Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 8 August 2024

Research Article

The Analysis of Cytotoxicity of Durban Residential House Dust Extracts on Human Lung Bronchial Epithelial Cell Line (A549) and Human Lung Bronchus Virus Transformed Epithelial Cell Line (ATCC No. CRL – 9482 BBM)

Bongani Nkala[1,2]* and Nceba Gqaleni[2]

[1]*Faculty of Applied Science, Eduvos, Glen Austin AH, Midrand, South Africa*

[2]*Department of Traditional Medicine, University of KwaZulu-Natal, South Africa*

***Corresponding Author:** Bongani Nkala, Faculty of Applied Science, Eduvos, Glen Austin AH, Midrand, South Africa.

DOI: 10.31080/ASMI.2024.07.1418

Received: May 10, 2024

Published: July 29, 2024

© All rights are reserved by **Bongani Nkala and Nceba Gqaleni.**

Abstract

There has been increased focus on house dust due to its potential to contain biological pollutants in indoor environments. Biological factors may include pollen, spores, moulds, bacteria, viruses, allergens, dust mites, and epithelial cells. This study aimed to analyse the cytotoxicity of house dust on the human lung bronchus carcinoma epithelial cell line (A549) and human lung bronchus virus-transformed epithelial cell line (BBM). One hundred and five (n = 105) house dust samples were obtained from households. Human cell lines were treated with five different dilutions of each house dust extract. Cell viability was assessed using the MTT assay. Toxic effects of house dust extract were analysed, following house dust extract treatment and cells were stained with double dye (annexin – V- and propidium iodide) and analysed with flow cytometry and fluorescent microscope. The results suggest that this study showed that dust extract is toxic to human cell lines and cells underwent a degree of apoptosis and necrosis at 62% (A549) and 99% (BBM). The findings in this study strongly suggest that house dust extracts are toxic to human cell lines.

Keywords: House Dust; A549 Cell Line; BBM Cell Line; MTT; Apoptosis; Necrosis

Introduction

Indoor air quality is described as the chemical, physical and biological characteristics of air in a residential or occupational indoor environment [1]. In residential settings, there are many contributions to indoor pollution levels namely; human activities, biological sources and outdoor air. House dust is a complex mixture of various bio-contaminants and a major source of allergens in non-industrial indoor environments [2]. Indoor house dust may contain biological pollutants such as pollen, spores, moulds, bacteria, viruses, allergens, dust mites and epithelial cells [2]. Previously, it was thought and understood that inhaled allergens were derived from pollen, but the latest developments point to dust extracts [3]. Dust can also carry a broad range of compounds such as polycyclic aromatic hydrocarbons, endotoxins and heavy metals which may damage biological systems [4].

Epidemiological studies have reported health problems associated with exposure to house dust. Its impact may range from slight irritation of the eye and mucus membranes of the throat, but these effects can also advance allergy, asthma, and bronchitis [5]. The exposure characteristics are not sufficiently understood to allow a reliable dose-response assessment of indoor exposure to toxigenic microbes or any single toxin. The possible presence of one of the inflammatory potentials of the dust may have multifactorial causes with no known single parameter being of major importance [6]. Furthermore, *in vitro* and *in vivo* tests have been used to assess dust toxicity [7,8]. The biological hazards of organic dust, regardless of its constituents and concentration, will assist in ranking individual samples according to their given parameters such as inflammatory potential [9].

House dust contains several undefined components which are difficult and very expensive to obtain a precise measurement of

each relevant component to estimate the risk of developing respiratory problems [9]. House dusts that are taken from different locations at different times may vary profoundly in composition which further complicates air pollution disease studies. Although there is no specific strategy and protocols in place for determining toxic effects in organic dust researchers have proposed a fundamental concept of toxic evaluation that is like that of a single substance [10]. Due to the house dust complexity, researchers have suggested a general test for toxicology and immunotoxicity such as LDH, XTT, MTT, Annexin-V etc, to quantify its effects [11].

Recently, epithelial cells have received attention in mechanistic studies of air pollution-induced airway disease. The findings of these studies suggest that cells are likely to play a fundamental role in the pathogenesis [12]. The reason for this is that bronchial epithelial cells play an important role as a physical barrier in protecting the underlying tissue and maintaining the local environment in the airways [12].

