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Full length Research Article

Cement Dust Exposure Alters Secretory Functions, Biochemical Profile and Morphology of Gastrointestinal Tissues in Rats

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Summary: Asides direct gastrointestinal exposure, inhalation route is another major xenobiotic exposure pathway to the gastrointestinal tract via mucociliary escalator. This triphasic study assesses the effect of cement dust inhalation exposure on gastrointestinal tissues and secretion. Seventy-two male, 16-week old Wistar rats were randomized into 3 different phases of 24 animals. Each phase comprised of three groups of 8 animals. Group 1 (control) were sham-operated with clean ambient air, group 2 (14-day exposed) were exposed to cement dust for 14 days, and group 3 (28-day exposed) were exposed to cement dust for 28 days. Biochemical indices including superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), sulfhydryl group, carbonyl group, Na^+-K^+ ATPase pump activity, Nitric oxide (NO) were investigated spectrophotometrically in gastric and hepatic tissues while histopathology was studied using standard procedure. There were significant increases in the levels of MDA, NO and carbonyl- an observation that contrasts with the level of CAT, SOD and sulfhydryl; no significant difference in Na^+-K^+-ATPase pump was observed in the exposed groups compared with control. Histopathological alterations in salivary glandular and gastric tissues includes edema, inflammatory cell infiltration and vascular congestion. There were significant alterations in basal salivary, gastric and biliary secretions; increased stimulated salivary and gastric secretions via cholinergic stimulation. Histopathological and spectrophotometric analyses revealed that exposure to cement dust significantly alters gastrointestinal secretions and predisposes the gastrointestinal tract to an array of deleterious effects via protein oxidation, antioxidant depletion and tissue peroxidation.

Keywords*: Cement dust exposure, gastric acid secretion, salivary secretion, biliary secretion, oxidative stress*

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INTRODUCTION

Cement dust has assumed the frontline of toxicological concerns in recent times having moved from an emerging toxicant to one of emerging concern, particularly with the array of multi-systemic damages traceable to the exposure to it. (Owonikoko *et al*., 2021). Cement kiln is a major depository of varieties of heavy metallic constituents that have been individually identified as environmental xenobiotics. They include Silicon oxide (SiO), Aluminiun oxide (Al_2O_3), Iron oxide, Lead, cadmium, Arsenic, Nickel, Cobalt, Chromium, Potassium, Sodium, Lead, Silica, Sulphur, and Magnesium oxide in varied toxic proportions (Fell *et al*., 2010; Chaurasia *et al*., 2013; Ogunbileje *et al*., 2013). Toxic elements and silica co-exposure, as obtainable in cement dust, are hazardous to human health due to significant systemic alteration during their chemical-biological interaction (Brzóska *et al.*, 2003). Typical of any xenobiotics, exposure to cement dust particles culminates in intestinal tissue internalization either through nasal or ingestion pathway, absorption from the gut into the circulation, and eventually tissue bioaccumulation (Cooper *et al*., 2018). These stimulates deleterious pathologies (Manjula *et al*., 2013; Olatunbosun *et al*., 2020) such as liver abnormalities, mutagenic, respiratory, pulmonary, and gastrointestinal disorders have equally been reported among occupationally/geographically exposed individuals (Adak *et al*., 2007; Aydin *et al*., 2010). Cement dust, a reported systemic toxin, induces multi-organ injuries (Tajudeen *et al.* 2011; Egbe *et al*., 2016) such as periodontal tissue disruption (Elagib *et al*., 2020), hematological alteration (Ogunbileje and Akinosun, 2011; Okonkwo *et al*., 2015), clastogenic (Fatima *et al*., 2001), immunotoxic effects (Akiibinu *et al*., 2019), genetic disorders and cancer (Jumat *et al*., 2021; Yahaya *et al*., 2022).

The most predominant reports on cement dust toxicosis have hitherto hinged on the respiratory effects (Ahmed and Abdullah, 2012; Kakooei *et al*., 2012; Egbe *et al*., 2016; Mbelambela *et al*., 2018; Rachiotis *et al*., 2018; Kim *et al*., 2020). The foregoing is due to the aerosolized particulate nature of the dust making it easily inhalable. Several segments of the lungs serve as potential sites for trapped particles as the entire conduit is lined with short hair covered with mucus which make up the structure called the mucociliary escalator. The latter makes it easy for particles that escape into the lungs to be appropriately trapped and evacuated by coughing or swallowing (Weupe *et al*., 2013;

Bustamante-Marin and Ostrowski, 2017) into the gastrointestinal tract.

Secretion is central to the overall function of the gastrointestinal tract. Buccal secretions including saliva and salivary lingual lipase from salivary and Ebner's glands assist in pre-digestion of food material at the cephalic phase of deglutition. This function differs from all other functions relating to protection, speech, antibacterial etc. Gastric secretion is central to the digestive functions of the gastrointestinal tract hydrolyzing food material and transforming it into chyme prior passage into the duodenum; extramural bile secretion emulsifies lipids and fat-soluble vitamins bioconverting them into micelles and chylomicron. These secretions have been reported to be sensitive to intra-systemic bioavailability of xenobiotics (Gerbino *et al*., 2010; Li *et al*., 2019). The bioavailability of heavy metals in soil was investigated in mouse model and reported to alter the pH of gastric acid (Kang *et al*., 2016). Recently, workers exposed to chromium- a major component of cement dust, demanded clinical attention after the development of salivary duct carcinoma (Secin *et al*., 2021). Environmental factors have been reported to induce salivary gland cancer (Horn-Ross et a., 1997). The ultimate goal of these functions is to advance the task of availing the body system the energy present in the chemical fuel ingested as food. The respiratory system, via the mucociliary mechanism, significantly contributes to wearing away of the gastric mucosa surface making it susceptible to dysfunction. Despite of the array of pathologies that have been reported to accompany exposure to cement dust particularly occupational exposure, there has been paucity of information on the physiological alterations that follow cement dust exposure. Hence, this work was designed to investigate the cytoarchitectural and biochemical changes in gastrointestinal tissues (salivary, gastric and hepatic) and secretory functions following exposure to cement.

