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Research Article

Evaluation of a Cement Dust Generation and Exposure Chamber for Rodents: Blood Heavy Metal Status, Haematological Variables and Gastrointestinal Motility in Rats

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ABSTRACT

Exposure to cement dust has been documented to cause various occupational and long-term health complications both in human and animal. However, investigations on the extent of toxicity associated with cement dust exposure have been limited by lack of suitable model for controlled laboratory exposures. In this study, a glass house animal exposure chamber was fabricated using a plexi-glass and a blowing fan of adjustable revolution. Model simulations were validated using experimental data showing the effects of cement dust exposure on haematological indices, trace element status and gastrointestinal motility in rats. Thirty male Wistar rats were randomly divided into three groups. The unexposed group (n = 10) served as control while the other groups were exposed for five hours daily to cement dust (200g) at a revolution of 2400-3000rpm. Blood collected was analysed for some haematological variables as well as plasma concentrations of cadmium, lead, silicon, aluminium, manganese, calcium, iron and magnesium. Organ weights were measured and histopathological features of the kidney, lungs stomach and liver were assessed to determine the degree of tissue damage. Intestinal motility was assessed *in vivo* using the Charcoal meal method while colonic motility was studied by measuring the distance travelled by beads inserted 2cm into the distal colon through the anal opening. Data were expressed as Mean ± SEM, analysed using one-way ANOVA and $p<0.05$ was significant. Blood analysis from exposed rats on days 14 and 28 showed significant increase in concentrations of Calcium, Silicon, Manganese, Iron, Lead, Cadmium, Aluminium and magnesium compared with unexposed animals. Significant reductions were observed in haematocrit values, red and white blood cell counts after cement dust exposure. Also, significant increases were observed in the neutrophil-lymphocyte ratio and erythrocyte sedimentation rate in exposed rats compared with control. There was a significant decrease in organ weights - stomach, lungs, kidney when compared with control. Rats exposed to cement dust had significantly decreased small intestinal motility but increased colonic transit time. Histopathological examination from exposed rats revealed peribronchiolar infiltration by lymphocytes in the lungs while gastric gland was severely infiltrated by inflammatory cells. The results from this study are comparable to data obtained from earlier reported on haematological and heavy metals in humans occupationally exposed to cement.

Keywords: *Cement dust, fabricated chamber, haematological indices and gastric motility*

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INTRODUCTION

Man's environment is constantly being polluted by remains of industrial activities which poses serious threat to human health. One of such activities in developing countries is increased construction of structures such as houses, roads, schools, factories, hospitals etc. for which usage of cement is inevitable. Cement is a powdery composition (limestone, laterites, clay and gypsum) used in making and holding blocks or bricks in-place during construction (Amodu and Egwuogu, 2014). The major components of cement are derived from toxic heavy metals such as nickel, cobalt, lead, chromium and Silica (Gbadebo and Bankole 2007; Baby *et al*., 2008; Ogunbileje *et al*, 2013). It also contains Thallium and many other impurities (Short and Petsonk, 1996). Many of these toxic compounds have been shown to cause damages at both cellular and organ levels in the lungs (Ade-Ademilua and Obalola, 2008); Akpan *et al*. 2011), blood (Goyer *et al*, 1973) gut (Olaleye *et al*, 2006, 2007; Adeleye and Olaleye, 2016; Adeleye *et al*, 2018) in addition to their roles in genetic

disorders and cancers. In most developed countries, dusts from cement factories is a major problem that both factory workers and nearby residents are faced with as it affects the quality of air they inhale. Apart from factory workers, cement dusts also pose environmental threat to the ecosystem with adverse impact on vegetation and aquatic life (Anda, 1986; Iqbal and Shafiq, 2000).

Several reports are available in literature which underscore the adverse effects of repeated and or prolonged exposure to cement dusts on the health status of most cement factory workers as well as those living around cement factories (Calistrusjudge *et al*., 2002; Fell *et al*., 2003; Lameed, 2008). Excessive exposure to cement dusts Cough and phlegm production, chest tightness, impairment of lung function, obstructive and restrictive lung disease, pleural thickening, fibrosis, emphysema, lung nodulation, pneumoconiosis and carcinoma of lung (Alakija *et al*., 1990; Meo, 2004; Baccarelli *et al*., 2014,). In the gastrointestinal tract, cement dust exposure is believed to cause mechanical trauma, mucosal inflammation, loss of tooth surface, periodontal disease, dental abrasion, dental caries, Stomach ache and cancer of stomach (Kolev and Shumkov, 1975; Struzak-Wysokinska and Bozyk, 1989; Jakobsson *et al*, 1990; Tuominen and Tuominen, 1992).

