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Toxicity of Indoor Dust-Derived Trace Elements and Organic Contaminants Impact on Normal Human Colon Epithelial Cells

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TOXICITY OF INDOOR DUST-DERIVED TRACE ELEMENTS AND ORGANIC CONTAMINANTS' IMPACT ON NORMAL HUMAN COLON EPITHELIAL CELLS

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Professor Shishir Shishodia, Ph.D., Advisor

Indoor dust stands out as a burgeoning challenge in society due to its implication on people's health. Exposure to toxic dust particles occurs in different ways that include inhalation, ingestion, and dermal contact. In the hindsight, many people spend most of their time indoors exposing themselves to high levels of dust. Notably, different groups of individuals present varied risk levels to the toxicity of particles with vulnerable persons being susceptible to adverse effects of these contaminants. This includes the elderly, children, and immunocompromised individuals. The study examines the impacts of two variables that are trace elements (TMD) and organic contaminants (OD) on a normal human colon cell (CCD841) that is exposed to indoor dust. In this case, cultures of the cells were made and treated with varying concentrations of TMD and OD. Then, different factors were observed to highlight the impact of each sample on the cultured cells. Consecutively, viability, apoptosis, cytotoxicity, oxidative stress, and inflammation were observed. The methods used in the experiment included the performance of MTT assay, protease cell viability, apoptosis 3/7 caspase, LDH release, reactive oxygen species, and

inflammasome caspase-1 assay. In this case, two variables were treated to these experiments while varying their concentrations.

The findings indicated significant figures when both variables had higher concentrations. This supported the research hypothesis where prolonged exposure to indoor dust results in huge damage to the body, specifically the normal colon cells for this study. The research points out to the importance of averting indoor air pollution. The effects of indoor dust samples in activation of oxidative stress by measuring the generation of reactive oxygen species (ROS), was noticeable which suggest weakening the immune system, and the human body becomes an easy target to invade. Inflammasome-Caspase-1 also was induced when being exposed by both dust samples. Although, both variables TMD and OD have a positive correlation, The results shows that a significant change in the culture cells meant an increase in the concentration of both TMD and OD, and it is hard to be determined which one of these two samples is more toxic to normal human colon cells; but if they both are combined and penetrate at a cellular level, there is a high chance to cause a serious harm.

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LIST OF ABBREVIATIONS

CCD841	Normal human epithelial colon cells
CD	Crohn’s Disease
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
DDT	Dichloro-diphenyl-trichloroethane
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EMEM	Eagle's minimum essential medium
FBS	Fetal Bovine Serum
GF-AFC	Glycylphenylalanyl- Amino fluorocoumarin
GIT	Gastrointestinal Tract
HMGBl	High Mobility Group protein Bl
H_2O_2	Hydrogen Peroxide
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IL-18	Interleukin-18
IL-ß	Interleukin 1 Beta
LDH	Lactate Dehydrogenase
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

NBF	Nitrogen Fixing Bacteria
NIST	National Institute of Standards and Technology
OD	Organic contaminants in Dust
PBS	Phosphate-buffered Saline
PM	Particulate Matter
PS	Phosphatidylserine
ROS	Reactive Oxygen Species
SOD_2	Superoxide Dismutase 2
TMD	Trace Metal Dust
UC	Ulcerative Colitis

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CHAPTER 1

INTRODUCTION

Dust is among the most exasperating annoyances often experienced by the building occupiers. This issue can be effectively addressed by identifying the sources of dust and suitable solutions to be introduced. Indoor dust can be classified into three categories. These include dust carried or walked inside, blown-in, and trapped and attracting dust like magnets. There are various sources of dust. Firstly, there are natural sources, which include outdoor and indoor. Outdoor dust usually contains organic particles caused by decomposing leaves, soil erosion, bacteria, viruses, insect parts, fragmented cockroaches, plant matter, household fireplaces, and bush fires (Ali, 2021). Indoor dust includes human skin, hair and flakes, dust mites, food crumbs, rodent excrement, papers and books, and cigarette smoke. Outdoor dust is usually from inorganic materials. The offending sources include heavy metals like lead, asbestos dust, arsenic, volatile organic compounds mainly from agricultural DDT, pesticides, fertilizers, herbicides, lawn mowing, and vehicle exhaust. Nonetheless, where there is reliable evidence of organic matter, the dust is likely to have originated from inorganic sources.

On the other hand, indoor dust combination indicates fabric in pillows, mattresses, curtains, flame retardants, and blinds. This dust particulates in the fax machine, photocopiers, and frying and barbequing smoke. Once individuals have established the

cause of dust, they should start confirming the problem source. Organic dust can be wiped using a damp microfiber cloth on surfaces. A vacuum with a HEPA filter and a powerhead can easily remove organic dust (Shi & Wang, 2021). In this case, a person can rinse the microfiber between rooms and surfaces. Consequently, inorganic dust reacts to the vacuum processes and microfiber cloth. Therefore, the best methods should be utilized and determine the best one that can remove dust effectively.

Exposure routes are how contaminants enter a person or people after contact. In essence, exposure can occur through various routes, including ingestion, inhalation, or dermal. Inhalation exposure results from breathing contaminated air with certain particles like dust, vapors, or aerosols (Wang et al., 2019). In this regard, a person may be exposed through inhalation route during various outdoor and indoor activities. Consequently, indoor receptors may also be exposed to outside contaminants that usually infiltrate the indoor surrounding.

In most cases, pollutant levels found in house dust, such as the past banned compounds, are the main sources of exposure for the population, specifically children (Lee et al., 2020). Indoor dust is a repository and sinks for semi-volatile organic compounds. Accordingly, indoor compounds and dust absorbed into the house can enter the human body by inhaling re-suspended and suspended particles via non-dietary dust ingestion (Zhang et al., 2020). This occurs through ingestion of elements adhering to surfaces in buildings, food, and on the skin by absorption. The amount of dust inhaled and left in various alveolar tract parts is reliant on the aerodynamic diameter. Exposure to indoor dust does not ultimately occur through inhalation. For example, ingestion of building dust

particles adhering to objects, the skin, and food or direct absorption via skin may be the main exposure routes.