MATERIALS AND METHODS

Cement material and experimental exposure: Full, intact and freshly supplied bag of Nigeria Portland cement was acquired from an accredited company depot within the neighbourhood of the University of Ibadan, Ibadan, Nigeria. The exposure was done daily by the introducing about 100g of cement particles into the sub-chamber every other day. Left over cement particles from the previous day's exposure were completely and consistently ridded off the chamber. The animals were experimentally exposed to cement dust according to the standardized method of Owonikoko *et al*., (2021) using a plexi-glass house chamber.

Experimental animals, groupings and ethical perspectives: A total of seventy-two (72) male Wistar rats weighing between 160-180g acquired from the central animal breeding house of the college of medicine, Faculty of Basic Medical Science, University of Ibadan, were used in this study. They were randomized into three (3) phases of 24 animals each. Each phase comprised of three groups of eight animals each according to the level of exposure to cement dust following two weeks acclimatization post-acquisition. Group 1 animals (control animals) were kept and raised in environment completely free of cement dust. Group 2 animals were exposed to cement dust for 14 days (14-day exposure) while group 3 were exposed to cement dust for 28 days (28-days exposure). All exposure was carried out 5 hours between 8 am and 1pm daily. All procedures used in this study are in line with the Animal Care Regulations and Standards as approved by the Institute for Laboratory Animal Research (ILAR, 1996) while the experimental protocols were approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan, Ibadan and allotted the approval number UI-ACUREC/18/0129. The overall experimental set up is shown in Figure 1.

Salivary secretion: Following the respective days of cement dust exposure, salivary secretion study was conducted in compliance with the method of Romero *et al*., 2012. Under 0.1ml/kg Xylazine and 1ml/kg ketamine anesthesia cocktail, 24-hour fasted animals were subjected to salivary stimulation using pilocarpine administered intraperitoneally (0.6mg/kg body weight). Standard cotton wool was used to carefully collect saliva from the buccal cavity. The animals were restrained on a dissecting board and the cotton wool were inserted sublingually for 40 mins. The total saliva collected within the time frame was estimated by subtracting the final weight of the cotton wool from the initial weight.

Gastric acid secretion: Experimental animals exposed to cement dust were anesthetized with the combination of 0.1ml/kg Xylazine and 1ml/kg ketamine and subsequently prepared for continuous perfusion for gastric acid secretion. Ghosh and Schild's (1958) method of continuous perfusion as modified by Amure and Ginsburg (1964) was used to assess gastric acid secretion in cement dust-exposed rats. Briefly, an opening was made on the trachea which was located by an incision on the neck of the rats to ensure undisturbed respiration. The latter was followed by an abdominal incision along the *linea alba*. This was done to localize the viscera (stomach) and another quasi-transection made at the pyloro-duodenal junction and a cannulation to allow the collection of the gastric effluent. Another cannula was inserted orally to allow orogastric perfusion with warm normal saline stabilized at 37^{o}C at a rate of 1ml/min (pH 7.0) with the aid of Langerdoff perfusion pump. We collected gastric acid intermittently (10 min intervals) through the pyloro-duodenal junction. Gastric perfusate (10mL) was titrated against 0.01M sodium hydroxide (NaOH) solution with phenolphthalein used as an indicator, to determine gastric acidity and titrable acidity expressed in mEq/L/10 mins. The steady effluent collection was followed by histamine administration (1.6mg/kg) through femoral vein canulation and reported as stimulated acid secretion.

Biliary Secretion: Biliary secretion was investigated in experimental rats exposed to cement dust according to the method described by Madrid *et al*., 1983. Briefly, a sterile cannula (22G) with internal and external diameters of 2mm and 3mm respectively. The cannula has a narrowing acute angle bifurcation produced by a stiff ring at 2cm from the anterior end. The latter moves along the inner cylindrical passage made from the polyvinyl tube previously introduced. The labile internal cannula may assume any of the two possible positions; cocked or open. While cocked,

the needle's inner lining pieces through the bile duct for cannulation such that at the exteriorization of the inner needle (at the open position), the bile flows into the cannula by capillary action.

Oxidative Stress Assessment

Tissue total protein: Gastric and hepatic protein concentrations were estimated according to the method of Lowry *et al*., 1951. 1 ml of the sample homogenate was to 3ml Biuret reagent and incubated (at room temperature) for 30 mins. Using bovine serum albumin as a standard, absorbance was read at 540nm with the aid of a microplate reader.

Lipid peroxidation: Malondialdehyde production is a bioindicator of biochemical plasmalemma oxidative damage from reactive oxygen species (ROS) indicating the level of lipid peroxidation. It is a well-harnessed methodology usually employed for the assessment of xenobiotics. Cytotoxic agents are known to primarily disrupt the cell membrane integrity before subsequent cytosolic perturbations. When they attack the polyunsaturated fatty acid content of the cell membrane, the resultant effect yields malondialdehyde (MDA)- the most studied product of peroxidation and then used to evaluate the extent of oxidative stress. This study assessed the extent of lipid peroxidation/MDA level in the gastric homogenates by measuring the thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990). At 100^{o}C and in an acidic medium, TBARS reacts with MDA precipitating a red/pink colour change which is extracted by butanol. The absorbance of the clear supernatant was read at 532nm using a microplate reader (Epoch BioTek Instruments, Inc)

