<u>Original Research Article</u>

EXPOSURE-TIME EFFECT OF CEMENT DUST ON LIVER ORGAN IN RAT

Abstract

The aim of this study was to compare the effect of duration of cement dust on the liver organ of albino rats. In this study, a glass house animal exposure chamber was fabricated using a plexi-glass and two blowing fans of adjustable revolution. A total of twenty five rats were used and categorized into five groups composed of 5 rats each: A group (without cement exposure); B group (exposed to 200g of cement dust for 1 hour daily for 15days); C group (exposed to 200g of cement dust for 1 hour daily for 30 days); D group (exposed to 200g of cement dust for 1 hour daily for 45 days) and E group (exposed to 200g of cement dust for 1 hour daily for 60 days). After the exposure periods, the animals were sacrificed, blood collected in plain bottle and analyzed for some liver function. The result showed that there were significant alterations ($p < 0.05$) in the levels of some liver function markers such as alkaline phosphatase (ALP), Total protein (TP), Albumin and Conjugated bilirubin (CB), and the alteration patterns were irregular. On the other hand, there were no changes ($p>0.05$) in the levels of Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Total bilirubin (TB) among the groups. This study has therefore shown that exposure to cement may cause some decrease of liver injury in rats regardless of the duration of the exposure.

Keywords: *Cement, exposure, liver, rats*

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Introduction

Even in the 21st century, millions of people are working daily in a dusty environment. They are exposed to different types of health hazards such as fume, gases and dust, which are risk factors in developing occupational disease. Cement industry is involved in the development of structure of this advanced and modern world but generates dust during its production.

Cement is a powdery composition (limestone, laterites, clay and gypsum) used in making and holding blocks or bricks in-place during construction [1]. Cement product which has wide application in the construction industry is a homogenous mixture of hazardous heavy metals such as Cobalt (Co), Iron (Fe), lead (Pb), cadmium (Cd), Chromium (Cr), Nickel (Ni), Manganese (Mn), and arsenic (As) at different relative proportions which have been considered to be toxic to the body system.

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. [2]. Many of these toxic compounds have been shown to cause damages at both cellular and organ levels in the lungs, gut in addition to their roles in genetic disorders and cancers [3-5]. 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 [6].

Pollutants such as particulate matters, Sulphur dioxide, nitrogen dioxide, volatile compounds, long lived dioxins and heavy metals all arise from the manufacturing and burning process of cement. Cement industries constitute a notable source of environmental toxicants encountered during the manufacturing, distribution, and utilization of cement product [7-8]. Occupational and environmental exposure to cement dust has been known to precede a number of systemic injuries with particular reference to the respiratory, gastrointestinal, and integumentary systems characterized by fibrosis, emphysema, cough, cancer, inflammation, and liver diseases among workers and host community residents of cement factories [9].

A single short-term exposure to cement dust is not likely to cause serious harm [9]. However, exposure of sufficient duration to cement dust can cause serious, irreversible tissue destruction in form of chemical burns, including third degree burns. It has been found that there was significant increase in ill-health among children in schools near cement kiln compared to those out of the area. Acute effects such as eye, nose, upper respiratory tract irritation, cough, expectoration, shortness of breath and wheezing have been recorded in humans due to exposure to cement dust [9].

Following the risk associated with cement exposure on human health and living organisms in the environment, this study was focus to assess the durational effect of cement dust on liver organ in rats.

Materials and Methods

Study area

The study was carried out in the Animal House of the Faculty of Basic Medical Sciences in PAMO University of medical science, Port Harcourt, Nigeria.

Ethical Clearance

Ethical clearance was obtained from The Ethics Committee of Pamo University of Medical Sciences.

Study Design

The total number of rats for the study was 25. These 25 rats were divided into five groups designated as A, B, C, D and E consisting of 5 rats per group. See the table 1 below for the description of each group.

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Table 1: Rat Grouping

Groups	Number of rats	Description
A	5	Without exposure to cement dust (Control group)
B	5	Exposed to 200g of cement dust 1 hour daily for 15 days
C	5	Exposed to 200g of cement dust 1 hour daily for 30 days
D	5	Exposed to 200g of cement dust 1 hour daily for 45 days
E	5	Exposed to 200g of cement dust 1 hour daily for 60 days

The animals were housed in a glass cage constructed with two fans set at 3000rpm for the circulation of the cement dust in the cage system. This circulation was needed for adequate exposure of the rats to cement dust.