Previous work conducted in South Durban households reported high asthma prevalence in children [13]. Thus, this study aimed to assess the cellular stages that cells undergo when treated with house dust extract from settle dust. This will provide a better understanding regarding house particles inhaled through the respiratory tract system. Human bronchial epithelial cell lines were selected due to their direct contact with dust particles.

Materials and Methods

Household conditions

The nature of the household sampled was built using bricks, concrete and asbestos cement sheeting and households were not air-conditioned. The households were generally in good condition taking into account that 50% were built before 1978 and 39% were built after 1978.

Sample selection

The households for this study were drawn randomly South Durban Health Study (SDHS), which had 823 school children participating from 7 schools in Durban communities [14]. In total 136 households, from the main SDHS were recruited to participate in this study. Of the 136 households visited, dust samples were collected from 126 homes. Unfortunately, no samples were collected in 21 homes due to the unavailability of electricity as required by the equipment used for collection. Therefore, only 105 samples were collected and used in this study.

Dust collection

A portable vacuum cleaner (Wap Combi Cleaner Vs 300s, Germany) with a specialized unit that collects house dust into the filter paper (Macherey-Nigel, Germany) with particle retention of 8 µm was used. House dust was collected from both children's sleep areas and/or playrooms. Sleep area samples were collected from children's Pillows and mattresses for 2 minutes and if there was a soft floor in the room a 1.0 m^2 area was vacuumed for 2 minutes as well. From the play area, samples were collected from the couches and carpets or rugs for 2 minutes over a 1.0m^2 area. The collected dust samples were folded and covered with aluminium foil before being placed into a ziplock bag before transportation and stored at 4°C until analysis. For this study, 1 gram of dust sample was needed for the analysis. Consequently, one hundred and five dust samples (n = 105) were used in this study.

Dust extraction

The house dust samples were extracted according to Indoor Biotechnologies, Charlottesville, US for allergen protocol as previously used by [15]. Allergen is the final product that was suspended, stored at -20°C awaiting laboratory analysis and has been reported to be prevalent in indoor households [16].

Cell line and culture

Human lung bronchus carcinoma epithelial cell line (A549) and human lung bronchus virus transformed epithelial cell line (BBM) were obtained at the Medical Microbiology Laboratory University of KwaZulu-Natal School of Medicine. A549 (ATCC No. 185-CCL) was maintained in Eagle's Minimum Essential Medium (EMEM) and Ham's F-12 (Cambrex/Lonza) which was as previously described [17]. BBM (ATTC NO. CRL - 9482) was maintained in Bronchial Epithelial Growth Medium (BEGM) coated with 1% albumin from bovine serum minimum 98% and grown medium (Cambrex/Lonza) as previously described by [18]. Cells were incubated at 37°C in a humidified environment with 5% carbon dioxide (CO_2). The fresh medium was used to feed cells which were changed every second day and sub-cultured when confluent growth of 80-90% was reached, and thereafter cells were trypsined with 0.25% trypsin-versene.

3-(4, 5-Dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT)

The viability of treated cells and controls were evaluated using MTT assay (Roche) in triplicates. After trypsinazation, cells (1.4 x 10^5 cells/ml) were seeded into 96 wells microtitre plate containing

100µl growth media (Cambrex/Lonza) incubated in a humidified atmosphere at 37°C and cells were allowed to adhere for 12 to 19 hours. After the incubation period, wells were washed with phosphate buffer (PBS) and 100µl of house dust extract (in culture medium) was added into 96 wells with five different dilutions (1:200; 1:40; 1:10; 1:4; and 1:2) and incubated at 37°C in humidified environment with 5% CO_2. After the incubation period medium was removed from the wells and replaced with MTT salt as per manufacturers' instructions (Roche) and incubated for 4 hours. Cell viability was measured with an ELISA plate reader at 550-600nm (Bio-RAD, 350 Microplate reader).

Analysis by fluorescence microscopy

After trypsinazation, cells (2.5 x 10^5 cells/ml) were seeded in 24 well tissue culture plates already pre-inserted with sterile round coverslips (SMM Instruments) which contained 1000 µl of growth medium incubated at 37°C in the humidified environment and cells allowed to adhere for 12-19 hours. After the incubation period, media were removed and cells were washed once with PBS and treated with 1:10 dilution of house dust extract; and incubated together with controls (untreated cells) at 37°C in humidified environment. After the incubation period, media were removed, and cells were washed once with PBS and fixed with ice-cold 70% ethanol for an hour. Cells were washed once with PBS, and stained with annexin-v-fluorescein and propidium iodide as per manufacturer's instructions (Roche). Cover slips were taken out of 24 well plates and allowed to dry in dark room temperature and mounted on DPX in glass slides. Specimen was analysed by fluorescence microscopy at 515 – 565 nm (green).