Superoxide Dismutase (SOD) Activity: Oxygen is considered a dangerous chemical species to most cellular processes in the body as it is also able to generate superoxide radicals by binding and ripping off the electron from them. SOD is an endogenous antioxidant enzyme that scavenges for the superoxide radicals. Total SOD activity was assessed in the homogenate samples by the indirect method described by Oyanagui, 1984. This principle is based on the xanthine-xanthine oxidase complex which causes the reduction of tetrazolium salts. Xanthine oxidase enzyme catalyzes the production of superoxide anion. The latter reacts with hydroxylamine producing nitroso ion which combines with sulfanilic acid and n-(1- naphthyl) ethylenediamine. The reaction precipitates a characteristic colour which was determined by spectrophotometry at the wavelength of 560nm. A unit of SOD is taken as the amount of the SOD enzymes required to inhibit tetrazolium reduction

Catalase: Catalase, an essential antioxidant enzyme responsible for the degradation of hydrogen peroxide (H_2O_2), is known to be most abundant in the gastric, liver and kidney tissues. Its level was assayed according to the method of Goth 1992. Perhydrol in 50 mM TRIS/HCl buffer was added to the homogenized and buffered gastric samples and the pH adjusted to 7.4. The reaction was primed by the addition of fresh hydrogen peroxide (H_2O_2; BDH). Since water and Oxygen do not absorb at 240nm as H_2O_2 does, the rate of H_2O_2 decomposition was spectrophotometrically read at that wavelength using UV Vis Spectrophotometer **(**Model: UV1700).

Nitric Oxide (NO): NO plays significant roles in wide range of physiological and pathological processes in the body. The level of NO has been shown to alter its levels in various pathophysiological processes determining homeostasis such as inflammation and apoptosis. It is particularly known to stimulates cytotoxicity in heavy metal intoxication (Wu *et al*., 2019). Greiss reaction (Sigma, Poole, UK) and the level of nitric oxide were assessed based on the level of its metabolites (Nitrite and nitrate) in the tissue supernatant according to Karampour *et al*., 2019. Gastric and hepatic homogenates samples diluted in phosphate-buffered saline- An isotonic buffer comprising of NaCl; KCl, anhydrous Sodium phosphate dibasic and Potassium phosphate anhydrous.

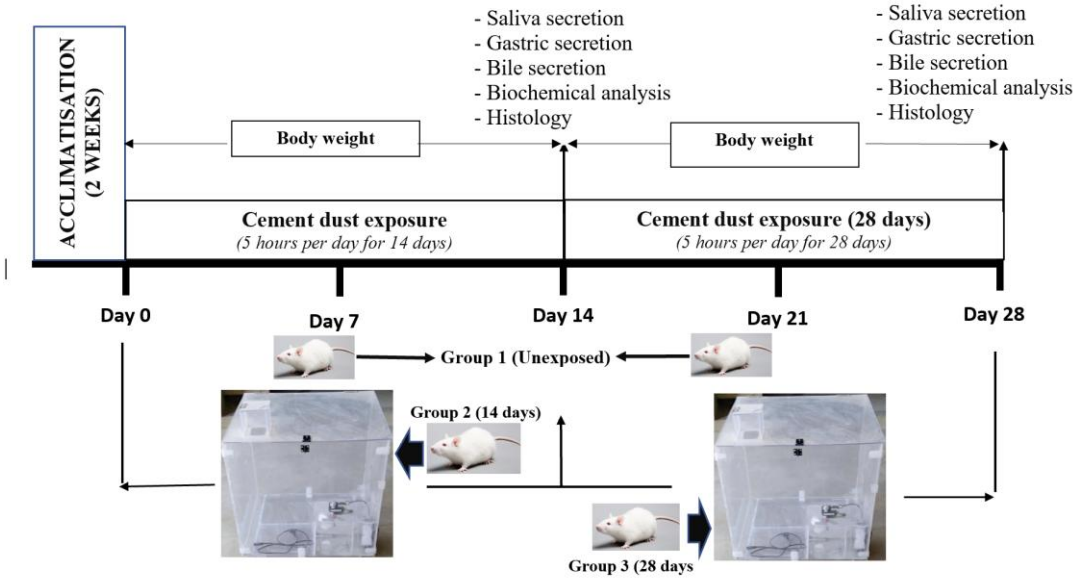

Figure 1:
Experimental design showing the treatment timeline. CDE = Cement dust exposure

Nitrate content was converted into nitrite with the aid of nitrate reductase- a process followed by the addition of Greiss reaction 1 [sulfanilamide (1%)] in conc. phosphoric acid (5%) (BDH) which ultimately yields azo product with a color change in the presence of 0.1% N-(1-Naphthyl) ethylenediamine (Greiss reaction 2) in a photo-proof container. The endpoint absorbance was read at 520nm and values were recorded.

Sulfhydryl: Total gastric and hepatic homogenate thiol content was evaluated according to the method by Ellman *et al.*, 1959. Aliphatic thiol compounds are known to react with Bis(p-nitrophenyl) disulfide (I) at pH 8.0 to produce one mole of p-nitrothiophenol anion per mole thiol. 95% 5-5-dithiobis-(2-nitrobenzoic acid) (DTNB; Central Drug House (P) LTD batch no: 040718) also known as the Ellman's reagent was prepared by dissolving it in 1ml ethanol (95%) in the presence of N_2-deficient Tris buffer and de-ionized water. The reaction yielded 5-mercapto-2-nitrobenzoate (MNB) and was aliquoted for reading. The absorbance of the resulting anion was read using the microplate at the wavelength of 412nm.

Na^+-K^+ATPase activities: Gastric Na^+-K^+-ATPase activities were assessed in the experimental animals exposed to cement dust according to the blend of the methods of Rezin *et al*., 2014. A 200µL cocktail of NaCl (80mM), $MgCl_2$(5.0mM), Tris-HCl (40mM) and KCl (20mM) stabilized at a pH of 7.4 made up the reaction mixture which was first incubated at 37°C for 10min and then the reaction initiated with the introduction of 3.0mM ATP. It was later re-incubated for the period of 20mins. Absorbance was read at 725nM.