Indoor dust is a common household issue that people deal with daily (Cao et al., 2020). It is unlikely to do away with it permanently since it constitutes things from the outside and within. Some of the known causes of indoor dust include fallen insects, food particles, fibers, bacteria, smoke, and lead (Zhou et al., 2020). These elements accumulate in their most miniature form, making them hard to recognize at times. Not only do they make the place dirty, but they are associated with health and environmental problems (Rahman et al., 2021).

As mentioned above, individuals' environmental well-being and physical health are at risk due to the presence of indoor dust (Entwistle et al., 2019). Physically, exposure to indoor dust is attributed to respiratory issues, eye problems, and throat complications (Soleimani et al., 2020). Living in an environment dominated by such threats could be hazardous due to the impending dangers on one's health and wellness. Air is another factor that is polluted by indoor dust because when the particles are blown, they interfere with breathing air. Finally, soil pollution is also a probable cause of indoor dust, especially when contaminated particles such as lead land on it.

As much as indoor dust is exposed to external body parts, it can also be ingested. In its digestible form, it can result in various health complications depending on what is consumed. For example, eating exposed foods can cause food poisoning, a digestive complication (Dingle et al., 2021). Also, the consumption of lead, one of the renowned harmful metals, could result in life-threatening issues (Hu et al., 2019). Finally, as an allergic stimulator, indoor dust is even more dangerous to people with allergic reactions.

Aims and Objectives

Hypothesis

If normal human colon (CCD 841) cells are exposed to dust particles, there will be a reduction in cells viability, increase in cytotoxicity, cellular death, reactive oxygen species, and inflammation. The aims of this study were to:

1. Examine the effects of dust samples on CCD841 cell viability using MTT and GF-AFC Assays
2. Examine the effects of dust samples on CCD841 cell cytotoxicity using LDH release Assay.
3. Examine the effects of dust samples on CCD841 cell activation of reactive oxygen species.
4. Examine the effects of dust samples on CCD841 cell inflammatory using inflammasome caspase 1 Assay.

CHAPTER 2

LITERARY REVIEW

Millions of bacteria make up the gut microbiome. Scientists have engaged in extensive studies on the effect of the bacteria on human health. The influence of the gut microbiome bacteria on the disease risk and general human health is quite significant. In addition, the effect of the bacteria on essential organs like the heart is an area under research. Environmental factors such as diet and pollution are known to affect the gut microbiome. Air pollution is the main environmental factor that alters the activity of the gut microbiome (Sbihi et al., 2019). This can lead to debilitating diseases. It means that the air quality across the cities affects the health of the guts. Exposure to environmental pollutants leads to a change in the microbiome. Such changes can lead to Inflammatory Bowel Disease (IBD). The IBD includes serious conditions like ulcerative colitis and Crohn's disease. These are lifelong conditions that do not have a particular cure. That is to say, air pollution can lead to life-threatening diseases. Weak body immunity leads to the above illnesses. As a result, the body starts self-attacking, and this leads to inflammation and ulcers in the gut. Ulcerative colitis influences the large intestine. On the other hand, Crohn's disease mainly affects the gut. This means that the two conditions can lead to poor quality of life. Smoke, soot, pollen, dust, sulfur dioxide, nitrogen oxide, and carbon monoxide are the pollutants known to cause the disease and even death in extreme cases. Air pollutants lead to serious health conditions including asthma, diabetes, Alzheimer's

disease, strokes, heart attacks, and lung diseases. Cigarettes are the common causes of air pollution in the household. The act of inhaling cigarette smoke exposes one to the risk of developing Crohn's disease. This is the major environmental risk that leads to IBD.

Inflammatory Bowel Disease, Ulcerative Colitis Disease, and Crohn's Disease

Inflammatory Bowel Disease (IBD) is an intestinal condition that affects many people across the globe. The ulcerative colitis (UC) and Crohn's disease (CD) are the major IBD forms. These conditions are known to originate from the interplay between gut microbiota and the immune system. Environmental pollutants contribute to chronic disorders associated with IBD. In Western countries, the IBD incidence has been relatively stabilized. On the other hand, the disease has emerged in developing worlds, where it was not witnessed. This is attributed to controls concerning environmental pollutants in the West. The developing worlds have not developed proper protocols to address the problem of pollution. This has led to an increase in the incidence of IBD risk. The popularity of the Western lifestyle across the globe and industrialization have contributed to the UC and CD. The fumes produced by factories increase the level of air pollution and this exposes people to high risks of developing diseases. Moreover, aspects like diet contribute to the worsening of the IBD symptoms. The environmental changes associated with the exposure to particulate matter from industries have increased the incidence of IBD. Industrialization has contributed to the increased release of particulate matters and toxic substances to the atmosphere. There is enough evidence which suggests that air pollution contributes to cardiorespiratory disorders. Moreover, the effect of air pollution on deleterious gastrointestinal effects has been established. Air pollution can lead to intestinal injury through immune response alterations, epithelial cells adverse effects, and gut microbiota

modulation. Epidemiological evidence indicates that there exists a relationship between IBD and air pollution. Although it is an area under research, the few studies available indicate that air pollution contributes to IBD. It has been established that exposure to high amounts of sulfur dioxide and nitrogen dioxide leads to an increase in the risk of developing UC and CD. Industrial fumes contain high amounts of sulfur dioxide and nitrogen dioxide. This is the main reason why industrialized countries record high levels of UC and CD. The risk of developing the two conditions is high in young adults and children. This means that age-related constraints affect the incidence of gut illnesses.