Most of the reports on the effects of cement dust exposure on body functions have been on human studies carried out on industry workers or on animals taken to production areas. In such studies, quantification of the extent of exposure are practically impossible. The implication of this is that information on mechanistic and detailed laboratory-based exposure models of cement dust exposure in animals are not available, leading to limitations in the knowledge of cement dust toxicity in the body. In this study, the efficacy of a fabricated cement dust exposure chamber was tested and validated by assessing and comparing the effects of cement dust exposure on hematological indices, trace metal status and intestinal motility in rats.

MATERIALS AND METHODS

The Exposure chamber: The fabricated plexiglass house animal exposure chamber consists of a square shaped box made up plastic glass with two compartments. One of the inner compartments houses the experimental animal during exposure (Plate 1) while the second inner smaller compartment contains two industrial fans which is been connected to electricity to blow the cement dust been deposited into the compartment to the other compartment that houses animal during the exposure at a revolution of 2400-3000rpm

The bigger compartment has a height of 60cm and a width of 59.9cm. The smaller inner chamber has a height of 19.6cm and a width of 26.1cm. The chamber also contains outlet opening (vent) which regulates the temperature of the chamber during exposure at every 30minutes interval to prevent suffocation of the experimental animal. It has a height of 9.9cm and a width of 10.6cm.

Animals: Fifteen male Wistar rats (100 – 110g were randomly divided into three groups viz: 1-unexposed group (control) while other groups 2 and 3 were exposed to cement dust for 14 and 28 days respectively. Animals were acclimatized for two weeks with free access to standard commercial rat chow and tap water *ad libitum* before commencement of studies. The animals were housed under standard conditions of temperature (23 ± 2ºC), humidity (55 ± 15%) and environmental 12hour light and dark cycle in the Animal house of Department of Physiology, University of Ibadan, Ibadan. They were kept in plastic cages with beddings which were adequately changed throughout the study period. They were exposed for five hours daily to cement dust (200g) at a revolution of 2400-3000rpm in the enclosed plexiglass exposure chamber between 8:00 am to 12 noon.

Plate 1
Fabricated enclosed cement dust chamber before exposure (plate 1a) and during exposure (plate 1b)

Exposure procedure: 200g of cement was weighed daily and kept in the inner compartment of the exposure chamber for circulation. A thick transparent hollow glass plate was placed inside the chamber filled with 50ml of distilled water in order to ascertain the level of cement dust that will be diffused in to the water during exposure so as to measure the effectiveness of the chamber and also to compare the concentration found in water and that in the blood throughout the period of fourteen days and twenty eight days of exposure. Afterwards, animals were placed in the outer compartment (as shown in plate 1b) and the fan switched on.

Hematological examination: Blood collection was through the retro-orbital sinus using heparinized capillary tubes and EDTA bottles on days 14 and 28. The blood parameters (PCV, HB, WBC and RBC) were determined according to the method described by Dacies and Lewis (1994).

Histological Analysis: On sacrificing the animals, the lungs and stomach were harvested, weighed and a small section were fixed in formalin before Histological evaluation was carried out on them.

Metal analysis in blood: After collecting 1 ml of blood into a test tube, 2 ml of Nitric acid (HNO_3) was added and left overnight after thorough mixing. The digested blood was placed in water bath and heated for 30 minutes at 98-100°C. After cooling, 12mL of distilled water was added to the digested blood and filtered. The filtrate was then analyzed for the major heavy metals found in cement viz: Calcium, Silicon, Manganese, Iron, Lead, Cadmium, Aluminum and magnesium using atomic absorption spectrophotometry (Awad *et al*, 2013).