Histopathology
Excised gastric, hepatic and salivary tissues having been carefully examined for any gross pathology were fixed in adequate solution of formol saline, and immediately processed for the assessment of possible cytoarchitectural changes. They were thereafter embedded in paraffin wax; sectioned at 5µm and stained with Haematoxylin and Eosin before viewing under the light microscope for any pathological alterations according to Haber and Lopez, 1999.

Statistical analysis: Statistical analysis was performed using GraphPad prism 8.0® software for windows with data presented as Mean ± SEM for n=5 per group. One-way ANOVA was used for group comparison with Dunnette posthoc test; different levels of significance were stated in each case. However, $p<0.05$ was considered significant.

RESULTS

Salivary secretion in control and cement dust-exposed rats: Salivary secretion was assessed in the experimental animals exposed to cement dust with consideration of both basal and stimulated phases of secretion. Results that there was a significant increase in basal salivary secretion in the 14-day cement dust-exposed rats when compared with the control. The pilocarpine-stimulated secretion shows significant increases in both 14- and 28-day cement dust exposed rats when compared with the control (Fig 2).

Gastric secretion in control and cement dust-exposed rats: Basal and stimulated gastric acid secretion was investigated in the rats exposed to cement dust. The result is as presented in figure 3 which shows significant decrease in basal gastric acid secretion in the 14-days and 28-days exposed when compared with the control. However, there was an increase in histamine-stimulated gastric acid secretion in the 28-days exposed group than the control (Fig 3). This was the basis of our investigation of the Na-K-ATPase discussed below.

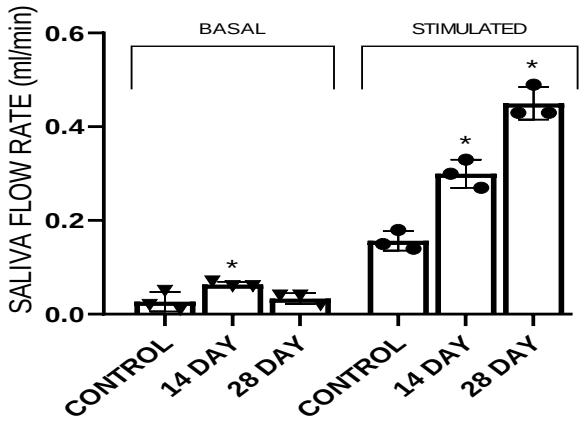

Figure 2:
Effect of exposure to cement dust on salivary secretion.
** Significant when compared with the control

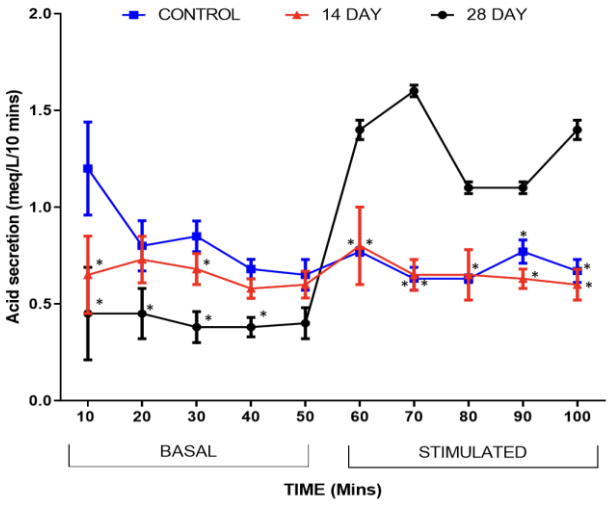

Figure 3:
Effect of exposure to cement dust on gastric acid secretion.
**Mean values with asterisks are significant (p <0.05) when compared with control*

Biliary secretion in control and cement dust-exposed rats: Biliary secretion was also studied in the basal and stimulated phases. There was a steady state of biliary secretion in the control animals while there was an increase in basal biliary secretion in the 14-days exposed when compared with the control. There was decrease in basal biliary secretion in the 28-days exposed group when compared with control. However, pilocarpine-stimulated

biliary secretion was inhibited in the 14-day and 28-days exposed groups when compared with the control (Fig 4). This decrease in the 28-day bile secretion in the exposed groups shows that there is a major effect of the exposure to cement dust on the liver. In an earlier investigation from our laboratory, we found decrease in liver function tests, increase oxidant and decrease antioxidant levels of experimental animals exposed to cement dust. This study hereby confirms that cement dust does not only affects the gastrointestinal tract but also its accessory/associated organs

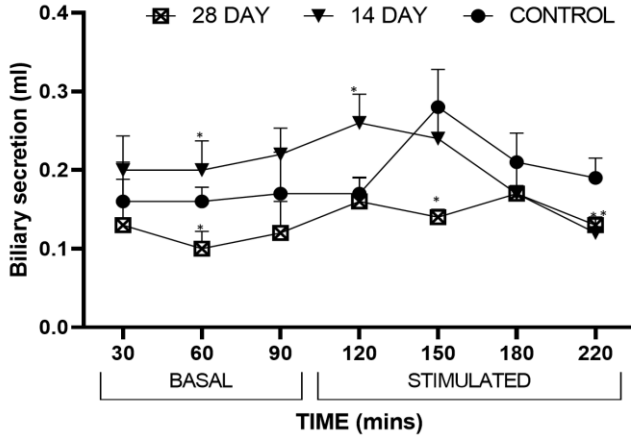

Figure 4:
Effect of experimental cement dust exposure on Basal and Stimulated Biliary flow
** Mean values with asterisks are significant (p <0.05) when compared with control*