Sample Collection Method

As described by Okolonkwo and his colleagues in 2022, the blood was collected via cardiac puncture after the rats were anaesthetized using chlorofoam [10,11]. Blood samples collected were dispensed into plain bottle and separated to get serum needed for liver function test. After blood collection, the liver organs were harvested, preserved in 10% formal saline for histological examination.

Laboratory Analysis

Aspartate aminotransferase (AST/SGOT) method: by Reitman and Frankel as described by Okolonkwo [10,11]

Procedure: In estimating the activity of this enzyme, 0.5ml of buffered-L- aspartate and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

Calculation: Obtain the activity of the enzyme AST in the serum from the table of values previously plotted against activities. Haemolysis interferes with the assay.

Alanine aminotransferase (SGPT) by Reitman and Frankel as described by Okolonkwo [10,11]

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Procedure: In estimating the activity of this enzyme, 0.5ml of buffered-L- alanine and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

Alkaline phosphatase (ALP) method as described by Okolonkwo [10,11]

Procedure: Fresh double distilled water (ddH_2O) was aspirated and used to perform a new Gain calibration in flow cell mode. This zero the equipment from previous sample run. ALP was selected in the Run Test Screen and a water blank test run was carried out, after which 0.02ml of sample and 1.0 ml of reagent (Diethanolamine buffered p-nitrophenylphosphate) was dispensed into a test tube and mixed for 2 minutes. The mixture was then aspirated into the Rx Monza. After about 2 minutes the result of the test sample was then printed out from a printer connected to the machine.

The advantage of this machine procedure is that up to 200 samples can be processed and results produced within one hour in S.I. unit = IU/L.

Manual calculation: To calculate the ALP activity, using the manual method, the following formula was utilized: IU/L = 2760 x ΔA 405 nm/min.

Total protein: Biuret colorimetric method as described by Okolonkwo [10,11]

Procedure: 1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 25μL each of Standard (7g/dL) and Sample added to the 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at room temperature, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A(Test) ÷ A(Standard)] X 7(Standard concentration)

= Result in g/dL

Albumin: Bromocresol green method as described by Okolonkwo [10,11]

Procedure: 3mls of Bromocresol green reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 10μL each of Water, Standard (7g/dL) and Sample added to the 'Blank', 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at 20 – 25^0C, after which, the absorbance (A) of the 'Test' and

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‘Standard’ were read against the ‘Blank’. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A (Test) ÷ A (Standard)] X 7 (Standard concentration)

= Result in g/dL

Bilirubin method as described by Okolonkwo [10,11]

Procedure:

Total Bilirubin: 1.5mls of reagent-1 (Sulphanilic acid, HCl and Dimethylsulphoxide) was added to two glass-tubes labeled ‘Blank’ and ‘Test’ respectively. $50\mu L$ of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently $100\mu L$ of sample was added to the ‘Blank’ and ‘Test’ tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 19.1 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (μL/L).

Direct Bilirubin: 1.5mls of reagent-2 (Sulphanilic acid and HCl) was added to two glass-tubes labeled ‘Blank’ and ‘Test’ respectively. $50\mu L$ of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently $100\mu L$ of sample was added to the ‘Blank’ and ‘Test’ tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 14 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (μL/L).

Histopathological Procedure of the Liver

Tissues (liver) was fixed in 10% formal saline and were dehydrated in four (4) concentrations of Isopropyl alcohol, i.e. 70%, 80%, 90%, 100% for 1hr each and then cleared in xylene before embedded in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues was stained using Hematoxylin and Eosin (H&E) to demonstrate histomorphological effects. The Tissues sectioned were examined and interpreted using compound Light microscope with photomicrographic facilities and then photomicrographed by a histopathologist.

Hematoxylin and Eosin Staining Method and Principle

The histomorphology was determined using Hematoxylin and Eosin Regressive staining method and principle (Li *et al*., 2018). [12]

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Statistical Analysis

Descriptive and inferential statistics were performed with the data gathered from the study using SPSS 23.0. The descriptive statistics were represented as Mean±SD while inferentially ANOVA and Post hoc analysis were performed such that p-value<0.05 was considered significant.

