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Investigation of the Effects of Resveratrol and Epigallocatechin Gallate on Woodsmoke-Induced Inflammation

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By

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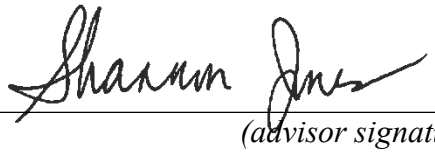

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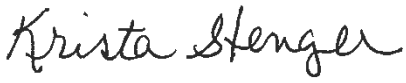

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Abstract

Until a few years ago, most scientific investigations related to the health effects of air pollution focused on outdoor air pollutants. But in recent years, the concerns over indoor air pollution has increased. People can spend up to 90% of their time in indoor environments, especially their homes, even more so since the beginning of the COVID-19 pandemic. The concentrations of some air pollutants are five times higher than what is found outdoors.

Indoor air pollution exposure remains a significant cause of morbidity and mortality worldwide. Approximately half of the global population is exposed to abnormally high concentrations of household air pollutants due to the burning of biomass fuels, accounting for 4 million annual deaths globally (Martin et al. 2011). Biomass fuel is defined as the byproduct of the combustion of plant or animal material. The combustion of wood, charcoal, dung and crop residues accounts for more than half of the energy source in most developing countries and 95% in countries with lower incomes (Torres-Duque et al. 2008). Biomass smoke exposure can occur in multiple ways, including the use of wood stoves or cookstoves, and exposure to forest fires or agricultural burning. Several epidemiological studies have shown that wildland firefighters have temporary reductions in respiratory function associated with their firefighting activities (Betchley et al., 1997). Burning of agricultural residue in rural communities has worsened respiratory symptoms among individuals, especially those with preexisting conditions. Inhalation of biomass smoke is correlated with chronic, inflammatory respiratory diseases including asthma, COPD, lung cancer, and microbial infections. Populations that were temporarily exposed to wildland fire smoke also exhibited an increase in hospital visits compared to those living in smoke-free environments (Mott et al., 2002).

Although there is evidence linking dung biomass smoke exposure with pulmonary diseases, there are few studies investigating the inflammatory effects of biomass smoke on human lung cells.

The use of alternative medicine is increasing in the United States and worldwide. Many people have sought herbal medicine to help with chronic inflammatory diseases and autoimmune disorders to help combat their symptoms. Most of the people directly impacted by biomass smoke are located in developing countries, with limited resources. Inexpensive and readily available naturally occurring compounds may be beneficial in reducing the pro-inflammatory health effects caused by biomass smoke exposure. In this study, we investigated two naturally occurring compounds: resveratrol and epigallocatechin gallate (EGCG). Resveratrol is found in dark-skinned fruits, while epigallocatechin gallate is predominantly found in green tea. Both resveratrol and EGCG are known to have antioxidant, anti-viral, anti-inflammatory, cardioprotective, and chemopreventive properties.

This study aimed to examine the underlying molecular and cellular events that result from the exposure of human airway epithelial cells to biomass smoke and show that pretreatment of the epithelial cells with resveratrol and epigallocatechin gallate inhibits the pro-inflammatory effects caused by wood smoke.

Background and Significance

Three billion people worldwide use a form of organic material to cook and/or heat their homes (Kodgule & Salvi, 2012). The burning of organic matter like wood leads to biomass smoke, which risks the respiratory health of those exposed, especially long-term exposure (Driscoll 1994) . People in developing countries use organic material more due to the limit of natural gases in these areas and the financial burden. Due to the poor ventilation, women and children are constantly exposed to the particulate matter found in the biomass smoke emission.

Studies have examined respiratory symptoms in a community that primarily uses small, wood cook stoves, compared to a non-cook stove-using communities. These studies showed that there is more wheezing among children under five years old in the cookstove-using community. These findings suggest that young children are more susceptible to respiratory effects due to wood smoke exposure compared to other groups (Browning et al., 1990). People in developing countries are not the only individuals affected by biomass smoke exposure. Those in the United States who still use wood burning fireplaces in their homes and outdoor wood fire pits are also at risk for exposure to biomass smoke. Firefighters and farmers who perform seasonal crop burning can be affected as well (Scott & Reilly, 2019).

When organic matter is burned, harmful chemicals are present in the particulate matter due to the thermal decomposition of the cellulose, lignin, lipids, waxes, and plant resins (Olloquequi & Silva O, 2016). When biomass smoke is inhaled, these components of the particulate matter make their way into the deepest regions of the respiratory tract. This can lead to inflammation and injury in the epithelial barrier of the lungs. Particulate matter has the ability to induce oxidative stress by modifying biological macromolecules and electrophilic chemicals. In addition, biomass smoke exposure can also result in the production of reactive oxygen species that leads to further oxidative stress (Scott & Reilly, 2019) . Particulate matter exposure causes activation of the innate immune system (Figure 1). This results in inflammation, the downregulation of antioxidants, and upregulation of the aryl hydrocarbon (AhR), toll-like receptor (TLR), and scavenger receptor pathways (Schuller et al. 2020).