Intestinal motility: In another experiment, intestinal transit was determined following the method described by Teke *et al*. (2007). Briefly, Healthy Wistar rats were grouped into 3 (n=5) and exposed to cement dust as described earlier. A control group was not exposed. All animals were fasted for 18 h prior to the administration of charcoal meal. The charcoal meal (1 mL) (10% charcoal and 5% acacia gum suspended in distilled water and made up to 100 mLs of solution) was administered by oral gavage to all the groups. The animals were sacrificed 30 minutes after charcoal meal was given by ketamine overdose (100 mg/kg) followed by cervical dislocation. The small intestine was removed carefully and lengths of intestine, as well as the leading end of the charcoal meal were measured. The percentage of distance covered by the charcoal was computed to calculate percentage inhibition.
% transit = (distance traveled by charcoal meal / total length of the intestine) x 100 .

Colonic motility: The effect of cement exposure on altered gastrointestinal motility was also studied in Wistar rats fasted for 24 hours prior to experiment. The animals were grouped into 3 and exposed to cement dust as described earlier. Beads of about 2mm were be inserted 2cm into the distal colon through the anal opening using the nasogastric tube (NG) which was well lubricated. The animals were then placed in different plastic cages lined with white tissue rolls and the time at which each animal expelled the beads was be noted. Colonic motility was be calculated by computing the time between bead placement and expulsion of the bead (Osiniki *et al*.,1999).

Ethical considerations; This study was conducted in accordance with the current Animal Care Regulations and standards approved by the Institute for Laboratory Animal Research (ILAR, 1996) and the experimental protocol approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan. Ibadan, Nigeria

Statistical analysis: All values are expressed as Mean ± SEM of the animals used in each group. Independent T-test and one-way ANOVA were employed to compare differences among variables. Comparisons between groups were done using appropriate post hoc test and the statistical differences was taken to be significant at $p<0.05$.

RESULTS

Body and organ weight changes

The changes in body weight of the animals after 14- and 28-days exposure to cement dust are shown in Figure 1. Significant decreases in the body weight of exposed animals were apparent when compared with the unexposed (control) animals. Table 1 shows that the relative weights of the kidneys, stomach and lungs were significantly decreased in cement dust-exposed rats. Liver weight was not significantly affected.

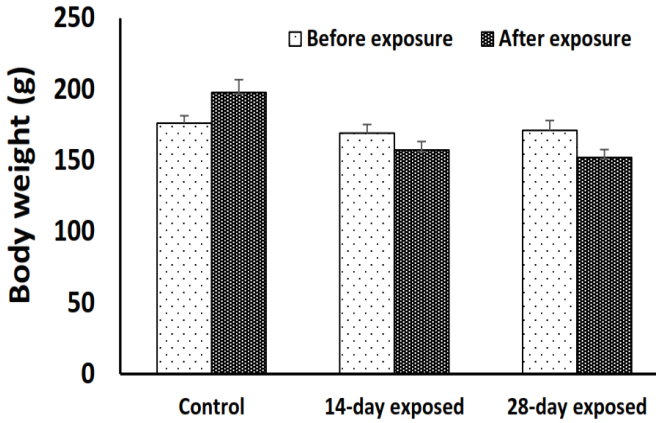

Figure 1:
Boy weight profile of rats before and after exposure to cement dust. Each vertical bar represents mean ± SEM of 10 rats per group.

Blood parameters:

The results of studies on the effects of cement dust exposure on blood variables are shown in Table 2. The values of red and white blood cell counts, platelets as well as Packed Cell Volume (PCV) and hemoglobin were significantly decreased in rats exposed to cement dust. The effects were marked more on the 28-day exposed animals.

Table 1:
Effect of cement dust exposure on body organ weight.

Groups	Organs			
	Lungs (g)	**Liver (g)**	**Kidney (g)**	**Stomach (g)**
Control	1.56 ±0.06	6.20 ± 0.17	1.28 ± 0.04	1.42 ± 0.10
14 days CDE	1.057 ± 0.06**	5.25 ± 0.25	1.11 ± 0.02*	1.14 ± 0.02*
28 days CDE	1.37 ± 0.09	6.80 ±0.39	1.07 ± 0.01*	0.96 ± 0.02**

Values are presented as Mean ± SEM, n=5. *Significant when compared to control (P<0.05). ** Highly Significant when compared to control (P<0.01)

Differential white blood cell counts revealed significant decreases in the lymphocyte and eosinophil counts in exposed rats while neutrophil and monocyte counts were significantly increased after 28 days of exposure. However, the decreases observed in lymphocyte, neutrophil and eosinophil counts on day 14 were not significant when compared with the control groups (Table 2).