Table 1:
Effect of cement dust exposure on gastric oxidative stress markers

Biochemical Assays	Control	14-Days	28-Days	P-Value
MDA {nmol/mg (10^6)}	6.85 ±0.51	16.97 ±1.18*	36.07 ±0.30*	<0.0001
SOD (nm/mg protein)	18.50 ±0.06	16.23 ±0.66*	15.53 ±0.03*	0.0039
CAT (nm/mg protein)	506.1 ±89.24	686.5 ±114.50	615.3 ±315.80	0.7053
SULF (µmol/g wet weight)	0.39 ±0.02	0.27 ±0.01*	0.24 ±0.04*	0.0190
CARBONYL {nmol/g of tissue ($X10^{-6}$)}	1.308 ±0.06	1.376 ±0.08	1.918 ±0.21*	0.0155
Na^+-K^+-ATPase PUMP {nmol/g of tissue ($X10^{-6}$)}	2.423 ±0.00	2.587 ±0.14	2.177 ±0.12	0.0870

*Significant when compared with control P<0.05
Keys: MDA: Malondialdehyde; CAT: Catalase; SOD: Superoxide dismutase; Sulf: Sulfhydryl

Effect of cement dust exposure on gastric and hepatic biochemical indices: Effect of cement dust exposure on gastric and hepatic biochemical indices are represented in Table 1 and table 2 respectively. Values of gastric and liver malondialdehyde show that the 14-days and 28-days cement dust exposed group has significantly higher MDA levels when compared with the control. The level of gastric and hepatic SOD follows a contrasting trend with a decrease in the level of SOD in the 14-day and 28-day cement dust-exposure groups compared with the control (Table 1). There was no statistically significant difference in gastric catalase level in the cement dust exposed (14-days and 28-days) groups (p=0.7053) while hepatic catalase levels were significantly decreased in the exposed groups when compared with the control (p=0.0011). This study equally assesses the effect of cement dust exposure on gastric carbonyl content. result shows no significant difference between the 14-days exposed and the control. however, there was significant increase in the 28-days exposed when compared with the control (Table 1).

Gastric Na^+-K^+ATPase pump activities were also assessed in gastric tissues (Table 1). The result show that there is no significant difference between the exposed groups and the control (p =0.0870). Gastric and hepatic sulfhydryl level in the exposed groups significantly decreased when compared with their respective controls (p-values=0.0190 and 0.0251 respectively).

Table 2:
Effect of cement dust exposure on hepatic oxidative stress markers

Biochemical Assays	Control	14-Days	28-Days	P-Value
MDA {nmol/mg (10^6)}	3.50 ±0.94	9.21 ±1.23*	10.43 ±0.45*	0.0182
SOD (nm/mg protein)	17.90 ±0.0.42	10.22 ±0.80*	11.00 ±1.00*	0.0201
CAT (nm/mg protein)	17012 ±402.16	1493 ±651.72*	12010 ±152.00*	0.0011
SULF (µmol/g wet weight)	1.56 ±0.165	0.45 ±0.17*	0.60 ±0.02*	0.0251

*Significant when compared with control P<0.05
Keys: MDA: Malondialdehyde; CAT: Catalase; SOD: Superoxide dismutase; Sulf: Sulfhydryl

Nitric oxide levels in the gastric and hepatic tissues were assessed as shown in Fig. 5. The result show in the gastric tissue that the 14-days and 28-days exposed groups were significantly higher than the control. the same trend of result was observed for the hepatic tissue (P-Values=<0.0001; 0.0238 respectively).

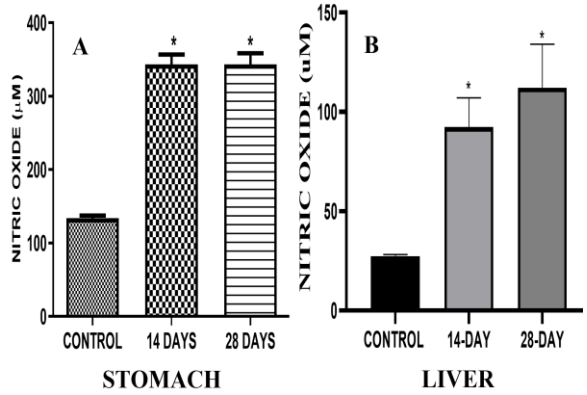

Figure 5
Effect of exposure to cement dust on (A) gastric nitrite and (B) liver nitrite content.
** Significant when compared with the control*

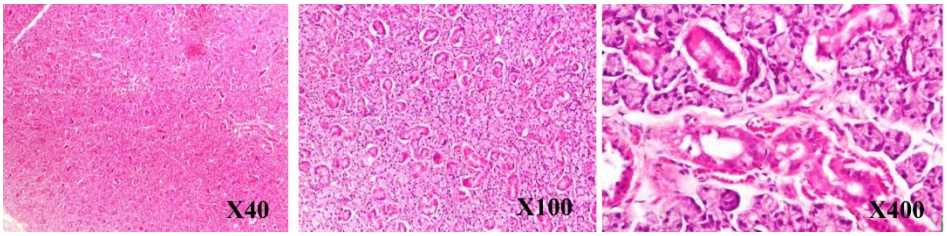

Plate 1A:
Representative photomicrographs of the salivary duct of control animals showing normal serous acini and interlobular ducts. No significant lesion seen.

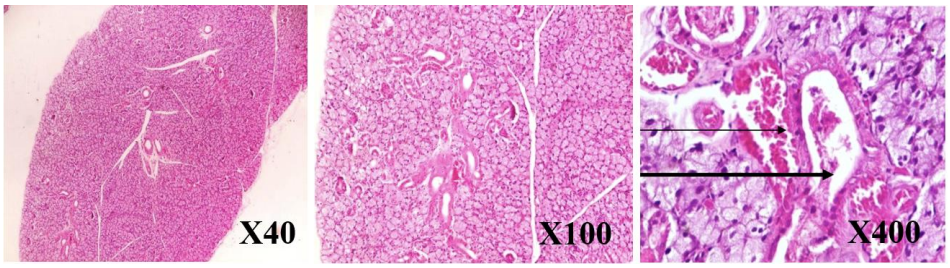

Plate 1B:
Representative photomicrographs of salivary gland of experimental animals exposed to cement dust for 28 days (28-day exposed group) with evidence of dilated interlobular ducts (black arrow) with stratified epithelial lining, congestion of blood vessels (thin arrow) and slightly thick septa.