Results

Table 2 and 3 represent the comparison of liver function parameters (AST, ALT, ALP, TP. Albumin, TB and CB) levels among the studied groups (A, B, C, D and E). The result showed that there were significant differences (p-value<0.05) in the levels of ALP, TP, Albumin and CB while there was no significant difference (p-value>0.05) in the level of AST, ALT and CB

Table 2: Comparison of liver function markers levels amongst groups.

Groups	AST iµ/l	ALT iµ/l	ALP iµ/l	Total Protein iµ/l	Albumin µmol/l	TB µmol/l	C.B µmol/l
A (Control)	18.0±6.3	7.0±3.8	18.0±6.3	71.5±7.4	55.5±5.5	6.3±0.9	1.9±1.4
B	10.3±9.0	7.0±3.8	96.8±16.1	74.0±5.4	44.25±1.7	47.5±41.8	8.0±1.8
C	10.0±2.4	10±2.3	88.8±42.9	74.3±4.4	43.5±1.3	54.8±44.2	11.8±2.2
D	19.0±2.9	6.5±1.9	37.5±17.9	53.3±5.2	45.5±0.6	35.4±6.2	24.1±3.3
E	17.0±4.2	9.0±3.8	17.0±4.2	69.0±7.5	74.8±8.3	5.1±1.6	3.4±1.8
F-value	2.5	0.9	11.9	8.2	33.9	2.8	63.7
P-value	0.09	0.51	0.00	0.00	0.00	0.06	0.00
Remark	NS	NS	SS	SS	SS	NS	SS

Table 3: Posthoc analysis

Post hoc							
A VS B	NS (0.64)	NS (1.00)	**SS (0.00)**	NS (0.98)	NS (0.09)	NS (0.44)	NS (1.17)
A VS C	NS (0.29)	NS (0.68)	NS (0.16)	NS (0.96)	NS (0.08)	NS (0.37)	NS (1.32)
A VS D	NS (0.99)	NS (0.99)	NS (0.39)	**SS (0.04)**	NS (0.13)	**SS (0.01)**	NS (1.82)
A VS E	NS (0.99)	NS (0.94)	NS (0.99)	NS (0.99)	NS (0.05)	NS (0.67)	NS (1.15)
B VS C	NS (1.00)	NS (0.68)	NS (0.99)	NS (1.00)	NS (0.95)	NS (0.99)	NS (1.44)
B VS D	NS (0.46)	NS (0.99)	**SS (0.02)**	**SS (0.01)**	NS (0.67)	NS (0.97)	NS (1.90)
B VS E	NS (0.68)	NS (0.94)	**SS (0.01)**	NS (0.81)	**SS (0.02)**	NS (0.42)	NS (1.23)
C VS D	SS (0.09)	NS (0.26)	NS (0.33)	**SS (0.01)**	NS (0.18)	NS (0.89)	NS (2.00)
C VS E	NS (0.16)	NS (0.99)	NS (0.16)	NS (0.75)	**SS (0.02)**	NS (0.35)	NS (1.42)
D VS E	NS (0.93)	NS (0.77)	NS (0.35)	NS (0.80)	**SS (0.02)**	**SS (0.01)**	NS (1.89)

Data was analyzed using one-way ANOVA followed by Games-Howell and values were considered significant at $p<0.05$.

KEY

SS = Statistically significant
NS = Not statistically significant

PLATE 1; Photomicrograph section of control of liver tissue shows morphology consistent with normal liver histology. The central vein (C), sinusoids (S) and hepatocytes (white arrows) are normal with no obvious sign of injury (H&E x100 & x400).

PLATE 2; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 15 days shows morphology consistent with normal liver histology. The central vein (C) and sinusoids (S) are normal. However, there are focal areas of degeneration of the hepatocytes (black arrows) and increasing fat cell deposition in the sinusoids (yellow star) (H&E x100 & x400).

PLATE 3; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 30 days shows morphology consistent with normal liver histology. The central vein (C) is normal. However, there are areas of degeneration of the sinusoids (yellow star) and fading of the hepatocytes (black arrows) (H&E x100 & x400).

PLATE 4; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 45 days shows morphology consistent with normal liver histology. The central vein (C) is normal. However, there are areas of degeneration of the sinusoids with fat cell depositions (yellow stars), and extensive inflammation of the hepatocytes (Red arrows) (H&E x100 & x400).