Celiac Disease

Continuous exposure to pollutants that are organic in nature is the major cause of celiac disease. The disease is known to lead to small intestine damage. This is because the immune system of the people with the disease reacts to gluten. This leads to an autoimmune response. The celiac disease results in abdominal bloating and pain, constipation, gas, nausea, and diarrhea. If the problem persists for a long time, it can lead to nervous system problems. It can also result in osteoporosis and problems relating to reproductive health (Salim et al., 2014). Exposure to pesticides can lead to celiac cases. In areas where agricultural activities are common, pesticides are the major air pollutants. People breathe pesticides as fumes when they are sprayed on crops. High-level exposure to pesticides leads to gluten intolerance. Moreover, the increased use of non-stick chemicals among women exposes them to the risk of developing celiac disease. The risk of this illness is high when one is exposed to dust particles from products such as Teflon. Moreover, fire-retardant chemicals are the major cause of the disease among males. Toxic chemical substances have a direct effect on celiac disease. The substances also have a great influence on the hormone

levels. This leads to developmental disorders and thus affecting proper growth and development.

Irritable Bowel Syndrome and Allergy

Irritable Bowel Syndrome (IBS) affects about 20% of the world population. It is mainly classified as a gastrointestinal disorder. It refers to a group of conditions that present similar symptoms. IBS is mainly assessed through the symptom-based criteria. IBS is associated with discomfort and chronic abdominal pain. Moreover, it is characterized by irregular bowel movement (Bonenberger et al., 2011). IBS can lead to food intolerance, mucosal inflammation, allergic disease, and hypersensitivity. It should be noted that allergic disease is mainly caused by pollutants such as pollen and dust mites. Exposure to allergens leads to the worsening of the disease. Allergic disease and IBS are closely related to the effect of dust particles on IBS. The release of mast cells leads to hypersensitivity in allergic patients. Such kind of reaction is associated with the binding of antibodies, especially immunoglobulin, to the mast cells. Allergic diseases have a great effect in terms of anaphylactic reactions and severity (Loo et al., 2020). This can lead to atopic dermatitis, allergic rhinitis, and recurrent asthma. Allergy is mainly diagnosed based on the allergen-associated symptoms. Mucosal inflammation and immune activation can lead to IBS which mainly involves inflammatory reactions that interfere with the functioning of the epithelial cells. As a result, this can lead to the permeability barrier of the epithelium which causes inflammation (Mutlu et al., 2018). Atopic IBS is the major subcategory of IBS that has a close link to allergic diseases. The severity of IBS is known to be high in the environment with numerous air pollutants. This is because pollutants increase the particulate matter concentration leading to the worsening of allergic reactions and IBS. Pollutants can lead to

the destruction of delicate internal organs. The intestines and the lungs are the most affected internal organs when one is exposed to dangerous dust particles. The fine dust particles can get into the bloodstream where they are circulated to other body parts. The dust particles that contain heavy materials can lead to multiple organs failure. Therefore, dust particles can be toxic to the body (Wildenberg & van den Brink, 2016). People need to stay in an environment that is free from dust. This can lead to effective control of IBS and allergic diseases. Every year people use a lot of resources to treat such kind of illnesses. This has resulted in slow financial growth as the available resources are used for the treatment of various ailments.

Asthma is a major recurrent condition that is characterized by hyperresponsiveness, obstruction, and inflammation. There is a close link between IBS and asthma. Patients suffering from asthma have a high risk of developing IBS. Those who have asthma experience hyperresponsiveness. This is the main factor that leads to the worsening of IBS symptoms. Bronchial hyperresponsiveness and reflux symptoms occur more often in IBS patients. There is a high likelihood of IBS patients developing asthma and vice versa. This attributed to prolonged exposure to dust particles. Moreover, the inhalation of dust from oral steroids can lead to the development of asthma and IBS. Asthmatic patients are highly sensitive to dust in the environment. Exposure to indoor dust can lead to reactions like sneezing and coughing. In extreme cases, when asthmatic patients are exposed to dust particles, they may experience difficulties in breathing. This means that asthmatic people need more clean air than an average human.

Gut Microbiota

Microbiota comprises several species of microbial organisms that have a specific host. Microbiota may include bacteria, protozoans, archaea, and fungi. The highest exposure to environmental toxins occurs in the gastrointestinal tract (GIT). The GIT has an approximate area of the 400-meter square. This means that the effect of environmental allergens is high in the GIT. There is a wide range of microorganisms in GIT. There are more than 12 phyla and 2,000 species of microorganisms in the GIT (Lazar et al., 2018). Such organisms have a great effect on the functioning and DNA of the GIT. GIT microbiota has great diversity. The variations are affected by factors such as sanitary conditions, geographic factors, immune system, age, gender, genetics, and health conditions. A wide range of microbiota is present in healthy individuals. The GIT is mainly colonized during birth. The exposure to the placental microbiome leads to transfer for fusobacteria, Bacteroidetes, tenericutes, proteobacteria, and firmicutes. The genera available in the oral cavity, vagina, and amniotic fluid lead to full-term infant meconium.

Microbial communities play a great role in mammalian processes. These include the aspects like metabolism and the ancillary barrier function of mucosal. It also helps in the host immune response modulation. This is common in the gastrointestinal tract. The adaptive immune environment can be altered dramatically by the bacterial species. Fecal samples of infants may indicate the risk of clostridium difficile and Escherichia coli. This is the major risk factor for allergic diseases in infants. This implies that the exposure of parents to dust may lead to complications that are passed to the children. The health of the mother determines the health of the infant. Allergic infant disease can lead to adaptive immune responses (Jin et al., 2017). Childhood asthma has also increased due to exposure

to antibiotics. The interaction of children with animals like cats and dogs can lead to allergic reactions. This is because infants are influenced by high levels of pet dander dust. The inhalation of such dust leads to allergic reactions. The immune response of an infant is lost when the child is exposed to harmful chemical pollutants in particulate matters. Exposure to environmental pollutants worsens allergic reactions. House dust contributes to the allergic reactions that people record. House dust poses a risk to asthmatic patients. To assess the effect of house dust, it is important to evaluate the amount of particulate matter in a room. This will help in the estimation of the level of pollution in it. It should be recognized that the house dust is mainly comprised of outdoor pollutants. The materials like pollen can get to the house and lead to indoor pollution. House dust increases the risk of gastrointestinal defects. This implies that house dust not only affects the lungs but the gut as well.