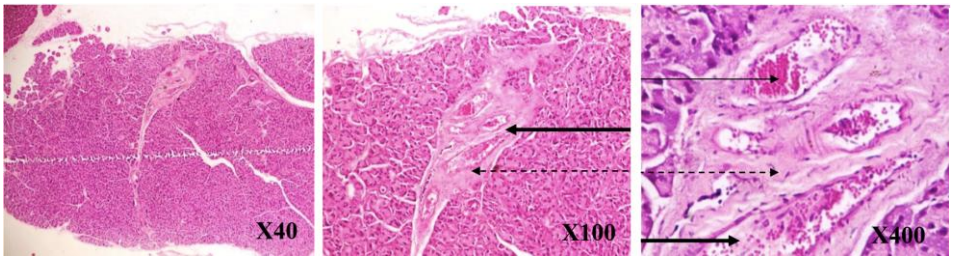

Plate 1C:
Representation photomicrographs of the salivary duct of experimental animals exposed to cement dust for 14 days (14-days exposed) showing dilated interlobular ducts (black arrow), congestion of blood vessels (thin arrow) and a mild increase in the connective tissue (dashed arrow) of the interlobular ducts

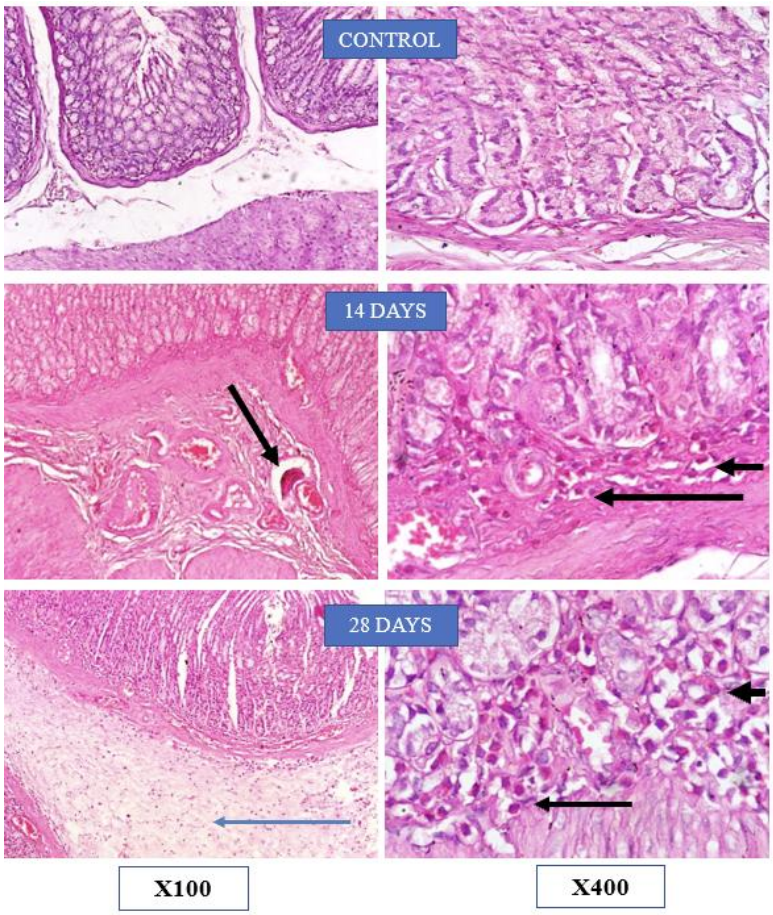

Plate 2A:
Effect of experimental exposure to cement dust on gastric architectural changes. 14-day group showed vascular congestion and inflammatory cellular infiltration (black arrow); the 28-day group showed edema (blue arrow) and inflammatory cellular infiltration. The control group showed normal architecture.

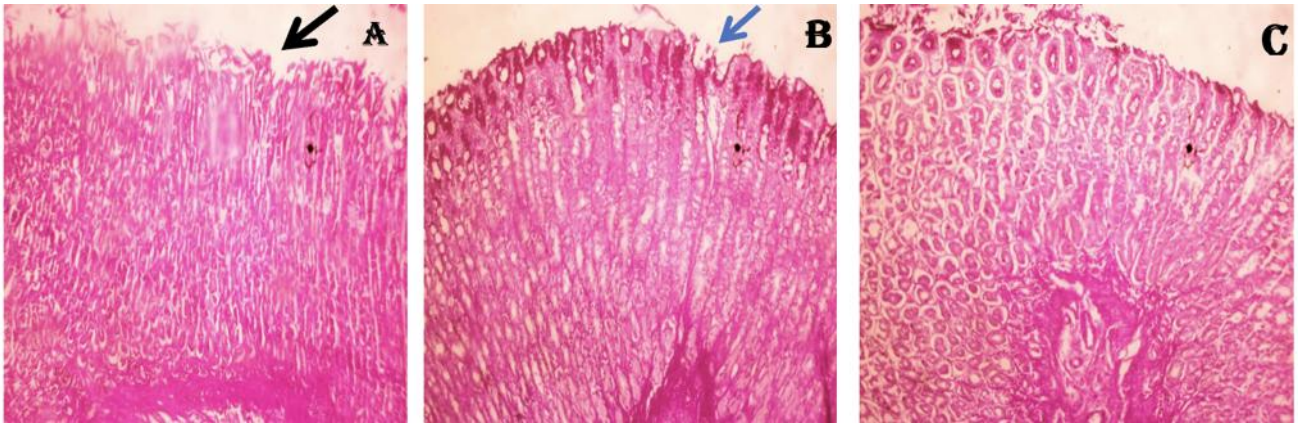

Plate 2b:
Representative Periodic Acid Schiff staining of the gastric tissue following exposure to cement dust. A: 14-days exposed group; B: 28-days exposed; C: Control group. The microscopic examination of the histopathological slide shows significant excoriation of the mucus neck cell layer in the 14-day group while 28-day group shows mild excoriation as compared with control.