As shown in Fig. 2, Erythrocyte Sedimentation Rate (ESR) was significantly increased after 14 and 28 days of exposure to cement dust. The figure also shows the relative increase in the Neutrophil-Lymphocyte ratio in the exposed rats when compared with the control.

Heavy metal levels in blood:
As shown in Fig. 3, the blood levels of lead and cadmium (3a), silicon, aluminum an manganese (3b) as well as calcium, iron an magnesium (3c) were all increased the end of the 28 day exposure period.

Table 2:
Effect of cement dust exposure on blood parameters

	Control	14 Days CDE	28 Days CDE
PCV (%)	40.40±0.67	39.80±1.16	33.80±1.72 *#
RBC (millions/mm^3)	6.53±0.18	6.88±0.19	5.72±0.23 *#
HB (g/dl)	13.56±0.27	13.68±0.34	11.14±0.58 *#
WBC (millions/mm^3)	8910±737.30	9050±1131	4770±94.34 *#
Platelet (x10^3/mm^3)	2.55±0.26	1.62±0.90*	1.66±0.89*
Lymphocyte	70.50±1.04	71.50±1.04	65.50±1.32 *#
Neutrophils	25.75±0.85	27.25±0.85	31.50±1.19 *#
Monocytes	1.25±0.25	2.25±0.25 *	2.75±0.25 *
Eosinophils	2.60±0.24	2.80±0.20	1.20±0.20 *#

Values are presented as Mean ± SEM, n=10
** Significant when compared to control (P<0.05).*
 # Significant when compared with 14 days CDE (P<0.05)
CDE stands for cement dust exposure

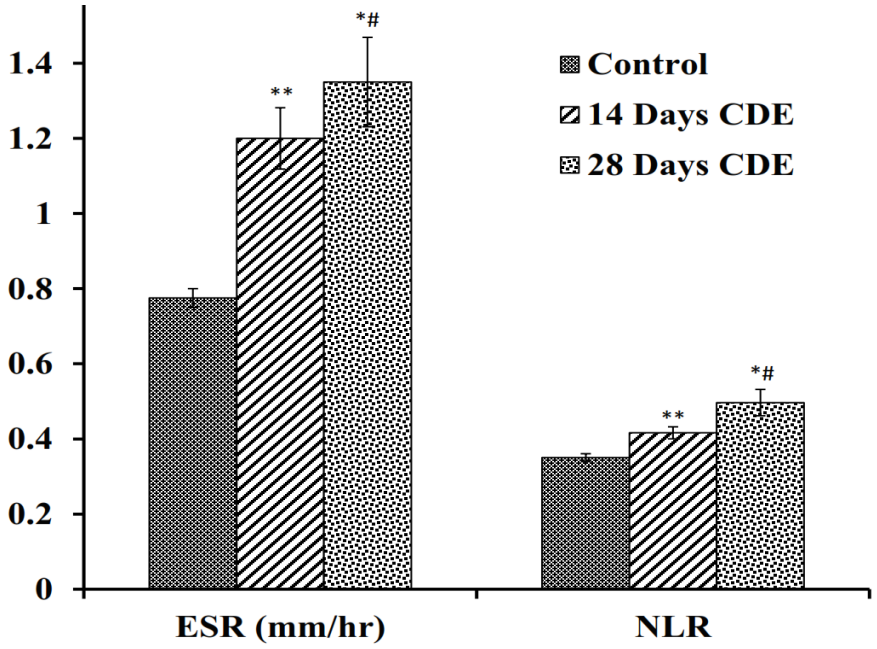

Figure 2:
Effect of cement dust exposure on erythrocyte sedimentation rate and neutrophil-lymphocyte ratio. Values are presented as Mean ± SEM, n=5
** Significant when compared to control (P<0.05). # Significant when compared with 14 days CDE (P<0.05) .*