House Dust Exposure

Humans experience a high-level exposure to microbes in the environment. Microbial colonization in the mammalian systems leads to immune homeostasis. The burden of chronic illnesses is high in the industrialized states due to increased exposure to environmental pollutants. The household environment is the major contributor to the microbes that affect humans. The indoor exposure to microbes leads to allergic reactions and inflammatory infections (Shan et al., 2019). The control of the level of indoor pollution can lead to the reduction of allergic reactions and adverse effects. The gastrointestinal microbiota is known to regulate CD8 T and CD4 cell generation. Exposure to house dust contributes to allergic reactions in the airways. Airway protection is mainly related to the activation of T-cells. This leads to the development of immune response against foreign

particles. However, the increased exposure to toxic dust can lead to serious damage to the respiratory system (Fujimura et al., 2014). This implies that humans require clean air in order to operate effectively. Host immune response is essential in the protection of organisms form adverse effects of dust. The exposure to bacteria, fungi, and viruses may affect the gastrointestinal tract. This can lead to a slow-down of body functions. The host immune responses can lead to a great decrease in the number of bacteria in the airways. The exposure to dust leads to immune responses that are essential in the protection of the body against foreign materials. Airway protection is mainly facilitated by protective microbiota. This implies that some form of bacteria is helpful in the protection of the airway. The main therapeutic strategy that can help to prevent people from allergic airway diseases and pulmonary diseases is gastrointestinal microbiome manipulation. This kind of therapy is very effective and can help people develop the desired immunity.

CHAPTER 3

DESIGN OF THE STUDY

Reagents

Eagle's Minimum Essential Medium (EMEM), Feta Bovine Serum (FBS), Phosphate-Buffered saline (PBS), and Antibiotic-Antimycotic (Anti-Anti) were purchased from Gibco by Life Technologies. Dimethyl Sulfoxide (DMSO) was purchased from Thermo Fisher Scientific (USA).

Indoor Dust Samples & Preparation

Trace metal indoor dust and Organic Contaminants of indoor dust are the two types that were used in the study. These dust samples were pursued from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). In each 1 ml of phosphate-buffered saline, a 50 mg of each dust types was diluted, vortexed, transferred to a dark glass vail, and finally stored -80°C refrigerator. Fresh Concentration of dust samples concentrations were prepared in 5 ml of a complete media. Eight 25 ml tubes were used for each dust samples and final the concentrations were as followed. (0, 10, 25, 50, 75, 100, 250, 500 µg/ml). After use, these concentrations were refrigerated in -20ºC for future use.

Cell Culture of CCD841

After receiving CCD841 cells (ATCC® CRL-1790™), the cells have been transformed from variants of primary cultures of normal human colon epithelial cells.

CCD841 cells were cultured in pre- prepared media Eagle's Minimum Essential Medium (EMEM) which has been supplemented with 5% Feta Bovine Serum (FBS), 0.5% EGF, and 1% antibiotic-antimycotic, and lastly incubated.

Cell Viability Assays (MTT & Protease Viability)

MTT Assay Overview: MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide designates tetrazolium salt which is usually used to identify the presence of reductive metabolism in cells for proliferation, cytotoxicity, and viability (Harris, 2019). Upon reduction, tetrazolium salts change into products that are deeply colored and can easily be measured using colorimetric. A purple formazan product is formed by MTT which has to be solubilized before any quantification (Lowry et al., 1951).

Protease Viability Assay Overview: (GF-AFC Assay): Protease Viability Assay (GF-AFC Assay) is a cell-permeable fluorogenic protease substance that is used to measure live-cell protease activity. It is worth noting that GF-AFC Assay is split by a live-cell protease to generate a proportional fluorescent signal that is used to determine the number of cells that are viable (Wang et al., 2017). This method has a shorter incubation time of 0.5-1 hour as compared to that of tetrazolium assays of 1-4 hours. This approach gives room for multiplexing with other existing assays in similar sample cells such as bioluminescent since it does not allow cells to lyse.

Cell Cytotoxicity Assay using Lactate Dehydrogenase Release
Lactate Dehydrogenase Release Overview

This is a colorimetric assay that avails a reliable and simple means of determining the cytotoxicity of cellular. Lactate dehydrogenase is a cytosolic enzyme found in different

types of cells that are usually released within the cell culture medium after the plasma membrane is damaged (Bonini et al., 2002).

Cell Death by Apoptosis Assay (Caspase-Glo 3/7 Activation) Overview

It detects and quantifies cellular events that are associated with cell death that are programmed including caspase activation, DNA fragmentation, and exposure of cell surface to phosphatidylserine (PS) (Hammerling, 2012). Types of these assays include the luminescent Caspase-Glo Assays which are used to identify the availability of caspases that are either involved in extrinsic or intrinsic apoptosis pathways.

Extracellular Reactive Oxygen Species Detection Using (ROS-Glo™ H2O2 Assay) Overview

Extracellular reactive oxygen species (ROS) are made by living cells as cellular metabolic by-products that are normal. Cells usually produce large quantities of ROS under conditions that are stressful (Banks and Peters, 2010). Additionally, living organisms develop continuous response mechanisms in order to adapt to the exposure of ROS and use it as a molecule for signaling. ROS molecules generally initiate oxidative stress as a feedback mechanism that involves biological activities such as necrosis, autophagy, and apoptosis. Evidence shows that ROS plays an important role that signals molecules in the entire cell death pathway.