Analysis by flow cytometry

After trypsinazation, cells (2.5 x 10^5 cells/ml) were allowed to adhere for 12-19 hours in 25 cm^2 tissue culture flask and incubated at 37°C in humidified environment. After incubation period cells were treated with 1:10 dilution of house dust extract and incubated at 37°C in humidified environment for 12 hours. After the incubation period, cells were washed once with PBS and trypsinized as described previously; and centrifuged at 2500 rpm for 5 minutes at 4°C (Multifuge 3 S/3R, Heraeus). Thereafter washed once with PBS and re-suspended in 1000 µl PBS. Concurrently, positive controls were conducted in parallel with samples and for the negative (unstained cells). The positive control used for A549 was actinomycin D (AD), and for BBM was cycloheximide (CHX). Actinomycin D (Sigma) induces apoptosis in tumour cells has been previously described by [19]. However, CHX (Sigma) induces apoptosis in normal bronchus cell lines has been previously described by [20].

Cells were stained with double dye (annexin-v and propidium iodide) as per manufactures instructions. The controls were also stained (positive being double stained, unstained negative and either with annexin or propidum iodide). Cells and controls were analysed by flow cytometry (LSR II, BD Science) at 488-617 nm within an hour after staining. The apoptosis and necrosis percentages were calculated using the equation that was previously described by [21].

Statically analysis

The data was analysed using software package of Microsoft Excel 2000 and SPSS for windows (15.1). Non-parametric test (Wilcoxon Signed Rank test) for pair data and $p<0.005$ was considered significant. Data was expressed as a mean ± S.D and median in some cases due to data being skewed.

Results

MTT assay

The toxic effect of house dust extract was analysed with MTT assay, and a dose-response curve was plotted (Figure 1) for both cell lines, and the mean and standard deviation were computed. The cell viability before the experiment was slightly above 80% in both cell lines. The results showed that cell viability for both cell lines went below 50% following exposure to various dilutions of house dust extracts as reflected in methods and materials. Furthermore, house dust was noticed to be responsible for lowering cell viability.

Determination of apoptosis and necrosis

The media was used instead of mean and the reason being results were not normally distributed (skewed). The percentage distribution for necrosis in both cells varies with the A549 median value below 30% while the BBM median value is just above 80%. It was observed that apoptosis was non-significant for A549 versus BBM ($p < 0.039$); while necrosis was noted to be significant ($p < 0.005$) for BBM versus A549. The positive controls for A549 (AD) and BBM (CHX) were observed to induce cell death with 97% necrosis of the BBM cell line and 93% apoptosis of the A549 cell line (Figure 2).

Human cell line apoptosis and necrosis were analysed by flow cytometry and results are given in Figure 3 for A549 and BBM cells. This confirms what has been observed in MTT assay that house