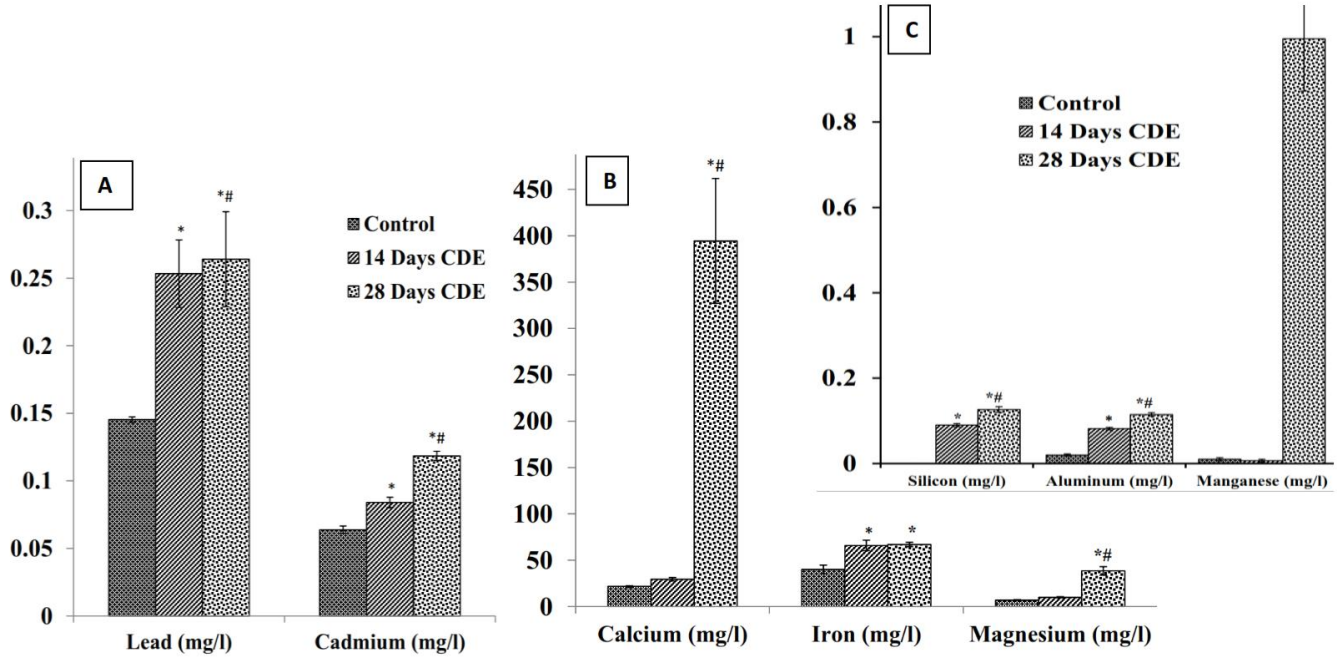

Figure 3.
Effect of cement dust exposure on Lead and Cadmium (A), Calcium, iron and magnesium (B) and Silicon, Aluminum and manganese levels. *Values are presented as Mean ± SEM. * and # Significant when compared to control and with 14 days CDE (P<0.05)*

Intestinal and colonic motility

Intestinal motility was significantly decreased 14 and 28 days after cement dust exposure compared with control group (Fig 4). Figure 5 shows the effect of cement dust on colonic transit time. Colonic transit time was significantly increased in all exposed animals

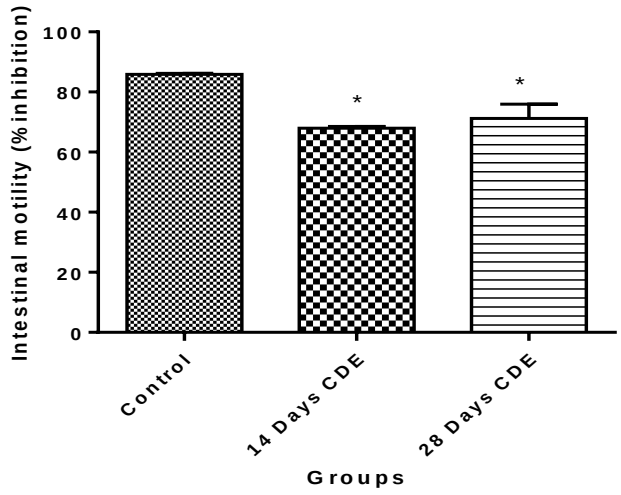

Figure 4:
Effect of cement dust on Intestinal motility
Values are presented as Mean ± SEM, n=10
* Significant when compared to control.