Inflammatory Assay Using Caspase 1-Inflammasome Activity Overview

Inflammasome-Caspase 1 assay is a simple, bioluminescent, and homogenous technique used to selectively quantify the caspase-1 activity of caspase-1 which is a crucial element of the inflammasomes. Inflammasomes refer to protein complexes that are induced by different inflammatory stimuli (Sittampalam, 2004). Inbred immune cells react to

pathogens and any other signal of danger with the formation of inflammasome and the change of procaspase-1 zymogen into caspase-1 that is catalytically active. Activation of caspase 1 leads to the production and release of cytokines IL-18 and IL-1β pyroptosis which is an immunogenic type of cell death (Sittampalam, 2004).

Software and Statistical Analysis to Obtain Experiment

Since the date obtained belong to a total of three independent experiment, standard deviation was used in statically analyze the data by using GraphPad Prism software generation 9, and probability value was calculated afterward to test the significance of experiments to determine if the hypothesis of the study was supported by the ran experiments or not.

CHAPTER 4

RESULTS AND DISCUSSION

Cell Viability Reduction of CCD841 After Treated with Indoor Dust Samples

A. MTT

The evaluation of cell viability is essential for determining the effectiveness of a novel medicine or treatment or effects of a component in this case pollutants. Cell viability is an assessment of a cell's health in general. Agents operating on cells, such as contaminants, have varying degrees of influence on the cell's survival and functioning. The vitality of cells is a good indicator of how they will react to pollutants (Adan et al., 2016). In this case, healthy human lung epithelial cells were exposed to two different forms of indoor dust, which were categorized based on their chemical contents. MTT test and GF-AFC analyses were used to examine cell viability 24 hours following exposure to dust at concentrations of 10, 25, 50, 75, 100, 250, and 500 g/ml. Both dust samples (Trace Elements dust and Organic pollutants dust) substantially decrease cell viability in a concentration-dependent manner (p0.5), as shown in Figure 1.

concentration-dependent manner (p0.5), as shown in Figure 1.

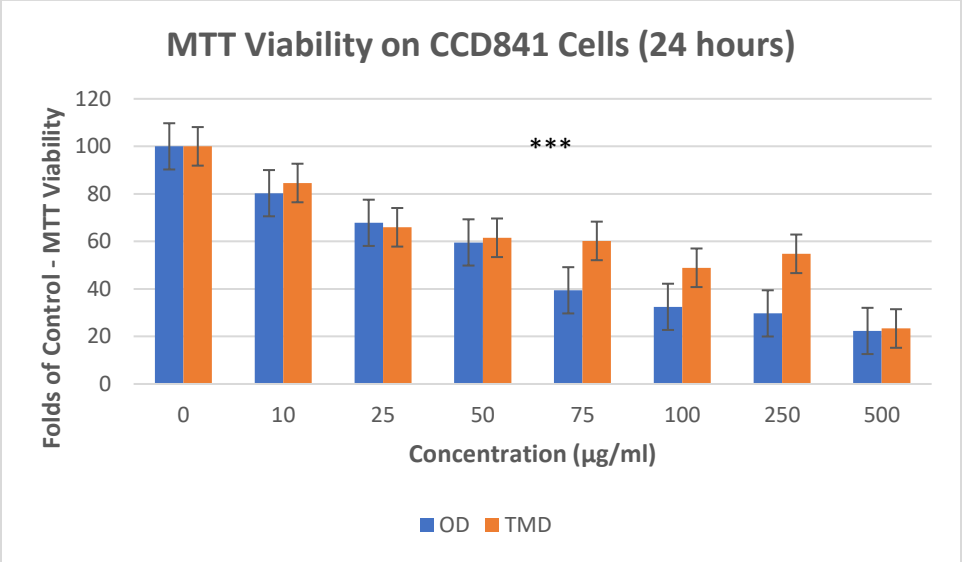

Figure 1: Cell Viability- MTT Assay
At higher concentrations cells rate are reduced

B. Triplex Protease

Cell viability was decreased by up to 85.5 percent for Trace Elements dust and 91.5 percent for Organic pollutants dust at higher concentrations (500 g/ml). The fraction of living, normal tissues in a population is called cell viability. Cell viability tests are used to assess the general health of cells, to improve culture or experimental conditions, and to assess cell survival after exposure to chemicals, including during a drug test. The study of cell viability is thought to be an important indication of cellular responses to contaminants according to Adan et al., 2016. After 24 hours of exposure to different quantities of indoor dust, the cells were examined using two methods: MTT assay and Protease analysis (GF-AFC) (Figure 2). The type of apoptosis was determined by annexin V and iodide propidium double staining. Fluorescent labeling and microscopy observations were used to assess the

reorganization of cytoskeletal proteins. Our findings reveal that the two forms of indoor dust reduced colon cell viability in a concentration-dependent manner.

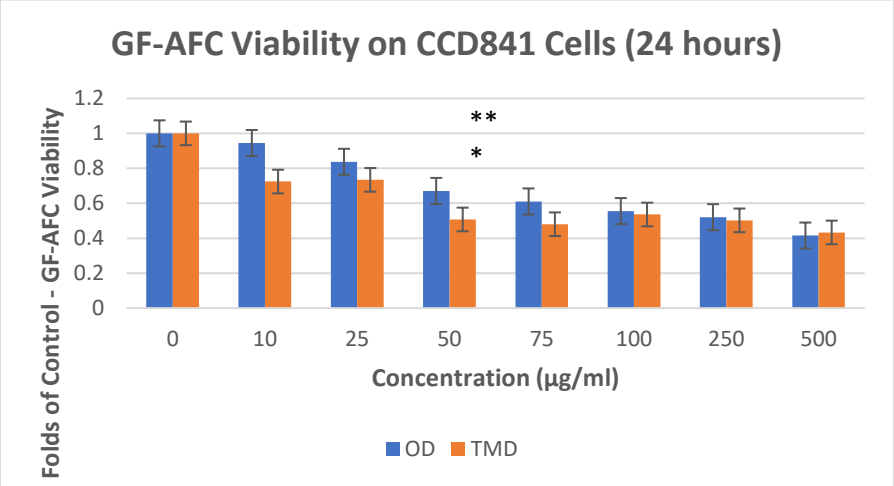

Figure 2: GF-AFC Viability Assay
At higher concentrations cells rate are reduced. Apoptosis Caspase 3/7

The stimulation of caspase-3 and -7 was examined after 24 hours of indoor dust exposure to investigate the process of CCD841 apoptosis. In comparison, dust exposure led to improved caspase activation beginning at 75 g/ml, proceeded by a concentration-dependent rise (Figure 3).