PLATE 5; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 60 days shows morphology consistent with normal liver histology. The central vein (C) is normal. However, there are areas of degeneration of the sinusoids (yellow star) and fading of the hepatocytes (black arrows) (H&E x100 & x400).

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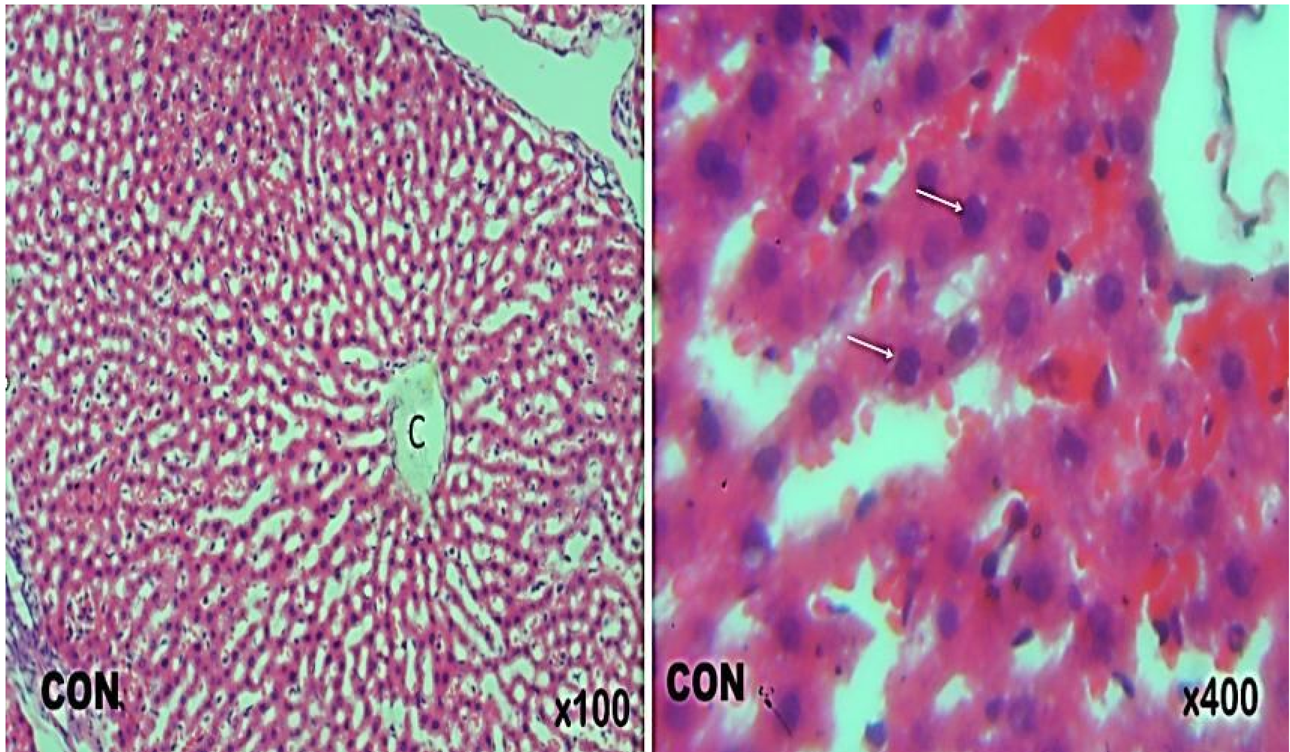

PLATE 1; Photomicrograph section of control of liver tissue shows morphology consistent with normal liver histology (H&E x100 & x400)

