

Figure 1: Plate 1- Histological sections of the lung of control group (Group A), composed of Alveoli (A), terminal bronchioles (B), interstitial space (C) bronchial artery (D) and lymphoid aggregate (E) (H&E x 40). Plate 2- Higher magnification of the Plate 1 (H&E x 100)

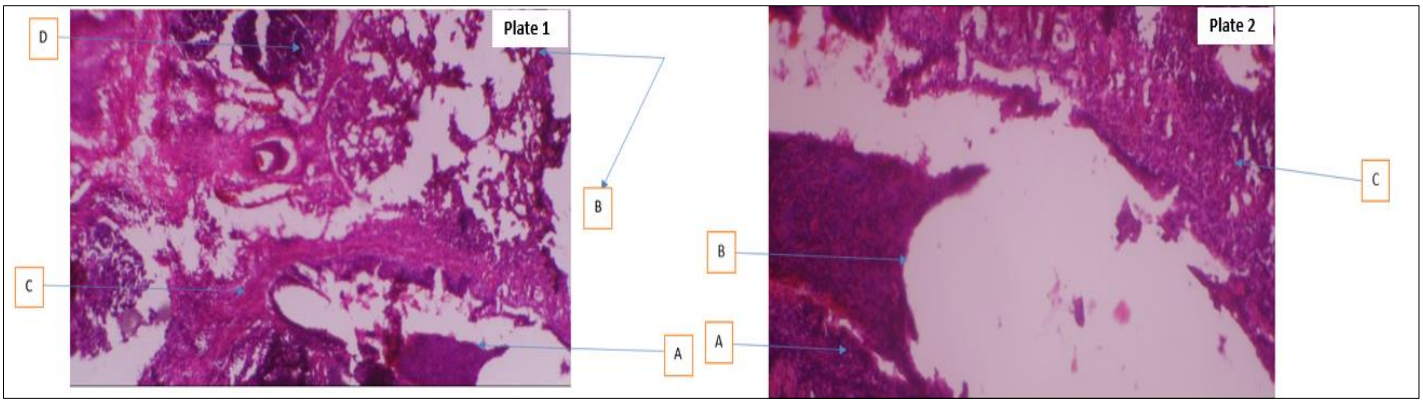

Figure 2: Plate 1- Histological sections of the lung of Group B; Rat given Cement dust only showing: distended alveolar sacs (A), particulate matter in bronchiolar lumen (B), inflammatory infiltrates (C) and florid activation (D) of the lymphoid tissue (H&E x 40). Plate 2- Higher magnification of the Plate 1 (H&E x 100)

As shown above in Figure 1, there was florid activation of the lung tissue of the rats that

were exposed to cement dust alone (Group B) which occurred as a result of the body sensing a foreign body leading to the activation of lymphoid tissues to get rid of it. The alveoli became distended and particulate matters were found in the bronchiolar lumen of the rats. These particles formed a clog in the lumen and they occur as a result of excess accumulation of cement dust leading to inflammation and secretion of mucus. Interstitial pneumonitis and oedema were observed. This is in accordance with previous studies done by Al-Hayali and Calistus *et al*. [22, 23]

As shown below in Figure 3, *Cymbopogon citrates* showed no negative effect on the histology of the lungs as the alveoli, terminal bronghiole and bronchial artery were found to be histologically normal in the rats that were administered only the extract (Group C). For rats in Group D (Figure 4), at low doses *Cymbopogon citratus* showed protective effect against cement dust-induced lung injuries. Interstitial pneumonitis was completely prevented and the accumulated particulate matters were cleared allowing the flow of oxygen into the alveolar sac and release of carbon dioxide from the blood. For rats in Group E (Figure 5), at high doses, C*ymbopogon citratus* showed some protective effects against lung injuries. Interstitial pneumonitis was partially prevented which also allowed the exchange of oxygen and carbon dioxide between inspired air and blood.

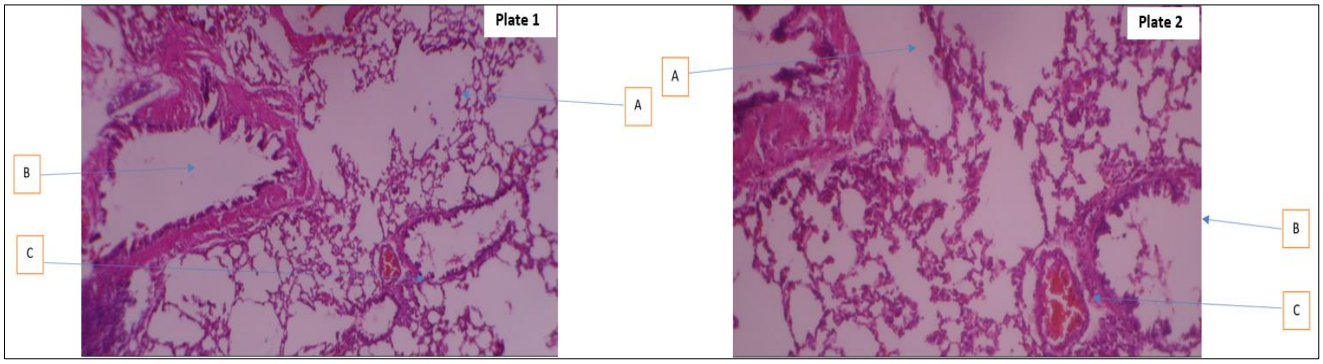

Figure 3: Plate 1- Histological sections of the lung of Group C; Rat given 500mg extract only showing: normal alveoli (A), terminal bronchiole (B), and bronchial artery (C) (H&E x 40). Plate 2- Higher magnification of the Plate 1 (H&E x 100)