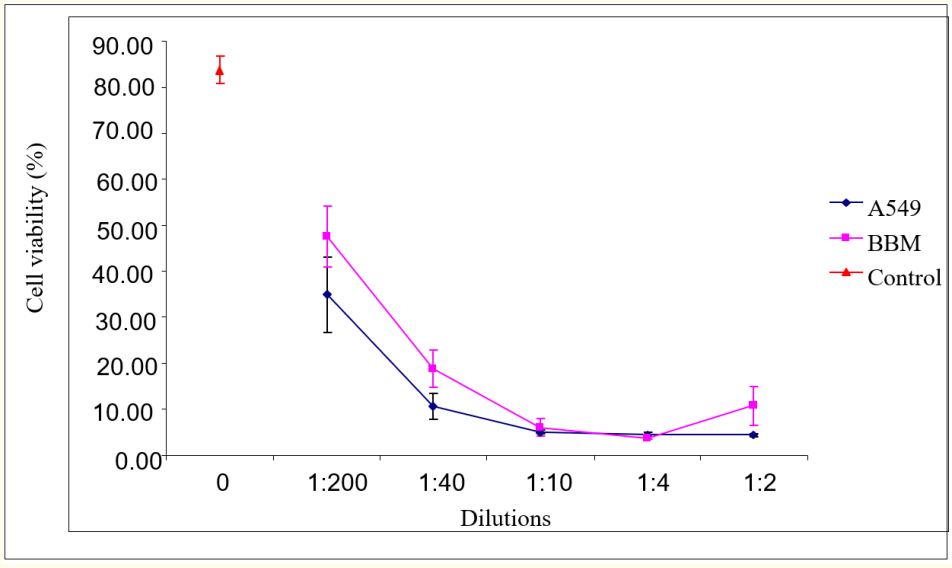

Figure 1: MTT dose response curve for A549 and BBM cell viability with different dilutions and represented in mean and 95% CI.

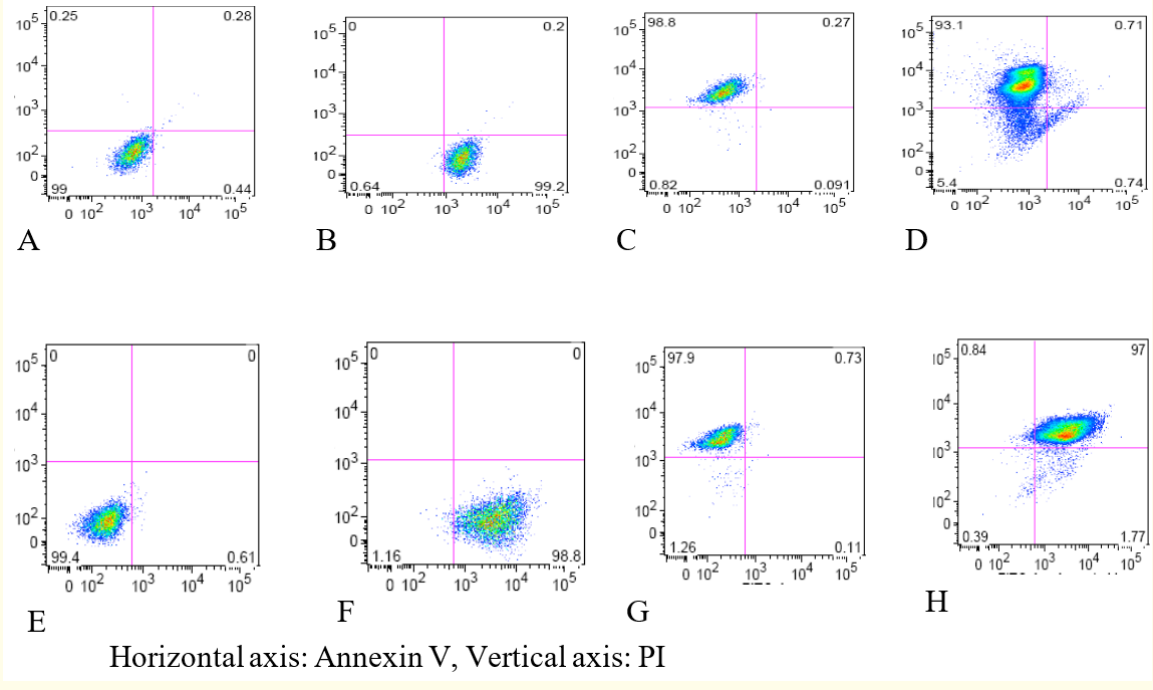

Figure 2: A549 flow cytometric analysis (A) Unstained cells. (B) Cells stained (Annexin). (C) Cells stained (PI). (D) Actinomycin D (positive control) stained (Annexin and PI). BBM flow cytometric analysis (E) Unstained cells. (F) Cells stained (Annexin-V). (G) Cells stained (PI). (H) Cycloheximed (positive control) stained (Annexin and PI).

dust is toxic to cell lines, where 87% (A549) and 99 %(BBM) were in the final stage of cell death (necrosis).

