

Original Research Report

SELECTED VEGETABLES AND SPICES IMPROVE DNA QUALITY AND HISTOPATHOLOGICAL ABNORMALITIES IN ROOF RATS (*Rattus rattus*) EXPOSED TO CEMENT DUST

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ABSTRACT

Conventional pollution control strategies in the cement industry have proven ineffective. As a result, effective and targeted complementary interventions are necessary. This study used roof rats (*Rattus rattus*) inhabiting the premises of a cement plant in Sagamu, Ogun State, Nigeria, to evaluate the ameliorative effects of moringa (*Moringa oleifera*), roselle (*Hibiscus sabdariffa*), fluted pumpkin (*Telfairia occidentalis*), and ginger (*Zingiber officinale*) on cement dust exposure. A total of 42 rats were divided into seven groups, with each group consisting of six rats. Group 1 served as the negative control group and was not exposed to any substances, while group 2 served as the positive control group and received standard feed throughout the experiment. Meanwhile, groups 3, 4, 5, 6, and 7 served as the experimental group. Rats in these groups were fed with 400 mg/kg of ethanolic extracts of *Z. officinale*, *M. oleifera*, *T. occidentalis*, *H. sabdariffa*, and a mixture of the four extracts with a composition of 1:1:1:1, respectively, for 90 days. The plasma DNA concentrations, DNA purity, and lungs of the rats were examined before and after the experiment. Prior to the experiment, the exposed rats had higher plasma DNA concentrations and lower DNA purity, as well as severe fibrosis and congested alveoli in their lungs, compared to the unexposed rats. At the end of the experiment, the experimental groups showed a significant increase in DNA purity ($p \leq 0.05$) and a decline in plasma DNA concentrations compared to the positive control group. In addition, the experimental groups showed fewer histopathological abnormalities than the positive control group. The mixture of the extracts yielded the most favorable results, followed by the extracts of *Z. officinale*, *M. oleifera*, *T. occidentalis*, and *H. sabdariffa*, respectively. These findings suggested that the selected vegetables and spices have the properties to ameliorate the effects of cement dust exposure. Therefore, individuals residing in close proximity to cement plants are encouraged to consume these vegetables and spices.

Keywords: Cement dust, congested alveoli, DNA purity, pollution, *Zingiber officinale*

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Highlights:

1. This study identified feasible and affordable alternatives to ineffective conventional cement production pollution control strategies.
2. *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale* are effective as personal interventions to ameliorate the effects of cement pollution.

INTRODUCTION

Humans can survive for some time without water and food, but only for a very limited time without air. Air is so essential to life that the average adult breathes in and out over 7 liters of air per minute

while at rest, or approximately 11,000 liters of air per day (Discovery Health 2022). Unfortunately, natural and anthropogenic phenomena are increasingly polluting the air, increasing the risk of several diseases. Breathing polluted air can cause illnesses such as eye and nose burns, throat irritation,

and difficulty breathing (National Institute of Environmental Health Sciences 2022). Particulate matter in the air can predispose humans to cardiovascular and respiratory diseases. It poses a greater risk, particularly for asthmatic individuals (Manisalidis et al. 2020, World Health Organization 2022). Heavy metals and volatile compounds such as benzene and vinyl chloride can initiate carcinogenesis, reproductive anomalies, lung injuries, and brain and nerve damage. In extreme situations, these effects can even be permanent and cause death (Lelieveld et al. 2020, Li et al. 2021). Pollutants can reach the upper atmosphere, causing the ozone layer to deplete. The gradual depletion of the ozone layer harms the environment and increases the risk of skin cancer and cataracts (United States Environmental Protection Agency 2022a).