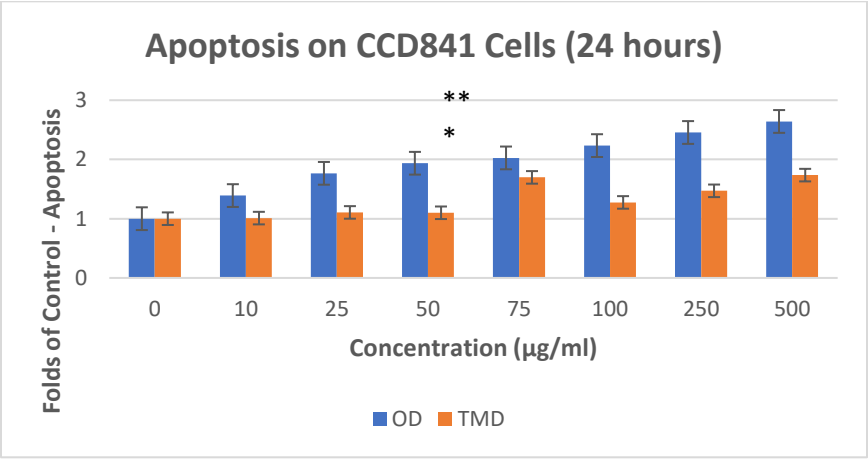

Figure 3: Apoptosis Caspase 3/7
At higher concentrations of dust samples cells death increased.

When the 100 g/ml concentration was employed, there was a considerable increase in caspase activity, which was accompanied by a drop-in activity until it recovered to the observed baseline level at the 500 g/ml concentration point.

These findings imply that apoptosis is the major method of cell death for moderate quantities of indoor dust, although high amounts might enhance alternative cell death pathways (necrosis). Caspases-3 and -7 can influence numerous morphological and biochemical modifications during controlled extrinsic or intrinsic cell death, including the creation of apoptotic cells and DNA fragmentation (Galluzzi et al., 2018). In the pathophysiology of respiratory illnesses and lung inflammation, apoptosis plays a significant role (Pierce et al., 2007). Investigating whether indoor dust causes apoptosis in lung cells might help researchers better diagnose and understand dust-related respiratory disorders.

The rise in caspase 3/7 signal after exposure to indoor dust indicates that cell death induction is the cause of the damage. Nevertheless, at higher dust dosage treatments, a drop in caspase 3/7 signal is found, along with an upsurge in cytotoxicity indicator (LDH), indicating an increased number of dead cells and suggesting apoptosis via the necrotic mechanism. This reaction demonstrates that, in addition to chemical content, dust treatment dosage impacts the sort of cellular death process as described by Nel et al., 2001. These findings back with the theory that the same substance can cause apoptosis at low quantities but necrosis at larger concentrations (Elmore, 2007). Reduced diesel fumes particles, for example, have been shown to cause cell apoptosis (Yun et al., 2009), whilst elevated doses have been shown to cause necrosis (Yun et al., 2009; Nel et al., 2001.

Increasing of Cell Cytotoxicity in CCD841 after Treated with Indoor Dust Samples (LDH Release)

LDH is a soluble cytoplasmic enzyme that is present in almost all cells and is released into extracellular space when the plasma membrane is damaged. In both healthy and disease pathologies, apoptosis and necrosis are two important mechanisms of cell death. Despite there being several methods for detecting apoptosis, there are just a handful for assessing necrosis. The softening of the plasma membrane is a crucial characteristic of necrotic cells. The release of the enzyme lactate dehydrogenase may be measured in tissue culture conditions to quantify this phenomenon (LDH). Testing LDH production is a good tool for detecting necrosis when used in conjunction with other methods.

In this experiment, following 24 hours of exposure to dust, the amount of LDH produced by CCD841 cells was evaluated. Low quantities from both forms of indoor dust did not produce cell membrane damage, as shown in Figures 4.

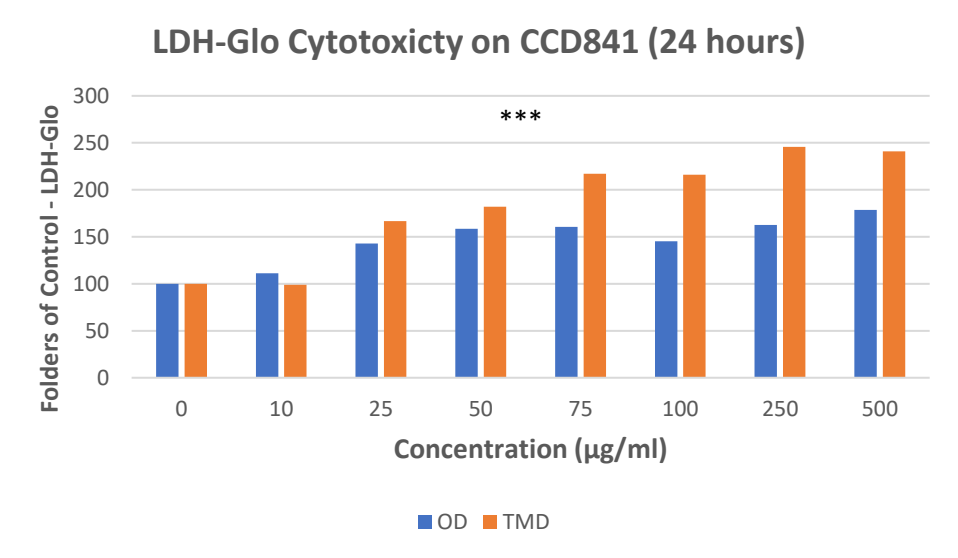

Figure 4: LDH-GLO Cytotoxicity

LDH-GLO increased with higher concentrations indicates that exposing to dust samples caused membrane damage of cells.