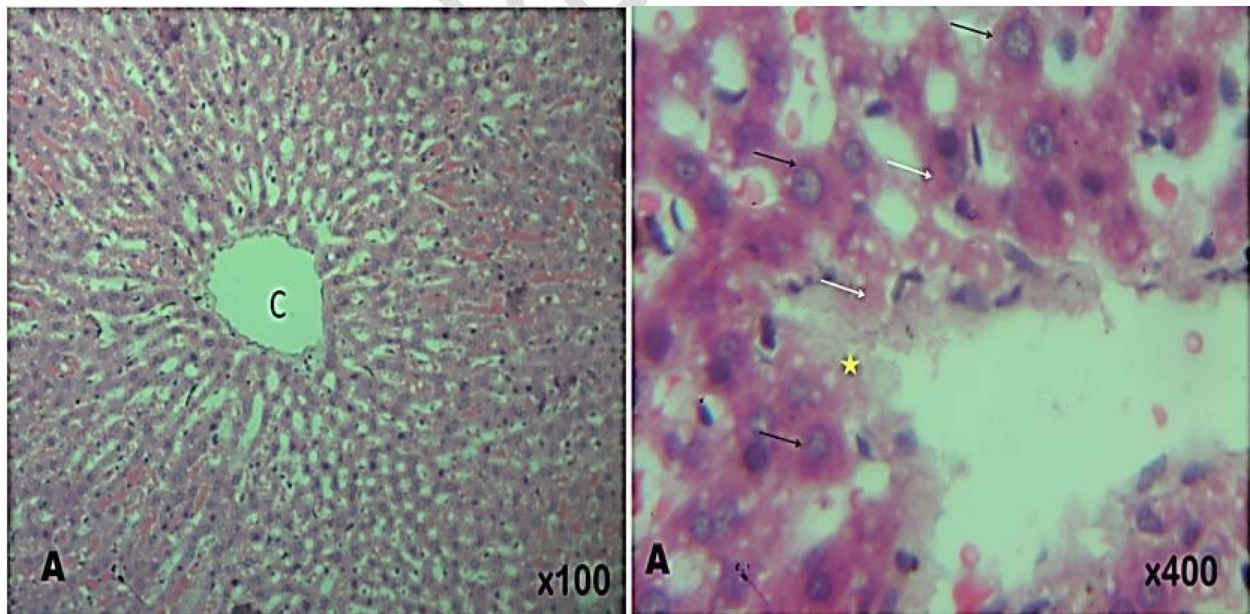

PLATE 2; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 15 days (H&E x100 & x400)

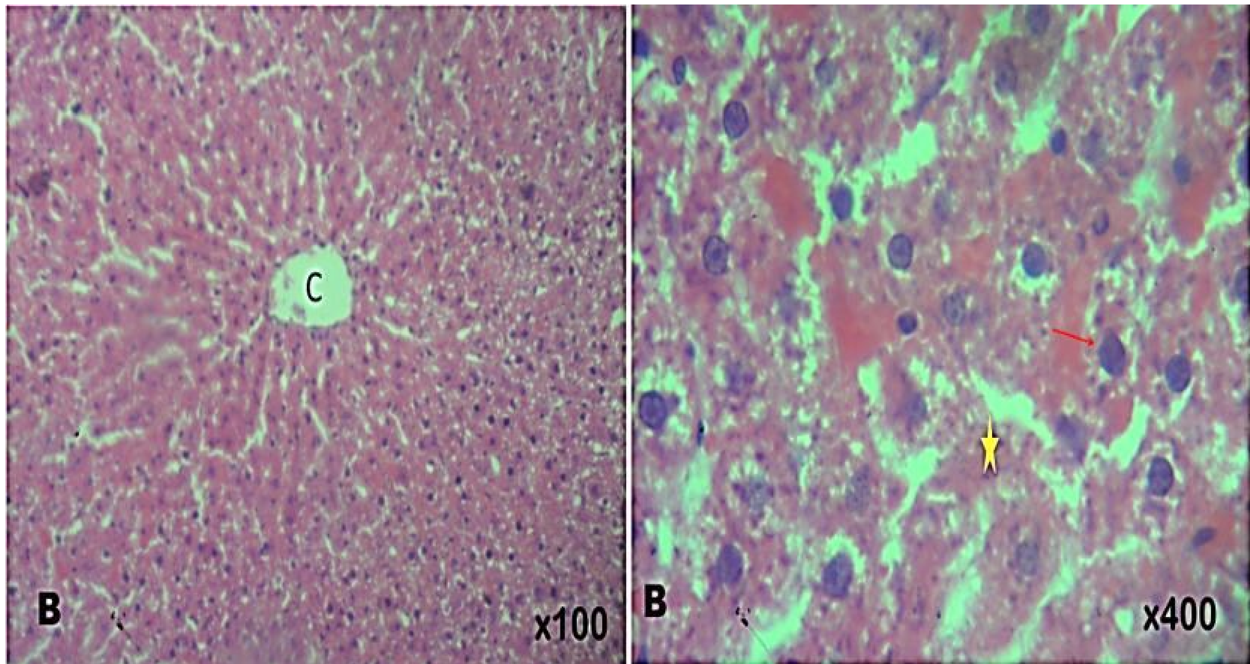

PLATE 3; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 30 days (H&E x100 & x400).