Nearly all industries pollute the air, and the cement industry is one of the top polluters. According to the United States Environmental Protection Agency (2022b), the cement industry ranked third on the most polluting industry list. The reason for this was that the cement industry emits over 500,000 tons of greenhouse gases annually. Moreover, particulate matter, heavy metals, and dioxin may be present in cement dust released by kilns powered by the burning of hazardous waste. Cement dust contains various contaminants that may be harmful if exposed to humans and animals in significant amounts. The contaminants typically include silicon oxide, aluminum trioxide, calcium oxide, ferric oxide, sand, and magnesium oxide (Yahaya 2014, Rahmani et al. 2018). Most of these substances can be hazardous to living organisms when they exceed regulatory limits. These substances in particular have been linked to cancer, organ injuries, skin and eye deformities, blood disorders, genetic problems, and respiratory disorders (Occupational Safety & Health Administration 2016, Ahmad et al. 2021). These and other health problems caused by cement dust pollution must be prevented or ameliorated, especially in Nigeria and other countries where the cement industry is booming because of growing demand.

In the cement industry, traditional pollution prevention and control measures include the use of dust filters and protective gadgets, as well as tree planting around cement plants. However, not so much success has been recorded from these strategies due to weak environmental protection laws, technical challenges, a lack of funds, and insincerity, among others (Yahaya et al. 2022). Therefore, a complementary, cheap, and simple strategy is needed to prevent or ameliorate the health consequences of cement dust pollution. Plant medicine is an area that has not been completely utilized in the management of pollutant exposure effects. This study assessed the effects of moringa

(*Moringa oleifera*), roselle (*Hibiscus sabdariffa*), fluted pumpkin (*Telfairia occidentalis*), and ginger (*Zingiber officinale*) on the DNA concentration and purity, as well as the lungs of roof rats (*Rattus rattus*) collected on the premises of a cement plant in Sagamu, Ogun State, Nigeria. People living in Nigeria frequently consume these plants as both vegetables and spices. These plants contain large amounts of antioxidants and phytonutrients, which are helpful in maintaining a healthy lifestyle. This study aimed to assess the use of nutrients in these plants to tackle health hazards caused by cement dust.

MATERIALS AND METHODS

This study utilized 42 roof rats (*Rattus rattus*) with a mean weight of 200.45 ± 4.30 g that were captured on the premises of a cement factory in Sagamu, Ogun State, Nigeria. The rats were kept in metal cages for seven days to acclimate to the ambient environment prior to commencing the study, according to the method used in a study by Yahaya et al. (2022). The rats had free access to standard animal feed and water. The plant samples were sourced from a farm in Lagos, Nigeria, and identified in the herbarium section of the Department of Botany, University of Lagos, Nigeria. The authenticated samples were retained in the herbarium under the code numbers LUH 4558, LUH 4394, LUH 4395, and LUH 4396 for *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale*, respectively.

The preparation to extract the bioactive components from the plants were carried out by following the guidelines in a study by Yahaya (2014). Powder (50 g) from each plant and the mixture were poured into 500 ml of 95% cold ethanol for 3 days (72 hours). The extracts obtained were filtered with muslin cloth and then dried to a constant weight at a $40 \pm 2^\circ\text{C}$ temperature. The dry extract of each plant was dissolved in water and used for the experiment. The 'classical LD50' protocols as demonstrated in Anyebe et al. (2021) were followed to evaluate the acute toxicity of the plant extracts. In their study, 36 mixed-sex albino rats weighing from 205 to 210 g were utilized and divided into 6 groups of 6 rats each. After 12 hours of fasting, the rats in the treatment groups received 200, 400, 500, 1000, 3000, and 5000 mg/kg oral doses of the crude extracts, while the control groups were only given distilled water. The rats in each group were monitored for signs of toxicity for 24 hours.

The rats' DNA purity and plasma concentrations, as well as the lungs, were examined before administering treatments in this experiment (Suguna et al. 2014). The 42 rats were thereafter divided into

7 groups, each containing 6 rats. The negative control group (group 1) consisted of unexposed rats obtained from an area free from cement dust. The positive control group (group 2) consisted of rats exposed to cement dust but did not receive any treatment. Rats in groups 1 and 2, respectively, were given standard feed and water only. Rats in groups 3, 4, 5, 6, and 7 were fed with 400 mg/kg extracts of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, *Z. officinale*, and the mixture (1:1:1:1) of the four extracts for 90 days, respectively. The rats' DNA purity and plasma concentrations, as well as the histopathology of the lungs, were examined at the end of this study (Griffiths & Chacon-Cortes 2014).