The LDH production, on the other hand, rose somewhat but not considerably at 100 g/ml. Cell membrane damage was mostly influenced by higher dust levels (250 and 500 g/ml). Treatments with 250 and 500 g/ml dust greatly enhanced LDH release levels by up to 4 times when compared to the untreated control.

Extracellular Reactive Oxygen Species Activation of CCD841 after Treated with Indoor Dust Samples

In CCD841 cells, hydrogen peroxides (H2O2) were utilized as additional oxidative stress indicators. In peroxisomes, H2O2 can be produced through maillard reaction of superoxide anion or by uncontrolled synthesis from molecular oxygen. Irrespective of its lower reactivity especially in comparison to other reactive species, H2O2 has a critical function in cancer development since it can dissipate across mitochondria and cell membranes, culminating in a diversified functioning result and potentially creating a range of cell injury or damage, for example, inflammasome creation, NFκB stimulation, and proinflammatory cytokines activation (Mates & Sanchez-Jimenez, 2000; Mittal et al., 2014; Ray & Husain, 2002).

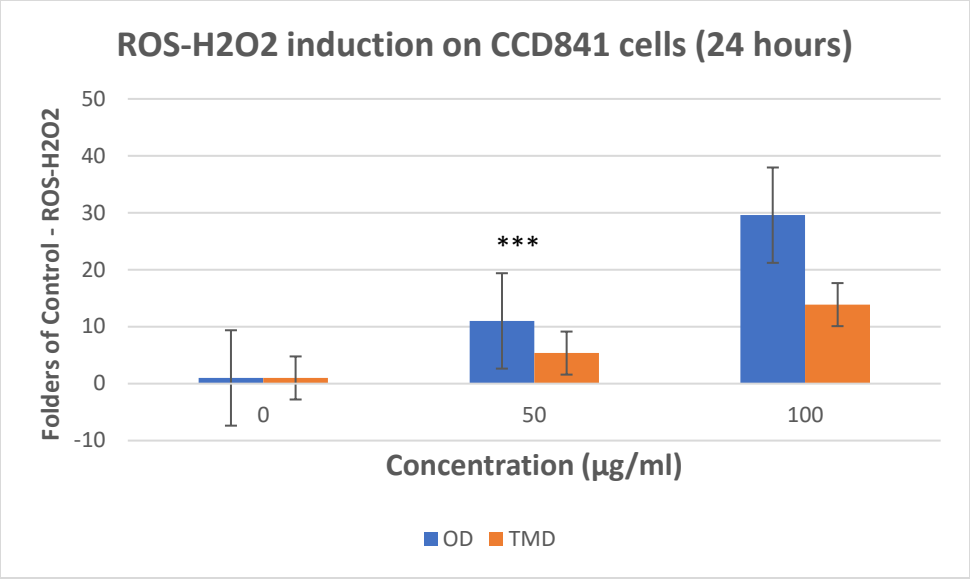

Figure 5: **ROS- H2O2 Induction in CCD841 cells.**

ROS-H2O2 release is significantly increased with increasing the treatment's concentrations.

When compared to untreated cells, indoor dust induced a considerable increase in H2O2 production after 24 h of treatment to 50 and 100 g/ml dust (Figure 5). The trace metal dust has a higher H2O2 emission capability compared to organic dust. Conversely, the cytotoxicity of cells exposed to organic dust was greater than that of cells exposed to trace metal dust at the very same dosages. Cells are affected by oxidative stress in a number of ways, including the creation of pro-inflammatory chemicals and structural induction of apoptosis. Because diverse ROS in the cell is interconverted to hydrogen peroxides, and hydrogen peroxide is the longest-lived oxidizing agent, evaluating chemicals for their potential to change hydrogen peroxide levels in cell cultures is advantageous.

Activation Inflammasome Caspase- 1 in CCD841 After Treated with Indoor Dust Samples

The stimulation of caspase-1 by signal transduction pathways is an important part of the host's reaction to microbial infections. In order to eradicate the infectious agent, it promotes the release of HMGB1, IL-1, and IL-18, as well as pyroptosis of diseased host cells. Based on the received signal and the specific inflammasome receptor domain protein that got it, an activated Caspase-1 might cause pyroptosis, a lytic form of apoptosis, as a result of an inflammatory response. Caspases are cysteine proteases that cause apoptosis and pyroptosis, two types of controlled cell death.

Glowing Caspase-1, the activity of caspase-1 in CCD841 cells exposed to indoor dust for 24 hours was detected using an inflammasome assay. These findings reveal that when cells are exposed to 50 and 100 g/ml of dust, Caspase-1 activation rises in a concentration-dependent way.

The polyprotein inflammasome complex contains caspase 1, which is a key component as described by Broz & Dixit, 2016. It can activate the pro-inflammatory cytokines IL1 and IL18 and cause pyroptosis (a kind of cell death that is triggered by inflammation) when it is active (Broz & Dixit, 2016; He et al., 2016; Mittal et al., 2014). After 24 hours of exposure to indoor dust, caspase-1 activity in CCD841 cells was detected using an inflammasome assay. Caspase-1 activity was dramatically elevated in colon cells following exposure to indoor dust (50 and 100 g/ml), suggesting that pyroptosis is likely involved in cell death.