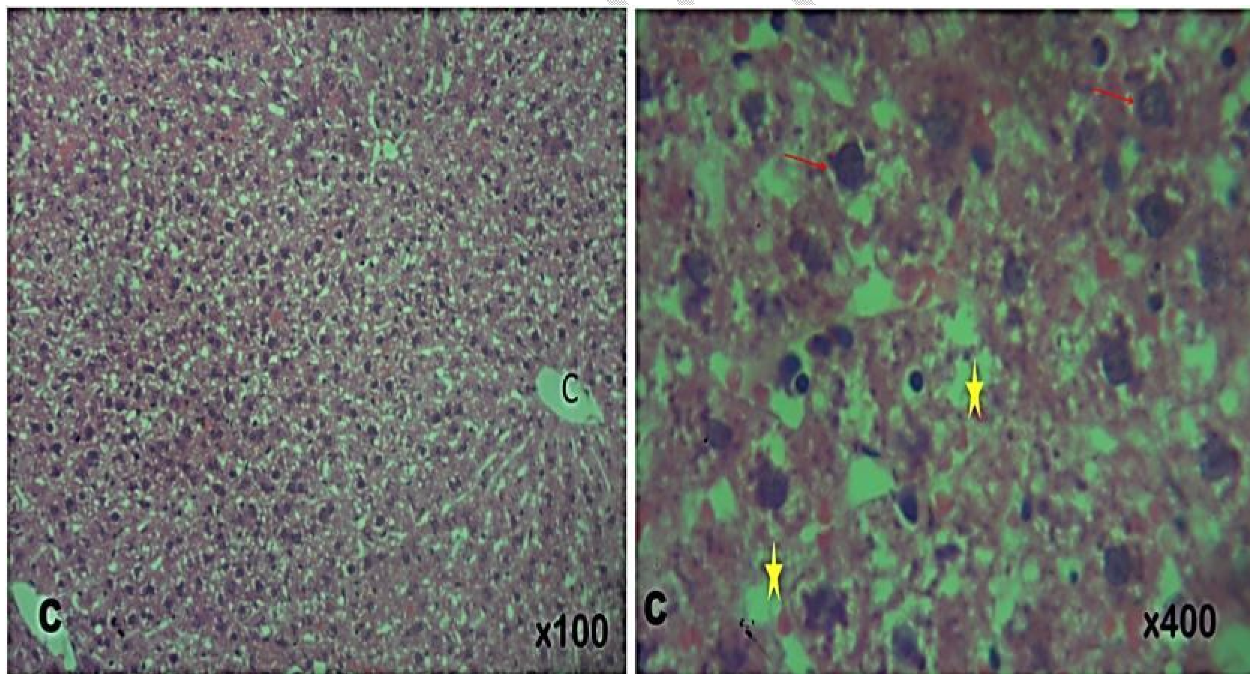

PLATE 4; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 45 days (H&E x100 & x400)