Fresh blood pellet samples from the rats were collected using small vials (sample bottles) to determine the DNA purity. The blood samples were temporarily refrigerated, centrifuged for ten minutes at 5,000 rpm, washed twice, and then centrifuged again in 1,000 µl of phosphate-buffered saline (PBS). Afterwards, the blood pellets were transferred into an Eppendorf tube using a side-mouth disposable pipette and centrifuged at 10,000 rpm for five minutes (Suguna et al. 2014). The supernatants were retained and stored in a refrigerator at -40°C until required.

DNA was extracted from the blood pellets for isolation following the procedures described by Griffiths & Chacon-Cortes (2014). After transferring 200 µl of blood pellets into each of the labeled (001-024) Eppendorf tubes, 200 µl of 20% sodium dodecyl sulfate (SDS) was added to each tube and properly shaken. The homogenized mixture was placed in a heated bath (65°C) and intermittently shaken for 30 minutes before being removed and left to cool. Thereafter, 500 µl of ice-cold 5 M potassium acetate was transferred into each tube and shaken several times for proper mixing. After incubating the mixture on ice for 30 minutes, it was processed in a refrigerated centrifuge at 12,000 rpm for 10 minutes. The mixture in the tubes separated into two layers: the liquid portion at the top containing DNA (supernatants) and the solid portion at the bottom (residue) (Griffiths & Chacon-Cortes 2014).

The supernatants from each sample were properly transferred into two tubes per sample. The tubes were then filled with 1,000 µl of ice-cold isopropanol and delicately shaken until DNA strands were visible. The tubes were left to stand for a while before being incubated for 24 hours at -20°C in a freezer to ensure complete precipitation, as characterized by the appearance of a white mass in the liquid mixture (Griffiths & Chacon-Cortes 2014). The supernatants were decanted, leaving the strands in the tubes. The tubes were placed face down on paper towels for an hour in order to remove

the final drops of isopropanol. The DNA strands were then washed by mixing them with 70% ethanol and centrifuging them at 12,000 rpm for 10 minutes at 4°C. The ethanol was discarded, and the tubes were inverted for ten minutes to remove the leftover ethanol. The tubes were then placed flat on paper towels for the pellets to dry completely over the course of four hours. The DNA was dissolved in 100 µL of double-distilled water and refrigerated before further analysis (Griffiths & Chacon-Cortes 2014).

Spectrophotometry was conducted to measure the rats' DNA quality and quantity using the BioPhotometer Plus (Eppendorf, A.G., Hamburg, Germany). The quantity was the concentration of the DNA in nanograms per microliter (ng/µl), while the quality (purity) was the absorbance of ultraviolet light through the cuvette measured in Armstrong (Å). The cuvette was an accessory that held the diluents (sterilized filtered water) and DNA material (Potter & Heller 2018). The equipment was activated and scrolled to the double-stranded DNA setting, which was calibrated to the quantity of materials being tested. The diluents used to elute or dilute the DNA were also used to reduce its concentration in order to obey the Beer-Lambert Law (concentration of double-stranded DNA = $A_{260} \times 50 \mu\text{g}/\mu\text{l}$) (Grasse et al. 2016). The dilution ratio for the DNA and diluents was 5:95 µL, with a total volume of 100 µL. The cuvette was rinsed with the same diluents and standardized prior to taking the reading for spectrophotometer analysis. The DNA was properly introduced and homogenized before pressing the sample button and obtaining a reading. On the liquid crystal display (LCD), the results for concentration (ng µL⁻¹), simultaneous absorbance at three different wavelengths (230A, 260A, and 280A), and a ratio of 260A/280A were shown to assess the purity of the sample.