The cellular apoptotic and necrotic effect was confirmed after cells were exposed to house dust extracts and measured using a fluorescence microscope. The image analysis was done at the University of KwaZulu Natal (UKZN), Optics and Imaging Unit and the Department of Microscopy; and the results are presented in Figure 4. Cells appear with double colours, the inner colour is orange while the outer colour is green. This is due to the double staining used, namely green (apoptosis) and orange (necrosis). The selected samples that are shown in Figure 4 are representative of cellular changes that A549 cells underwent (treated and controlled) during fluorescence microscope observation. Thus, one can say that the cells underwent apoptosis cell death stage and some necrosis stages. The limitation for BBM cells, which is not shown in this diagram due to the lack of cells to attach to cover slips. For this reason, we could not report whether BBM cells underwent any cellular changes during treatment. Despite the BBM results not being reported in this experiment, the toxicity results confirm that the house dust extract induced cell death.

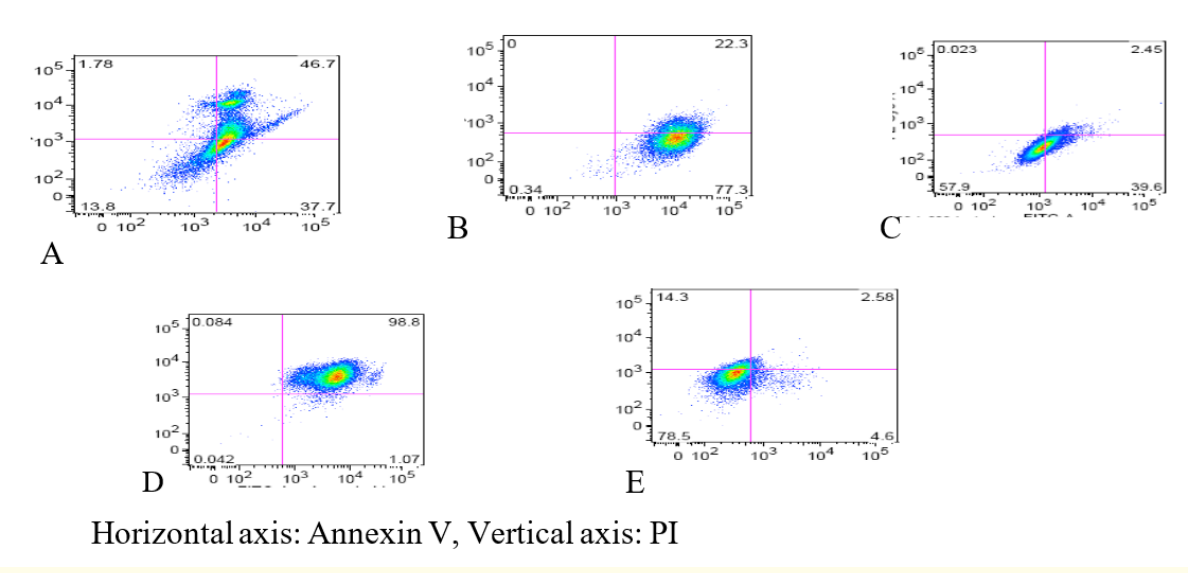

Figure 3: A549 cells were treated with house dust and stained with Annexin V and PI. (A) 25% necrotic. (B) 62% apoptotic. (C) Non-viable cells. BBM cells (D) are 99% necrotic. (E) Non-viable cells.

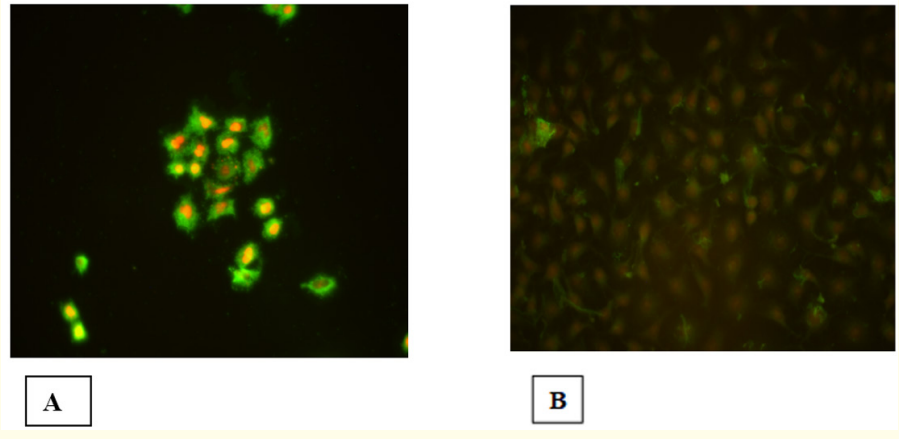

Figure 4: Fluorescent microscopic analysis of apoptosis A549 stained with Annexin-V-FLUOS (green) and propidium iodide (orange). (A) A549 sample. (B) A549 control.