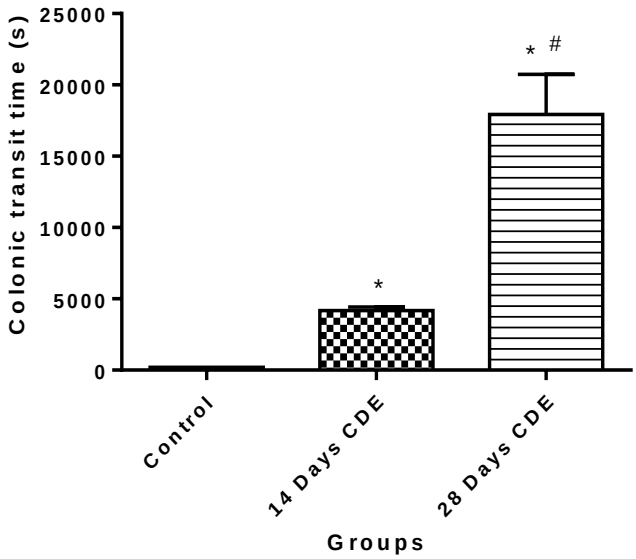

Figure 5:
Effect of cement dust on colonic transit time
Values are presented as Mean ± SEM, n=10
* Significant when compared to control.
# Significant when compared with 14 days CDE

Histology

The results of the histological examinations of the lung and stomach tissues of the exposed rats showed marked microscopic changes when compared the tissues of the control rats (Plates 2 and 3).

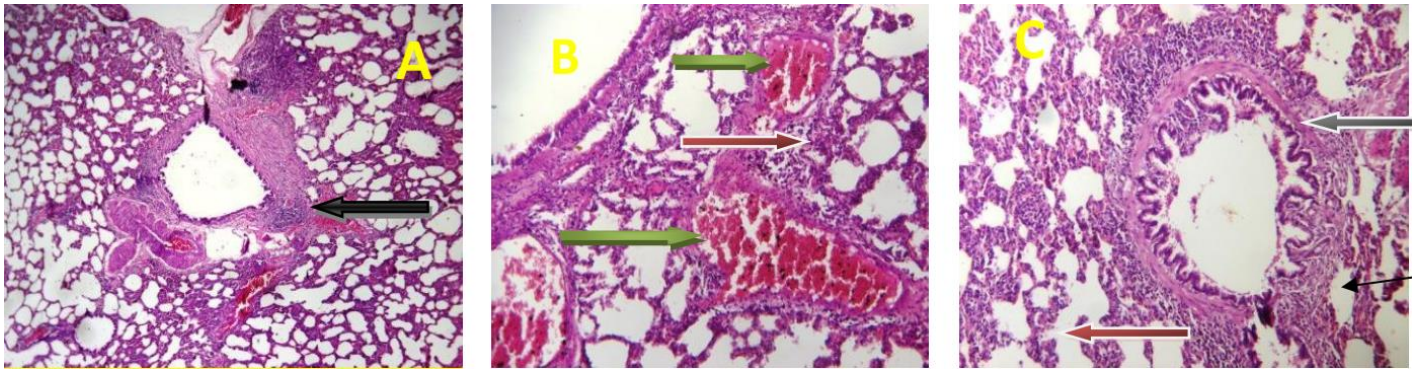

Plate 2:
Histology of The Lungs (H&E Stain MAG. X 100) showing A-Control: showing moderate peri-lymphocyte bronchiolar and vascular infiltration (black arrow), there is mild fat deposit at the perivascular region, no vascular congestion is noted. The intra alveolar spaces are not infiltrated and alveolar ducts appear normal, B-group 2, day 14: showing moderate lymphocyte follicle and severe peri-bronchial ar infiltration of lymphocytes (green arrow). There is moderate thickening of vascular wall and mild vascular congestion noted. The intra alveolar spaces and alveolar ducts (red arrow) are severely infiltrated. C-group 3, day 28: showing moderate fibrosis, severe peri bronchiolar infiltration of lymphocytes (black arrow). There is moderate thickening of vascular of vascular and mild vascular congestion noted. There is focal area of mild fat deposits. The intra alveolar spaces (slender arrow) and alveolar ducts (red arrow) are severely infiltrated (slender arrow)