The guidelines outlined in Anyebe et al. (2021) were followed to prepare the rats' lungs for histopathological examinations. After the tissues were excised, the routine hematoxylin and eosin staining technique was used for preparation. Following the staining, observation of the tissue was conducted under a light microscope (40X magnification), with emphasis on the cytology, architecture, and morphology of the tissues. In the data analysis, IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used to compute the values obtained as mean ± standard deviation. Comparison of the differences between the treatment and control groups was done using the F-test (ANOVA), and $p \leq 0.05$ was considered a significant difference.

RESULTS

The acute toxicity tests revealed that the extracts were non-toxic to the rats at all the doses administered. A dose of 400 mg/kg of body weight seemed to produce the best results. Therefore, the dose was chosen for subsequent analyses. General observations for 24 hours after extract administration presented no mortality. The administration of *Z. officinale*, *M. oleifera*, and *T. occidentalis* extracts, as well as the mixture of plant extracts, exhibited no signs of distress or excitement among the rats. The rats that received *H. sabdariffa*, on the other hand, licked the cannula aggressively, which was an indication that they relished the extract.

Regarding the effects of plant extract administration on the roof rats, Table 1 shows the DNA concentrations and absorbance (purity) with optical densities at 260/280. The DNA purity of the treated rats improved significantly ($p \leq 0.05$) compared to those that only received water. The absorbance was measured to be 1.65 in the negative control group (group 1), 1.39 in the positive control group (group 2), 1.51 in the group receiving *M. oleifera* extract (group 3), 1.43 in the group receiving *H. sabdariffa* extract (group 4), 1.52 in the group receiving *T. occidentalis* extract (group 5), 1.63 in the group receiving *Z. officinale* extract (group 6), and 1.60 in the group receiving a mixture of the plant extracts (group 7).

Table 1. DNA purity of the rats fed with plant extracts for 90 days.

Extract	DNA conc. (ng/μl)	260	Absorbance (A) 280	260/280
Group 1	2.11±0.91	0.028±0.001	0.018±0.006	1.65±0.08
Group 2	6.21±1.82	0.031±0.002	0.023±0.001	1.39±0.61
Group 3 (<i>H. sabdariffa</i>)	4.21±1.25	0.022±0.012	0.016±0.001	1.43±0.01*
Group 4 (<i>M. oleifera</i>)	3.32±0.73	0.018±0.002	0.012±0.001	1.51±0.11*
Group 5 (<i>Z. officinale</i>)	2.31±0.22	0.029±0.002	0.018±0.001	1.63±0.13*
Group 6 (<i>T. occidentalis</i>)	3.42±0.93	0.031±0.014	0.021±0.016	1.52±0.11*
Group 7 (Mixture of extracts)	2.35±0.53	0.048±0.001	0.030±0.002	1.60±0.16*
Rat baseline	7.56±2.51	0.052±0.007	0.039±0.003	1.30±0.02

Values were expressed as mean±SD; group 1: non-exposed rats (negative controls) obtained from a cement-dust-free area; group 2: exposed rats treated with only water; rat baseline: DNA quality of the exposed rats a day before treatment; if absorbance (A) at 260/280 is 1.5–2.0, it is normal; if absorbance <1.5 and >2.0, it is abnormal; values with asterisks (*) are significantly different from the negative control group (group 2), with $p \leq 0.05$ (ANOVA).

After administering the plant extracts to the rats, the lung tissue was examined to determine the extracts' effects. Figure 1 shows normal alveoli that were

found in the lungs of the rats that lived in an area free from cement dust. Figure 2 reveals the lungs of the exposed rats prior to extract administration, showing severe inflammation, vascular congestion, a congested alveolus, and severe fibrosis. Figure 4 demonstrates the improvement of the rats' lungs following the administration of the extracts. After the administration of *H. sabdariffa* extract, the lung tissue of the rats showed congested alveoli and moderate inflammation. There was moderate inflammation in the lung tissues of the rats after *M. oleifera* extract was administered. Mild inflammation was also present in the lung tissue of the rats that received *Z. officinale* extract. Mild vascular congestion was observed in the lungs of the rats treated with *T. occidentalis* extract. The lung tissue of the rats that received a mixture of the extracts showed mild inflammation. However, the exposed rats that received only water still revealed severe fibrosis (Figure 3).