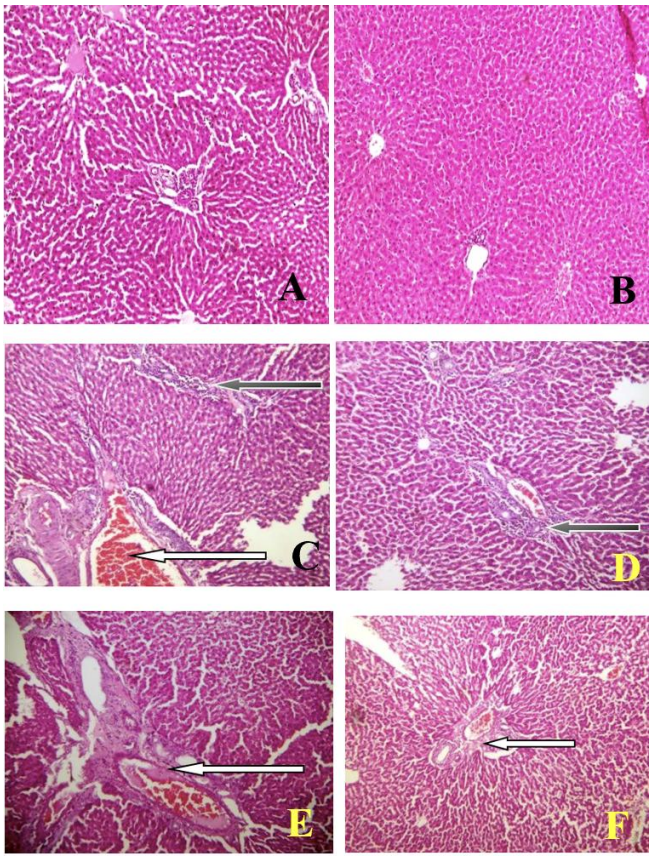

Plate 3
Photomicrograph of Liver Section of Control rats (A and B, with no lession Seen) and rats exposed to cement dust for 28-days (C,D,E,F) stained With H&E Showing Moderate Architecture. Exposed rats show moderate architecture as seen in lower magnification, the portal vein show very mild congestion (white arrows) and mild portal infiltration by inflammatory cells (black arrow) , the sinusoids show mild infiltration of inflammatory cells(slender arrow). The hepatocytes show normal morphology (blue arrow).

Histology
The cytoarchitectural changes following exposure to cement dust were also investigated histologically photomicrographs the plates above show the histopathological results of salivary, gastric and hepatic tissues as shown in plates 1, 2 and 3. the photomicrographs of the salivary, gastric and hepatic tissues show an array of pathologies including vascular congestion, infiltration of inflammatory cells in the 14 days exposed groups while oedema (blue arrow) and inflammatory infiltration were seen in the 28 days exposed group.

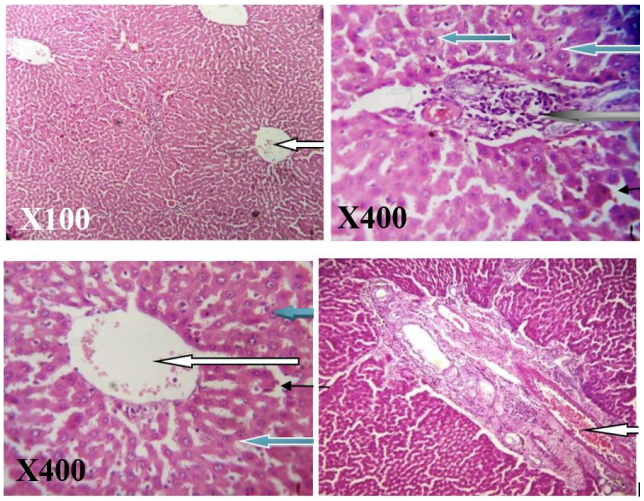

Plate 4:
Photomicrograph of liver section of 14-day exposed experimental animals stained with H&E showing normal architecture as seen in lower magnification (X100), the central venule appear normal (white arrows) while the portal vein show mild infiltration of inflammatory cells (black arrows). The sinusoids show no infiltration of inflammatory cells (slender arrow). The hepatocytes show normal morphology (blue arrow).

DISCUSSION

Gastrointestinal secretions are central to gastrointestinal functions and are sensitive to exogenous toxicants. There is an increase in 14-day salivary secretion while no difference was seen in 28-day group in relation to control. However, at pilocarpine-induced secretion, there was a significant increase in stimulation of saliva in both 14- and 28-day exposure groups. This shows that exposure to cement dust potentiates salivary secretion by stimulating aquaporin. The potentiated stimulation of salivary secretion following pilocarpine administration shows that cement dust might cause increased stimulation via pilocarpine administration- a direct-acting cholinergic agonist. Gastric acid secretion was observed in this study follows a considerable trend. The

14- and 28-day exposure groups featured decrease basal gastric acid secretion. Histamine-induced secretion shows an increase in secretion among the exposed groups in comparison to control. This shows that cement dust exposure inhibits parietal cell activity probably due to decrease cell mass or inhibition of proton pump activity.

The investigated biliary secretion shows an array of changes in the exposed compared with the control. For example, 14-days cement dust exposed group shows significant increase while the 28-day shows significant decrease in basal biliary secretion. After secretagogue administration, there were pan-exposure groups decrease in biliary secretion when compared with the control. This suggests cement dust inhibits biliary secretion in a mechanism not dependent on cholinergic stimulation.