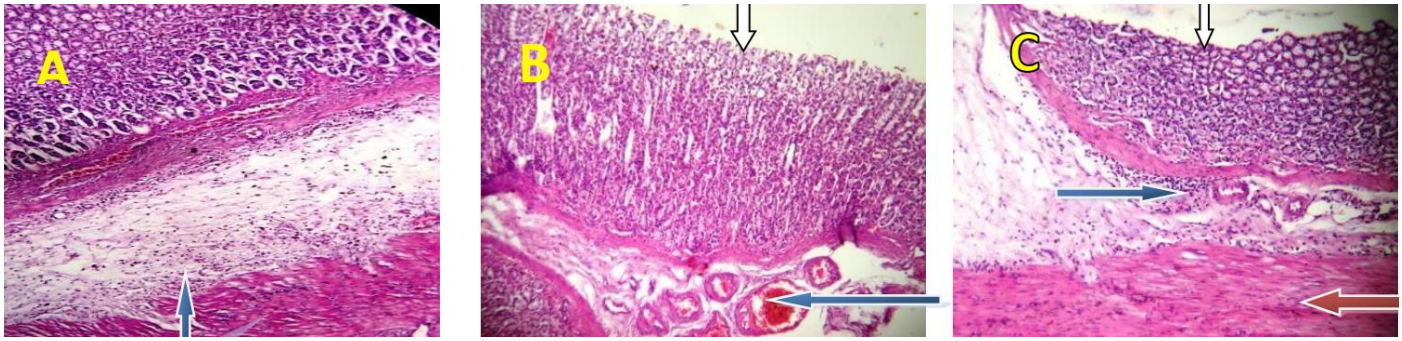

Plate 3:
Histology of the Stomach (H&E Stain MAG. X 100) showing Group 1 (A), Unexposed Control; moderate architecture, the mucosa layer shows scanty infiltration of the gastric gland and lamina propria. The submucosa layer shows mild infiltration of inflammatory cells (blue arrow). Group 2 (B), day 14 CDE: moderate architecture and poorly preserved mucosa epithelial cells layer (white arrow) which are severely eroded, there is moderate papillary infoldings, the mucosa layer shows no infiltration of the gastric glands and lamina propria. The submucosal layer appear mildly infiltrated by inflammatory cells and moderately vascularized with thickened vascular walls and mild congestion (blue arrow), the circular muscle layer (red arrow) appears normal. Group 3 (C), day 28 CDE: fair architecture, the mucosa epithelial cells layer is poorly preserved (white arrow), the mucosa layer shows moderate to severe infiltration of the gastric glands and lamina propria. The submucosal layer appears severely infiltrated by inflammatory cells (blue arrow) and also show moderate vascularization and mild fibrosis. The circular muscle layer (red arrow) appears normal

DISCUSSION

In this study, rats were exposed in the laboratory to cement dusts via a fabricated exposure chamber. The efficacy of the exposure chamber was tested by investigating the effect of the exposure on some indicators of toxicity.

The body weights of the animals exposed to cement dust in this study were decreased when compared with the unexposed control rats. Decreased body weights have been attributed to several factors such as impaired gastrointestinal functions (Chokshi, 2007) probably resulting from increased toxic end products during inappropriate food conversion (Klaassen *et al*., 2001). It could also be as a result of impairment or disturbances in the metabolic breakdown between carbohydrate, protein and fats (Klaassen *et al*., 2001) which can be linked to altered food appetite (Ezeonwumelu *et al*., 2011). Changes in body weight have been reported by several workers as an indication of toxicity (Lamanna and Hart, 1968; Kwan Yuet Ping *et al*., 2013). Decreased in body weight may be used as an index of toxicity or deleterious effect of certain substances (Hilaly *et al.,* 2004) which is evident in this study.

The weights of the liver were not remarkably altered by cement dust exposure in this study. This is in line with the work of Mojiminiyi *et al., (*2008) that reported that the liver function parameters remained similar in exposed workers compared to unexposed workers. Results of this study indicate that kidney and lung weights decreased as a result of cement dust exposure.