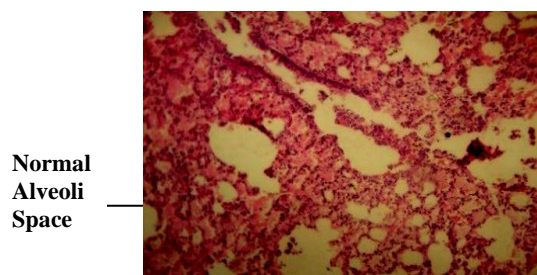


Figure 1. Lung histopathology of the rats obtained from an area without cement dust (400X magnification).

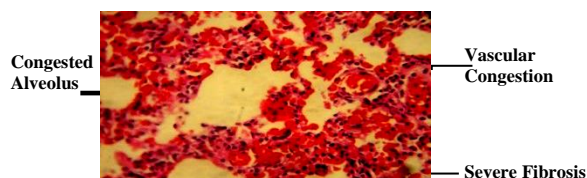


Figure 2. Lung histopathology of the exposed rats prior to extract administration (400X magnification).

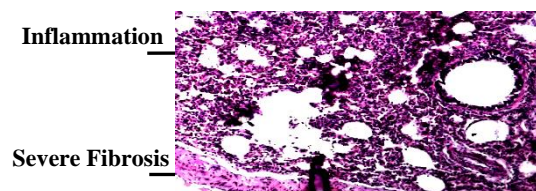


Figure 3. Lung histopathology of the control rats administered with distilled water (400X magnification).