MDA is the lipid adduct indicating lipid peroxidation (Liang and Yan, 2020) produced as a tissue response to xenobiotics. The higher level of MDA in this study indicates that exposure to cement dust stimulates tissue peroxidation. Cement dust is a repository of many heavy metals. When internalized either via inhalation, gastrointestinal or dermal exposure, the heavy metals are sequestered and bioaccumulated (Owonikoko *et al*., 2021; Ugbaja *et al*., 2020). The cement particles and heavy metal contents effectuate a cascade of biochemical reactions that culminate free radical generation which block electron transport chain in the mitochondrion and electrons to leak out. The latter attack polyunsaturated membrane lipids and cause the release of hydrogen peroxide which ultimately produces MDA. Hence MDA tissue concentration measures peroxidation. The increase in MDA level in gastric and hepatic tissues in this study suggests that cement dust induces lipid peroxidation in the organs.

Catalase is part of the general enzyme antioxidant store for the body system. It mops up free radicals sooner or later than they are made in the body (Melin *et al*., 2000; Szymonik-Lesiuk, 2003). This study assesses the level of catalase in gastric tissues following exposure to cement dust and the result shows that catalase level was not different while that of the liver significantly decreased in the exposed groups. Results from this work is at par with that already reported by Olaleye *et al*., (2007), particularly with respect to the liver, who showed that exposure to Pb; a toxic element known to be a constituents of cement dust, significantly decreased catalase level in exposed groups.

Superoxide dismutase is an enzyme antioxidant known to mop up free radicals. There was a decreased level of gastric and hepatic in the exposed (Table 1). The same trend had been earlier reported in other investigation from laboratory in another set of studies which focused on hepatic and renal dysfunctions following cement dust exposure viz liver and kidney (Owonikoko *et al*., 2022; in press). The decreased level of the antioxidant in the gastric and hepatic tissues shows that experimental cement dust exposure causes depletion of antioxidant status. Sheta and Zahran, (2015) demonstrated that cement dust components (particularly Fe and Sulphur) significantly decreased SOD levels in the cement dust exposed groups.

Sulfhydryl groups are also known as Thiol group; they are endogenous antioxidants ubiquitous in various tissues of the body. They are known to subserve the function of scavenging free radicals in the body (Prakash *et al*., 2009). The gastric and hepatic sulfhydryl group contents significant decreased. This observation is indicative that cement dust exposure depletes sulfhydryl group antioxidant level.

Carbonyl is a measurement of membrane protein oxidation level. The level of carbonyl group in the gastric tissue was also investigated and the result shows carbonyl level significantly increased particularly 28-day group. This suggests that cement dust culminates in overall oxidation of protein. Oxidative stress, particularly at the early-stage secondary to the exposure to xenobiotics, usually predisposes functional cellular proteins to modifications with functional implications (Breusing and Grune, 2010). This increase in carbonyl content affirms cement dust contain toxic contents that significantly affects the plasma membrane causing oxidative stress via increase in MDA and carbonyl content particularly considering the decrease in sulfhydryl group.

Sodium potassium adenosine triphosphatase (Na^+-K^+-ATPase) activity show no statistical difference between the cement dust exposed groups and control. There is stark dearth of scientific report on intragastric Na^+-K^+-ATPase activity in cement dust intoxication. However, Ray, (2013) explained that the normal gastric environment lacks Na^+-K^+-ATPase at normal physiological milieu but at altered hydrogen ion concentration state, the proton pump begins to assume the function of Na^+-K^+-ATPase or Ca^{2+}-K^+-ATPase depending on the cellular need albeit ouabain, a potent Na^+-K^+-ATPase inhibitor has been long shown to suppress gastric acid secretion (Davenport, 1962). The insignificant difference in the activity of Na^+-K^+-ATPase activity between the cement dust exposed groups and control when compared with decreased acid secretion observed in this study shows that cement dust exposure suppresses acid secretion most probably due to the suppression of parietal cell activity rather than selective destruction of parietal cells since there is no significant difference in the level of Na^+-K^+-ATPase activity.

Nitric oxide (NO) is a colourless, soluble, odourless and bifunctional gasotransmitter that play significant role in physiological, pathophysiological, and inflammatory processes. The role of NO in the biohandling of heavy metal stress has been extensively studied in the plant (Terrón-Camero *et al.*, 2019; Wei *et al*., 2020) but the dearth of such information in animals persists. In this study, we observed increase gastric and hepatic NO in cement dust exposed groups. Exposure to cement dust stimulated the cytokine-inducible Nitric Oxide Synthase (iNOS) via the essential cofactor tetrahydrobiopterin (BH_4). However, rather than consider the consequential increase NO bioavailabilty in the exposed groups as a biomolecular result of the exposure, we view the condition as a pathophysiological transition to a more deleterious injury. Excess NO secondary to iNOS upregulation has been known to exhibit cytotoxicity via activation of the inflammatory cell (neutrophil), potentiates the synthesis of nitrosamine; a carcinogen (Ribbon *et al.*, 1995; Ohshima and Bartsch, 1994) as well as form extremely toxic peroxynitrile radical by interacting with superoxides and culminating mutagenesis and DNA damage (Singer *et al*., 1996; Jourd'heuil *et al*., 1997).

Histopathology is usually considered a very important toxicological procedure used to analyse the cytoarchitectural changes difficult to be seen in biochemical assessments. The array of histopathological alterations seen

in the gastric tissues (Plate 2a) shows that exposure to cement-induced edema, vascular congestion and inflammatory response through infiltration of neutrophils. The gastric tissue appears excoriated following depletion of mucus content (Plate 2b) while the salivary tissues also show an array of related pathologies involving vascular congestion and neutrophil infiltration.

In conclusion, results of this study show that exposure to cement dust does not only affect the respiratory system as has been widely reported. It may also significantly predispose the gastrointestinal system including liver to an array of deleterious effects which include but not limited to induction of topical tissue pathologies. These pathologies are associated with protein oxidation and antioxidant depletion-dependent nitrosative is oxidative stress induction. This study also confirms that cement dust exposure alters gastrointestinal secretions via the cholinergic receptor stimulation pathway.

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