The proper functioning of the body system or cells is dependent on adequate nourishment, a factor determined by the efficacy of blood cells. Blood cells function in oxygenation, removal of waste products from organs and ultimately conferring immunity to the body system (Barrett *et al*, 2010). Anaemia has been documented over time as an index of toxicity. In this study, the blood cells- erythrocytes, leucocytes and platelets were significantly diminished in rats exposed to cement dusts. The adverse effect of cement dust on

hematological variables in humans (Mojimoniyi *et al*., 2007; Mohammed and Sambo, 2008) and animals taken to cement manufacturing sites (Yahaya *et al*, 2011) have been well documented. However, while Mojiminiyi *et al*., (2008) and Erhabor *et al*., (2013) increases in the platelet counts in exposed humans, Jude *et al*., (2002) observed decreases in the platelet count in exposed subjects. This disparity thus suggests for more investigations.

Erythrocyte sedimentation rate has been used clinically to denote presence of tissue damage during stress conditions. It is also a common haematological test used to measure non-specific inflammation (Gabriel *et al*., 2004; Punzi *et al*., 2005). Erythrocyte sedimentation rate is also an indirect measurement of fibrinogen level which are observed as acute phase protein in disease state (Husain and Kim 2002). In this study, the Erythrocyte sedimentation rate was elevated after cement dust exposure. This is similar to the observations of Erhabor *et al.,* (2013). An elevated ESR means fragile and reduced levels of red blood cell production which was observed in this study and suggesting adverse effect cement dust exposure may exert on erythrocyte formation, structure and function.

Ratios of blood cells- neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR), and mean platelet volume (MPV) have been projected as useful markers of disease conditions including cancers and systemic inflammation response (Liu *et al.,* 2011, Seretis *et al*, 2013; Lee *et al*, 2018). In this study, neutrophil counts remarkably increased in animals exposed to cement dust, similar to the report of Okonkwo *et al*., (2015) and Yahaya *et al.,* (2011). Also, Neutrophil/Lymphocyte ratio significantly increased in rats exposed to cement dust.

Similar to reports in humans occupationally exposed to cement and in studies involving animals taken to sites of cement production, our study shows significantly elevated levels in blood concentration of Cadmium, Calcium, Magnesium, Silicon, Manganese, Lead, Aluminum and Iron after 14 and 28 days of cement dust exposure. This finding is a confirmation of the previous where elevated levels of these heavy metals were detected in blood and lungs of exposed humans and animals (Abdul-Wahab, 2006; Gbadebo and Bankole, 2007; Akinola *et al*. 2008; Ade-Ademilua and Obalola, 2008; Akpan *et al*., 2011; El-Absay *et al*., 2011).

The decrease in the two types of intestinal motility tests (small intestinal transit and colonic motility) from this study did not present mechanistic evidence to trace the pathways of reported findings. However, the reduced motility is an indication of stasis in the gut. This slow dynamism of the gut movement in the presence of ingested heavy metals might be a signal suggestive of danger to the gut's health. The report by Manjula *et al* (2013) where factory workers exposed to cement dust presented with series of gastrointestinal problems ranging from diarrhea as well as constipation might suggest modulatory roles that the cement dust can play when exposed to the gut.

Chromium which is a component of cement was recently reported to possess decreased intestinal and colonic motility properties in rat models exposed to trivalent chromium (Odukanmi *et al*, 2017). This further buttress the reported role of delayed in motility adduced to cement dust exposure in this current study. Certain gastrointestinal cancers were also linked to exposure to cement in some factory workers (Jakobsson *et al*, 1990) and even though this could occur through series of pathways, delayed motility is certainly a strong precursor in development of gastrointestinal cancers. More importantly if the heavy metals have potentials of generating reactive oxygen species (Bishak *et al*, 2015).

The observed findings in this study which were very similar to animals taken to cement factory environment further confirms for the first time that this model mimicks pollutions as though in the cement factory. It also buttresses the fact that cement dust exposure can be performed experimentally using this exposure chamber model. Pathological observations of the lungs and kidney, adverse observations of the haematological variables of exposed rats as well as elevated heavy metal levels found in blood of cement exposed animals confirms that cement dust is pathogenic to rats as also observed in humans. This study is therefore in concordance with most of the research works conducted previously regarding the toxicity effects of cement dust exposure to animals and humans

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