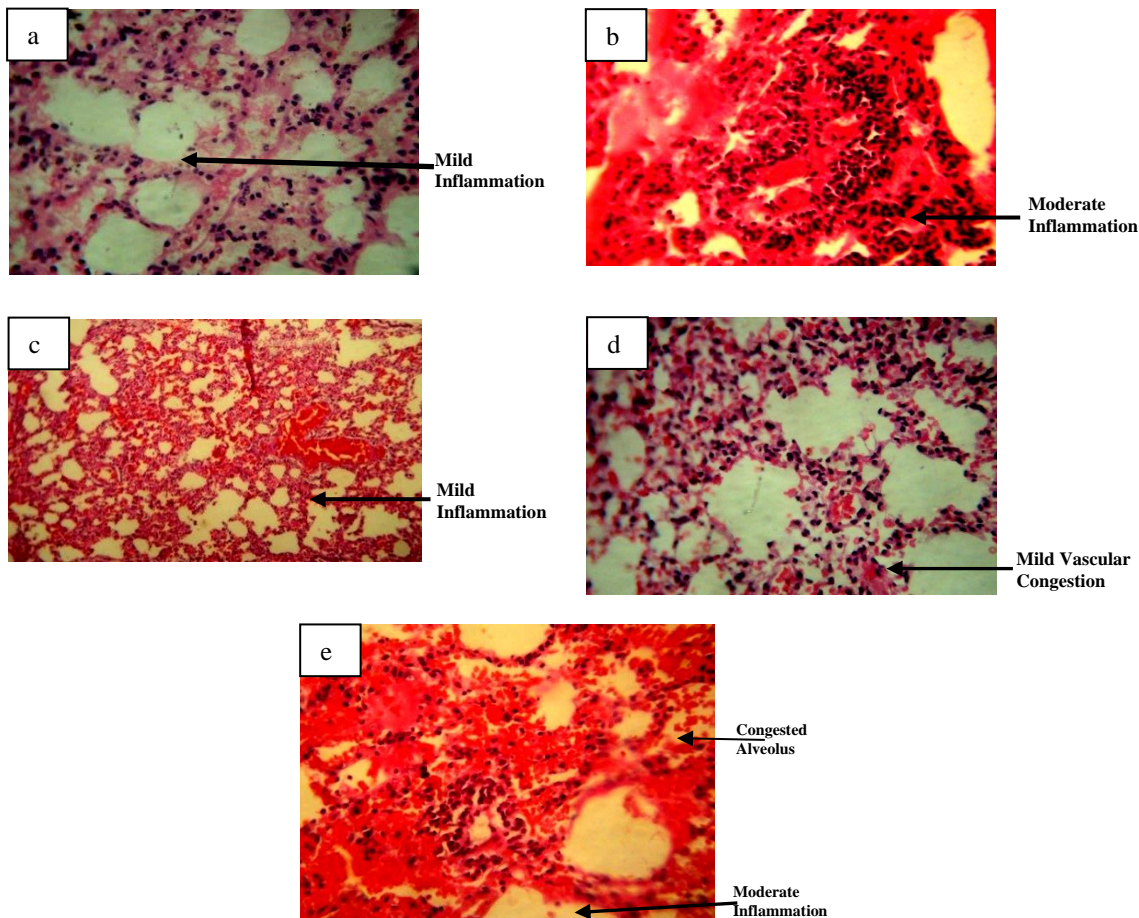


Figure 4. Lung histopathology of the rats administered with *Hibiscus sabdariffa* extract (4a), *Moringa oleifera* extract (4b), *Zingiber officinale* extract (4c), *Telfairia occidentalis* extract (4d), mixture of plant extracts (4e) at 400X magnification.

DISCUSSION

This current study evaluated the ameliorative effects of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale* extracts on roof rats residing in the vicinity of a cement plant. This study was conceptualized to find a nutrient-based and individualistic approach to the health hazards associated with cement dust exposure. Rats exposed to cement dust had high plasma DNA concentrations. This is an indication of oxidative stress-induced DNA damage, which is a biomarker for a number of diseases, including cancer, trauma, myocardial infarction, stroke, and sepsis, among others (Pizzino et al. 2014, Graille et al. 2020). Plasma DNA are DNA fragments that are detectable in extracellular fluid and are present at very low levels in healthy individuals. DNA fragments enter the circulation following cell death, either through cell necrosis or apoptosis (Pizzino et al. 2014, 2017).

The low DNA purity of the exposed roof rats indicated that their DNA had been contaminated and that their health had consequently been

compromised by the poisonous components of cement dust. A previous study reported that the bloodstreams of rats exposed to cement dust contained potentially toxic elements, mainly heavy metals, aluminum, and calcium (Yahaya 2014). These elements have been linked to several diseases. In rats exposed to chromium and lead in mining areas, overexpression of certain mRNAs was observed in the blood of the animals (Nakayama et al. 2013). An observation of the germinal cells of rats exposed to lead and cadmium revealed DNA damage in the spermatocytes. Exposure to aluminium oxide and silicon oxide nanoparticles has been demonstrated *in vitro* to cause DNA damage (Hashimoto & Imazato 2015, Adamkovicova et al. 2016). The results of the current study were in line with earlier studies that investigated cement dust exposure (Krishna et al. 2020, Akiibinu et al. 2016). Detected biomarkers in workers exposed to cement dust indicated DNA damage and increased cell death. Workers exposed to cement dust experienced an occupational hazard that led to DNA damage characterized by raised plasma 8-OHdG levels.

The improved DNA purity and reduced plasma concentrations following treatment using plant extracts could be attributed to the phytochemicals and phytonutrients in the plants. A phytoconstituent analysis of the examined vegetables revealed the presence of numerous vitamins and minerals (Yahaya et al. 2017). Health-boosting phytochemicals (such as alkaloids, flavonoids, tannins, phlobatanins, glycosides, and saponins) and reducing sugars were also detected. It has been demonstrated that plants containing the mentioned phytonutrients and phytochemicals, such as the vegetables and spices evaluated in this study, enhance health and prevent health risks. In an experiment, *H. sabdariffa* showed genoprotective effects against H₂O₂-induced DNA damage. Aqueous extracts of *M. oleifera* leaves, seeds, and fruits have also been demonstrated to inhibit oxidative DNA damage (Abdul et al. 2014, Karim et al. 2016). Other studies reported that *Z. officinale* extract reduced plasma DNA concentrations and damage in rats with heavy metal-induced oxidative damage. Oral administration of *T. occidentalis* seed oil has been shown to ameliorate DNA damage caused by azathioprine-induced oxidative stress (Shaban & Sahu 2017, Gabr et al. 2019, Okesola et al. 2020).