Discussion and Conclusion

The degree of cell activation membrane lysis and mitochondrial ultra-structural toxicity was observed when cells were treated with various dilutions of house dust extract. The ability of house dust extract to lower cell viability was observed in this study with cell reduction less than 50% following house dust extract exposure. In the dilution of house dust extract ability to low cell viability for human bronchial epithelial cells was observed to be about <40% for A549 and <50% for BBM. The suitable dilution (1:10) of house dust extract used was selected for further analysis in this study because both cell lines are congruent at this point.

The apoptotic and necrotic ability of cells when treated with house dust extract was further verified by Annexin-v-fluos assay as to confirm what has been reported in MTT assay. Apoptosis is the key feature of programmed cell death which leads to variety of cellular changes such as blebbing, changes in cell membrane and necrosis can be defined as the traumatic cell death that can results in acute cellular injury and noticeable by the destruction of cellular DNA [22].

In this study it was observed that human bronchial epithelial cell lines underwent a degree of apoptosis and necrosis. A549 cells responded differently towards the house dust extract with 62% apoptosis; whilst, 99% of BBM cells underwent apoptosis. The cellular changes were further confirmed (fluorescent microscope) for A549 cells, which was not seen in BBM cells due to the difficulties of cell attachment on coverslips after staining. Previous researchers have reported on cellular death characteristics such as poor cellular shrinkage, and round poorly adherent cells; unattached round shape cells were evident in this study [4,23]. The degree of cell death was different between the two cell lines. It's made sense due to fact that one is normal (BBM) and A549 is a cancerous cell line.

The ability of normal and carcinoma cells was tested with various test agents to assess the ability of the human cell line to activate the apoptosis pathway. It has been reported that the human cell line cytotoxicity effect is dose-depend on response which is regulated by different foreign dilutions. Another study has shown high mortality (84% -1%) for A549 when treated with organic dust for 24 24-hour incubation period and it is high when compared with what has been seen in this study [24]. Furthermore, [25] have reported a comparable cell mortality rate with this study, which was observed when A549 was treated with tyre debris after 24 hours which ranged (18%- 39.8%). In another study, [26,27] have further confirmed what has been reported in this study, when A549 was treated with *Scutellari barbata* and reported (2.34% -37.43%) mortality after 24 hours.

According to our knowledge, not much data has been published concerning BBM's role undergo apoptosis when induced by foreign materials. The limited data on BBM leaves us with no choice but to report on BEAS-2B findings since BBM was derived from it. The ability of BEAS-2B to mortality (40% -50%) has been reported which was noticed after 92 hours when cells treated with oxide nanoparticles [25]. The decline in cell mortality has been further confirmed which was seen to be just below 40% when BEAS-2B treated with DMSO [28]. Furthermore, [29] reported reduced cell viability which was below 50% when cells were treated with ultrafine titanium dioxide particles.

It can be concluded that human exposure to contaminated indoor air pollutants may be detrimental to humans and that the duration of exposure is critical. The study has shown us that house dust extract is toxic to cells and humans in the household studies are at risk from this house dust exposure. In future studies *in vivo* studies should be conducted with settled house dust particles as to further verify these findings.

Conflict of Interest

No conflict of interest

Acknowledgements

Many thanks to Dr Nkosana Jafta for his role during sampling, which was critical for the success of this study; as well the households that gave consent for this study to take place. National Research Foundation (NRF) is thanked for the funding which made this research possible.

Bibliography

1. J Laquatra. "Indoor Air Quality". in IntechOpen, (2018): 1-14.
2. P Kumar., *et al*. "Biological contaminants in the indoor air environment and their impacts on human health". Nov. 01, (2021).
3. LG Gregory and CM Lloyd. "Orchestrating house dust mite-associated allergy in the lung". (2011).
4. OM Morakinyo., *et al*. "Health outcomes of exposure to biological and chemical components of inhalable and respirable particulate matter". Jun. 14, (2016).