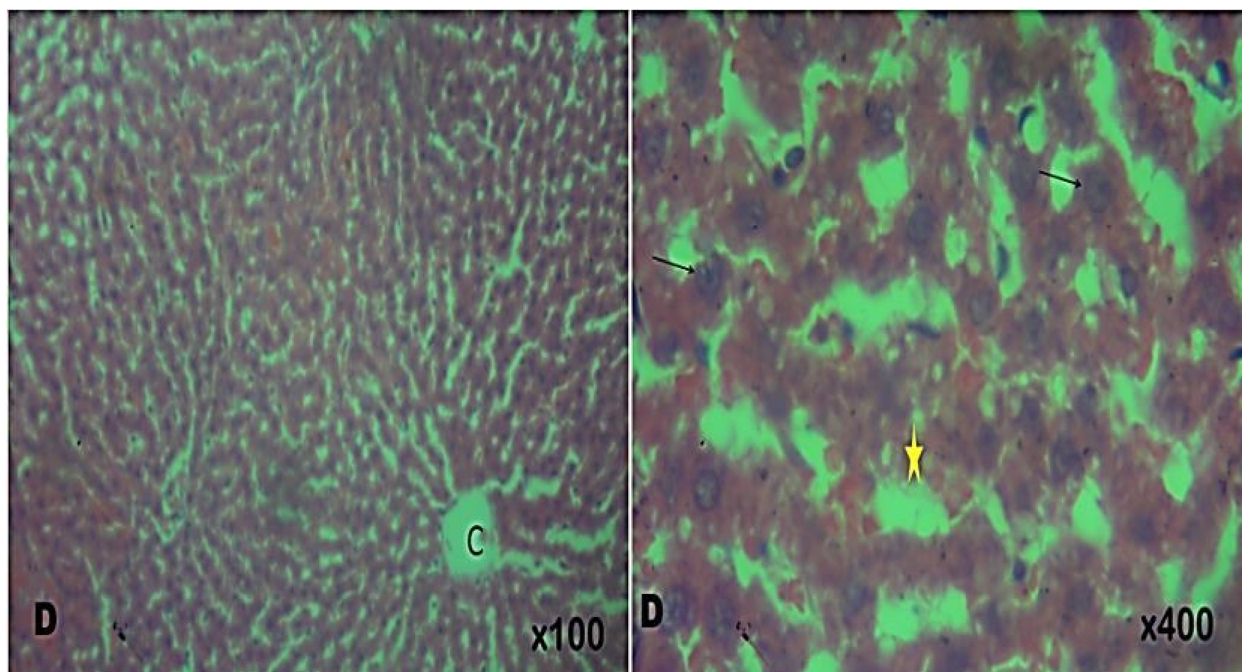

PLATE 5; Photomicrograph section of the liver tissue of wistar rat exposed to cement dust for 60 days (H&E x100 & x400).

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Discussion

In this research study, Wistar rats were exposed to cement dusts via a fabricated exposure chamber, to determine the histomorphological, haematological and liver function parameters of the exposed Wistar rats.

The result showed that there was significance (p<0.05) decrease in the Alkaline Phosphatase, Total Protein. Albumin and Conjugated Bilirubin, and no significant (p> 0.05) difference in Aspartate Aminotransaminase, Alanine Aminotransaminase and Total Bilirubin of the test groups compared to the control group. This result is not in agreement with the findings of Festus and his team in 2020 who reported that there was significance (p< 0.05) in Total Bilirubin and Alanine Aminotransaminase between cement handlers and non-cement handlers [13]. However, this result is in agreement with Ashwini *et al.,* (2016) who reported that there was no significant difference in total bilirubin of cement handlers compared with non-cement handlers [14]. Mojiminiyi and his colleagues in 2008 had also reported that there was significance (p< 0.05) in Alkaline Phosphatase and no significance (p> 0.05) in serum total bilirubin levels of cement factory workers compared with those of the control subjects [15]. This result agrees with the reports of the study carried out by Al Salhen in 2014 who reported a significant increase (p < 0.05) in the AST and ALT activities in cement handlers [16]. These variations in result may be due to the difference in exposure time. ALT is of value as it indicates the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in serum when

cellular degeneration or destruction occurs in this organ [17-19]. Increased Aspartate Aminotransaminase and Alanine Aminotransaminase shows sign of toxicity and hepatic damage.

The histological report following the exposure of cement dust showed no obvious sign of injury in the control, but showed focal areas of degeneration of hepatocytes and increasing fat cells deposition in the sinusoids after 15, 30, 45 and 60 days of exposure and extensive inflammation of hepatocytes after 45 days of exposure. This report shows that long term exposure to cement dust inhalation has a hepatocellular effect on the liver. This result is in agreement with Owonikoko *et al.,*(2021) who reported that research study Standardized experimental model for cement dust exposure; tissue heavy metal bioaccumulation and pulmonary pathological changes in rats the histopathology of this result showed necrosis, inflammatory cellular infiltration, and alveolar hyperplasia in the tissue [20].

CONCLUSION

The study revealed that term exposure to cement dust inhalation has a significant effect on the histological and biochemical activities of the liver of rats.

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