The histopathological damage observed in the exposed rats' lungs demonstrated the deleterious effects of cement dust since the lungs are the first to contact with an inhaled substance. Earlier studies revealed lung damage in humans and rats exposed to dust from cement plants (Rahmani et al. 2018, Owonikoko et al. 2021). Furthermore, Richard et al. (2016) reported reduced lung function in workers exposed to cement dust. As already mentioned earlier, the heavy metals in cement dust could be responsible for the histopathological damage. Studies have discovered that being exposed to silica can potentially cause damage to the lungs (Occupational Safety & Health Administration 2016, Almansour et al. 2018). Welders have been reported to suffer from interstitial lung disease, upper lobe fibrosis, and peripheral emphysema as they are occupationally exposed to aluminum dust. There was evidence of lung inflammation, injury, and proliferative responses in rats that were repeatedly exposed to hexavalent chromium (Feary et al. 2020, Zhang et al. 2023). Acute and chronic lead exposure have been demonstrated to cause lung damage. Calcium is a mineral that is essential for both humans and animals. However, a biologically foreign form of calcium, such as that found in cement dust, can cause organ damage when deposited in the wrong organs (Baccarelli et al. 2014, Offor et al. 2017, Attafi et al. 2022).

The improvement observed in the rats' lungs following the extract administration can be

attributed to the phytoconstituents mentioned previously. Protective effects of *M. oleifera* on lung functions, hematological damage, and lipid damage induced by heavy metals have been demonstrated using experimental rats. Ameliorative effects of *H. sabdariffa* on pulmonary fibrosis and chronic inflammatory interstitial lung disease have also been studied using rats exposed to a toxic chemical (Hemmeti et al. 2016, Ajibade et al. 2021). It has been demonstrated that pre-treatment using *Z. officinale* extract improves lung function and normalizes lung injuries in alcoholic humans and rats exposed to polychlorinated biphenyls (Alireza et al. 2017, Ahd et al. 2019). The administration of *T. occidentalis* extract to animals resulted in the repair of lung tissue damage. In addition, parameters of liver function revealed beneficial effects of the extract (Chukwuemeka et al. 2020, Saadat et al. 2022). This study complements the previous studies by demonstrating that the mixture of the four plant extracts in equal ratios worked better than each plant extract. This suggests that the phytoconstituents in the plants work synergistically to enhance the efficacy of the plant extracts.

Strength and limitations

The study reflects the practical implications of exposure to cement dust in a typical cement production environment by employing local rats that were cost-efficient on the premises. This implication was strongly supported at the molecular level, as the DNA purity check indicated the extent of cement pollution for anyone living in the vicinity of the cement factory. However, the limitation of this study was that only a representative sample was collected, which might not accurately reflect the entire population around the cement site.

CONCLUSION

The extracts of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale* can enhance DNA purity and alleviate histopathological damage in the lungs of rats exposed to dust from a cement plant. The therapeutic efficacy of these vegetables and spices may be enhanced by combining them in a mixed formula. Individuals working or residing in close proximity to cement plants are advised to incorporate these vegetables and spices into their diet.

Acknowledgment

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Conflict of interest

None.

Ethical consideration

This study was conducted in accordance with the ethical standards of European and German animal welfare legislation, the Declaration of Helsinki, and the National Institutes of Health guidelines for the care and use of animals in research. The local ethics committee of the Federal University, Birnin Kebbi, Nigeria, approved all protocols used in this study (Regulation CEE 86/609 dated 3/12/2022).

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None.

Author contribution

TOY conceptualized, designed, and drafted the manuscript. TFS analyzed and interpreted the data as well as performed a critical revision of the manuscript for important intellectual content. CO granted the manuscript final approval. AI provided the study materials. MNM collected and compiled the data. SSR conducted the statistical analysis. SA provided administrative, technical, and logistic support.

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