According to research, the organic components of particles appear to have a substantial role in inflammation and oxidative stress following particle exposure (Bonvallot et al., 2001; Baulig et al., 2004). Metals adsorbing to particulate matter, on the

other hand, might promote inflammation and oxidative stress (Kennedy et al., 1998, Zhang et al., 2008). Inflammasome stimulation was observed to be greater in cells exposed to trace metal dust than in cells exposed to organic dust in this research. Other researchers have found that air molecules with a greater concentration of metals and a lower organic content can produce considerable inflammatory mediator release, as well as more pronounced oxidative stress (Ghio, 2004; Hetland et al., 2005).

The control of inflammatory reactions has long been linked to oxidative stress, and new research is increasingly supporting this theory (Choi et al., 2010; Mittal et al., 2014). One possible link between excessive ROS generation and inflammation is the activation of superoxide dismutase-2. When SOD2 reacts with mitochondrial O2•, a less harmful byproduct is produced (H2O2). H2o2 can pass through the exterior mitochondrial membrane to reach cytosolic destinations, resulting in NFB stimulation, inflammasome formation, and proinflammatory cytokine production, among other functional effects (Mittal et al., 2014).

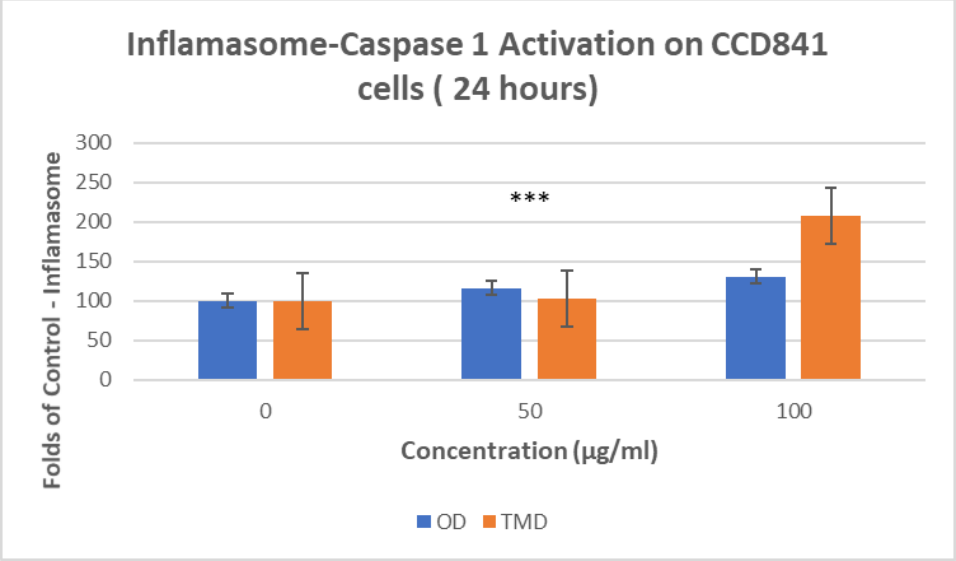

Figure 6: Inflammasome Caspase -1 Activation. Activation of caspase 1 was significantly high at higher treatment concentrations.

Data Results Interpretation

Given the results, it can be concluded that, both dust samples with different concentrations can change cell morphology of normal human colon cells by damaging their membrane. It reduced their density and detached them. Changing the shape lead in change in functionality.

Cell viability also was affected by indoor dust exposure. MTT assay along with protease assay clearly showed more than 75% reduction in viable cells at high concentrations. The higher the concentration, the cells were less viable (Figure 1 and 2).

Apoptosis was also induced by indoor dust treatment because the results shown in Figure 3 at higher concentration than both caspase 3 and 7 was activated as sign of increasing the apoptosis mechanism. Both indoor trace element and organic contaminates showed the elevation of caspase 3/7 starting from 50 μg/ml and continued to increase with higher concentrations.

On the other hand, cell cytotoxicity has a significant value; meaning that cytotoxicity marker did detect compromised membrane as shown in Figure4. An obvious increase of LDH marker at 500 μg/ml in trace element indoor sample, is shown, so the results this conclude that LDH is induced by the exposure of both dust samples.

As shown in Figure 5, there is an obvious increase of ROS generation markers after exposed to both indoor dust samples at which suggest that both trace element and organic contaminants of indoor dust samples has the ability in elevating pathogens and cytotoxic effects on these normal human colon cells.

Inflammasome caspase-1 assay was shown as a significant value if compared to untreated cells. Figure 6 illustrates the rate of effectiveness of dust samples on the

activation of inflammasome caspase-1 which increased due to bacterial or microbial inflammation. The media used had a percentage of antibiotic, and the dust samples were supplied, so they were exposed to rays to sterilize the dust from microorganisms and seem to be a reasonable explanation in this respect.

The question remains vail; which is more toxic between the two dust samples that were used; trace element or organic contaminants in indoor dust. The correlation between the two dust samples, and it is hard to determine which one of these two samples is more toxic to normal human colon cells; but if they both are combined and penetrate at a cellular level, there is a high chance to cause a serious harm.

CHAPTER 5

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

People worldwide spend their time mostly indoors, so indoor dust is an underling concern for all humans in general, but more importantly on those who are venerable who have weakened immune system such as children, elderly, and immunocompromised. Dust particles are everywhere, and these dust particles find their way through the human body through inhalation, injection, and dermal contact.

This study was designed to examine the effect of trace elements and organic contaminants dust in indoor dust on normal human colon cells. These cells were cultured, treated by both types of dust samples using different elevated concentrations, tested their viability, cell death mechanism, cytotoxicity, oxidative stress, and inflammation reaction by preforming MTT assay, protease cell viability, apoptosis 3/7 caspase, LDH release, Reactive oxygen species, and inflammasome caspase-1 assay respectively.

After preforming standard deviation to statically analyze the data by using GraphPad Prism software generation 9, and probability value was calculated ,the results were significant at higher concentration for the above reactions, and it supported the study hypothesis. Finally, the correlation between the two types of dust were used was about the same in terms of their toxicity affects, and it is left for future studies.

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