5. I Manisalidis., *et al*. "Environmental and Health Impacts of Air Pollution: A Review". Feb. 20, (2020).

6. M A Calderón., *et al*. "Respiratory allergy caused by house dust mites: What do we really know?". (2015).

7. F Kastury., *et al*. "In Vitro, in Vivo, and Spectroscopic Assessment of Lead Exposure Reduction via Ingestion and Inhalation Pathways Using Phosphate and Iron Amendments". *Environmental Science and Technology* (2019).

8. W Yuan., *et al*. "In vivo and in vitro inflammatory responses to fine particulate matter (PM2.5) from China and California". *Toxicology Letter* 328 (2020): 52-60.

9. K T Rim and C H Lim. "Biologically hazardous agents at2 work and efforts to protect workers' health: A review of recent reports". *Safety and Health at Work* 5.2 (2014).

10. I Silins and J Högberg. "Combined toxic exposures and human health: Biomarkers of exposure and effect". *International Journal of Environmental Research and Public Health* 8.3 (2021): 629-647.

11. K Kupcho., *et al*. "A real-time, bioluminescent annexin V assay for the assessment of apoptosis". *Apoptosis* 24.1-2 (2019): 184-197.

12. G D Albano., *et al*. "Impact of Air Pollution in Airway Diseases: Role of the Epithelial Cells (Cell Models and Biomarkers)". (2022).

13. R Mphahlele., *et al*. "Prevalence, severity and risk factors for asthma in school-going adolescents in KwaZulu Natal, South Africa". *BMJ Open Respiratory Research* 10.1 (2023).

14. RN Naidoo., *et al*. "Ambient pollution and respiratory outcomes among schoolchildren in Durban, South Africa". *SAJCH South African Journal of Child Health* 7.4 (2013): 127-134.

15. C D Earle., *et al*. "High-throughput fluorescent multiplex array for indoor allergen exposure assessment". *Journal of Allergy and Clinical Immunology* 119.2 (2007): 428-433.

16. TA., *et al*. "The relevance of allergen exposure to the development of asthma in childhood". *The Journal of Allergy and Clinical Immunology* 105.2 (2000): 503-508.

17. NQ Vuong., *et al*. "In vitro toxicoproteomic analysis of A549 human lung epithelial cells exposed to urban air particulate matter and its water-soluble and insoluble fractions". *Part Fibre Toxicology* 14.1 (2017).

18. Ashiru., *et al*. "Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes". *Cancer Research* 70.2 (2010): 481-489.

19. S Padilla-Arellanes., *et al*. "Cytotoxicity of a lipid-rich extract from native mexican avocado seed (Persea americana var. drymifolia) on canine osteosarcoma d-17 cells and synergistic activity with cytostatic drugs". *Molecules* 26.14 (2021).

20. AL Hodges., *et al*. "TNF family cytokines induce distinct cell death modalities in the A549 human lung epithelial cell line when administered in combination with ricin Toxin". *Toxins (Basel)* 11.8 (2019).

21. S A Lone., *et al*. "Cellular apoptosis and necrosis as therapeutic targets for novel Eugenol Tosylate Congeners against Candida albicans". *Scientific Report* 10.1 (2020).

22. S Elmore. "Apoptosis: A Review of Programmed Cell Death". (2007).

23. JP Cobb., *et al*. "Mechanisms of cell injury and death". *British Journal of Anaesthesia* 77.1 (1996): 3-10.

24. MG Dehcheshmeh., *et al*. "Effect of water-soluble PM 10 on the production of TNF-α by human monocytes and induction of apoptosis in A549 human lung epithelial cells". *Journal of Environmental Health Science and Engineering* 19 (2021): 143-150.

25. A Grilli., *et al*. "Transcriptional profiling of human bronchial epithelial cell BEAS-2B exposed to diesel and biomass ultrafine particles". *BMC Genomics* 19.1 (2018).

26. Y Gu., *et al*. "Advances in Anti-Cancer Activities of Flavonoids in Scutellariae radix: Perspectives on Mechanism". (2022).

27. H H Yin., *et al*. "Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning". *European Journal of Neuroscience* 19 (2004): 181-189.

28. K C Chang., *et al*. "TNF-α Mediates Eosinophil Cationic Protein-induced Apoptosis in BEAS-2B Cells". *BMC Cell Biology* 11 (2010).

29. MS Acar., *et al*. "Titanium dioxide nanoparticles induce cytotoxicity and reduce mitotic index in human amniotic fluid-derived cells". *Human and Experimental Toxicology* 34.1 (2015): 74-82.