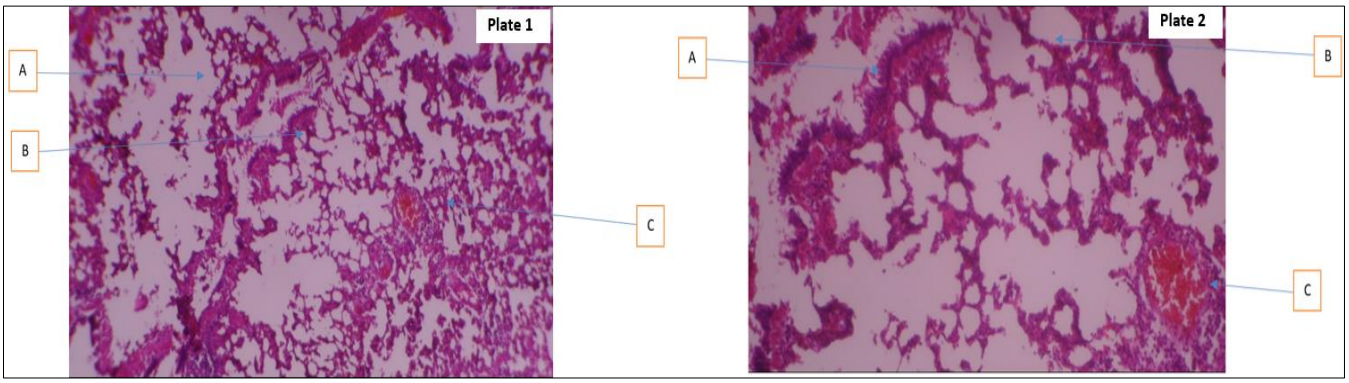

Figure 4: Plate 1- Histological sections of the lung of Group D; Rat given Cement dust + 250mg extract showing: normal alveoli (A), bronchiolar lumen containing small particles (B) and normal artery (C) (H&E x 40). Plate 2- Higher magnification of the Plate 1 (H&E x 100)

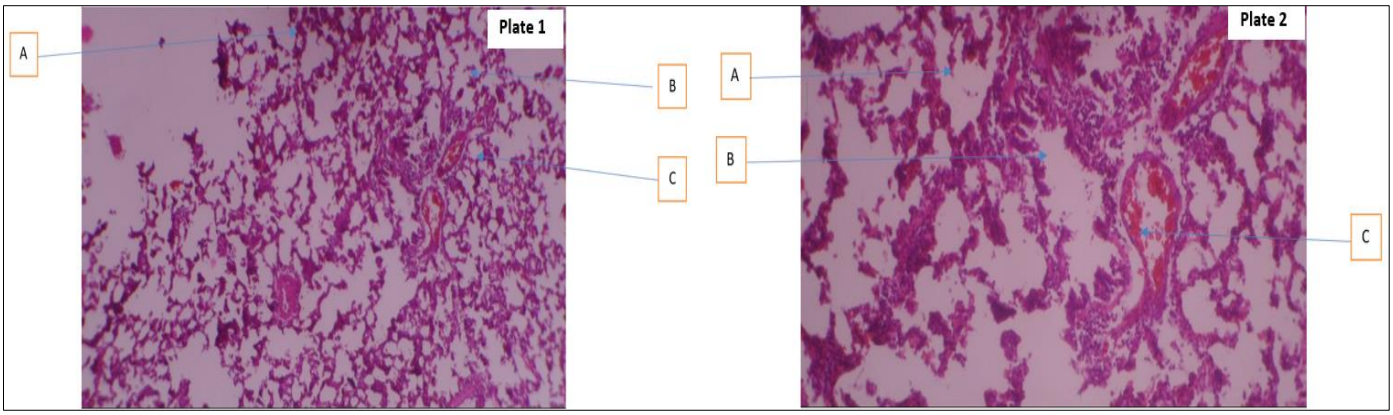

Figure 5: Plate 1- Histological sections of the lung of Group E; Rat given Cement dust + 500mg extract showing: normal alveoli (A), bronchiolar lumen (B) and, normal artery (C) (H&E x 40). Plate 2- Higher magnification of the Plate 1 (H&E x 100)

CONCLUSION

In conclusion, *Cymbopogon citratus* had ameliorative effects against cement dust-induced alveolar distension, pulmonary oedema, inflammation of the lung interstitial space, and activation of lymphoid tissue and its effect is inversely proportional to the doses administered. At low doses, the effects appear to be more potent and can be compared to that of the control group. Therefore, it can be used as a substitute to combat interstitial pneumonitis and other lung diseases.

Conflict of Interest

None declared.

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