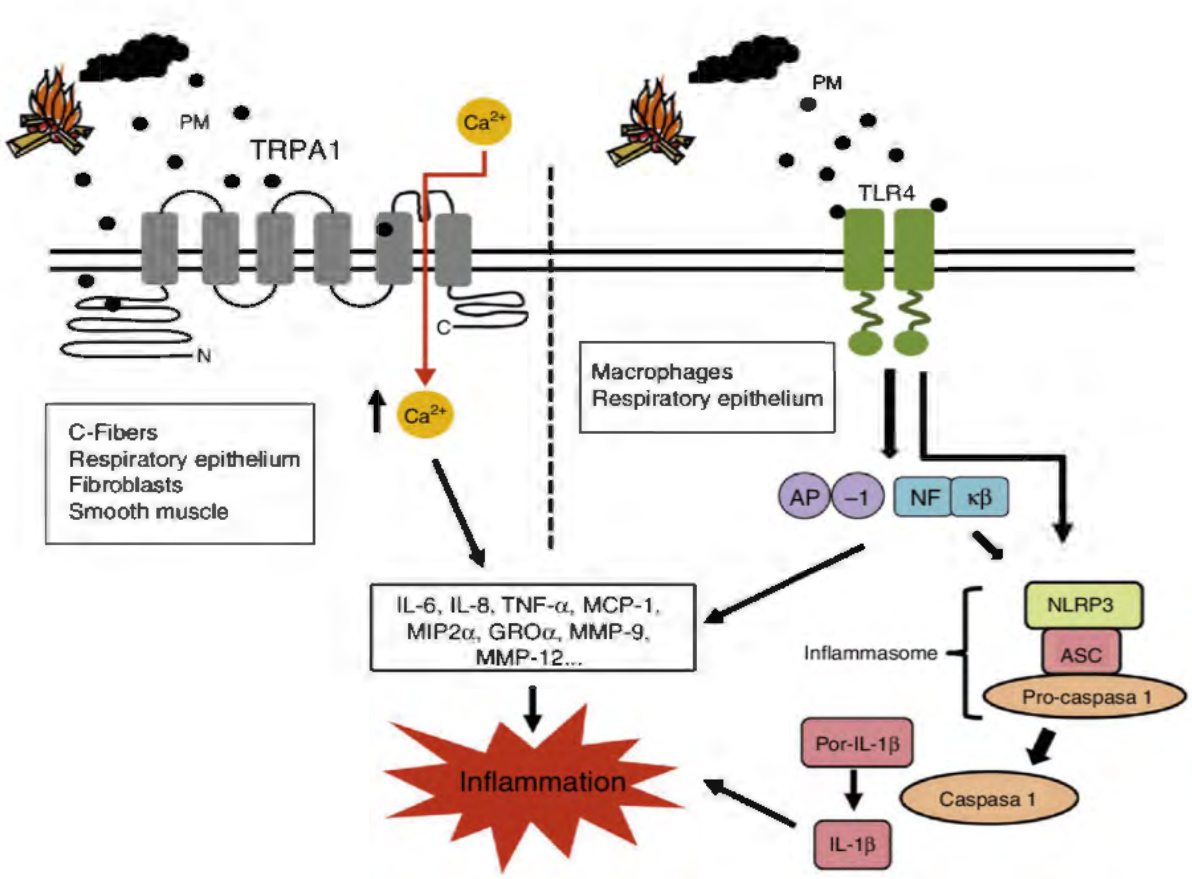

Figure 1. Biomass smoke induces inflammation through several mechanisms. First, the particulate matter calls for multiple inflammatory mediators such as IL-8. Next, a second group of mediators are produced like, MMP-12, which causes a change in the lung tissue. Biomass smoke increases intracellular signaling cascades that produce pro-inflammatory mediators along with inflammatory transcription factors which leads to ROS production. (Figure from Silva et al., 2015).

When breathing in biomass smoke, the first cells to be exposed are airway epithelial cells. Airway epithelial cells act as a major component of host defense mechanisms. Airway epithelial cells contribute to host defense by acting as a barrier, enabling mucociliary clearance, and by producing antimicrobial peptides, proteins, reactive oxygen species, and nitrogen species. When the particulate matter is detected by the epithelial cells, an extensive communication network of immune cells is initiated and acts to call leukocytes and mesenchymal cells to the site of inflammation. These events are major contributors to the innate immune response. (Hiemstra et

al., 2015). Persistent inflammation and activation of the innate immune system can contribute to chronic diseases such as heart disease, cancer, obesity, and diabetes (Furman et al., 2019). The particulate matter from the biomass smoke causes respiratory issues and other diseases throughout the body.

The inhalation of particulate matter can greatly increase one's susceptibility to asthma, a common inflammatory airway disease. Naturally occurring compounds might be able to reduce the morbidities associated with asthma and other inflammatory lung diseases. Resveratrol and 3,4', 5-trihydroxystilbene are found in the skins of red fruits such as grapes and raspberries. Resveratrol has been found to have anti-inflammatory properties within epithelial cells due to multiple mechanisms (Figure 2). Resveratrol is known to downregulate cyclooxygenase-2 (COX-2) transcription by inhibiting COX-1 and NF-kB activity. Pro-inflammatory cytokines such as COX-2 and IL-8 are expressed when NF-kB is activated. Reducing NF-kB expression, and oxidative stress, will also prevent inflammatory responses (Figure 2). Resveratrol has also been shown to activate receptors such as the peroxisome proliferator-activated receptor to counteract inflammation (Donnelly et al., 2004).

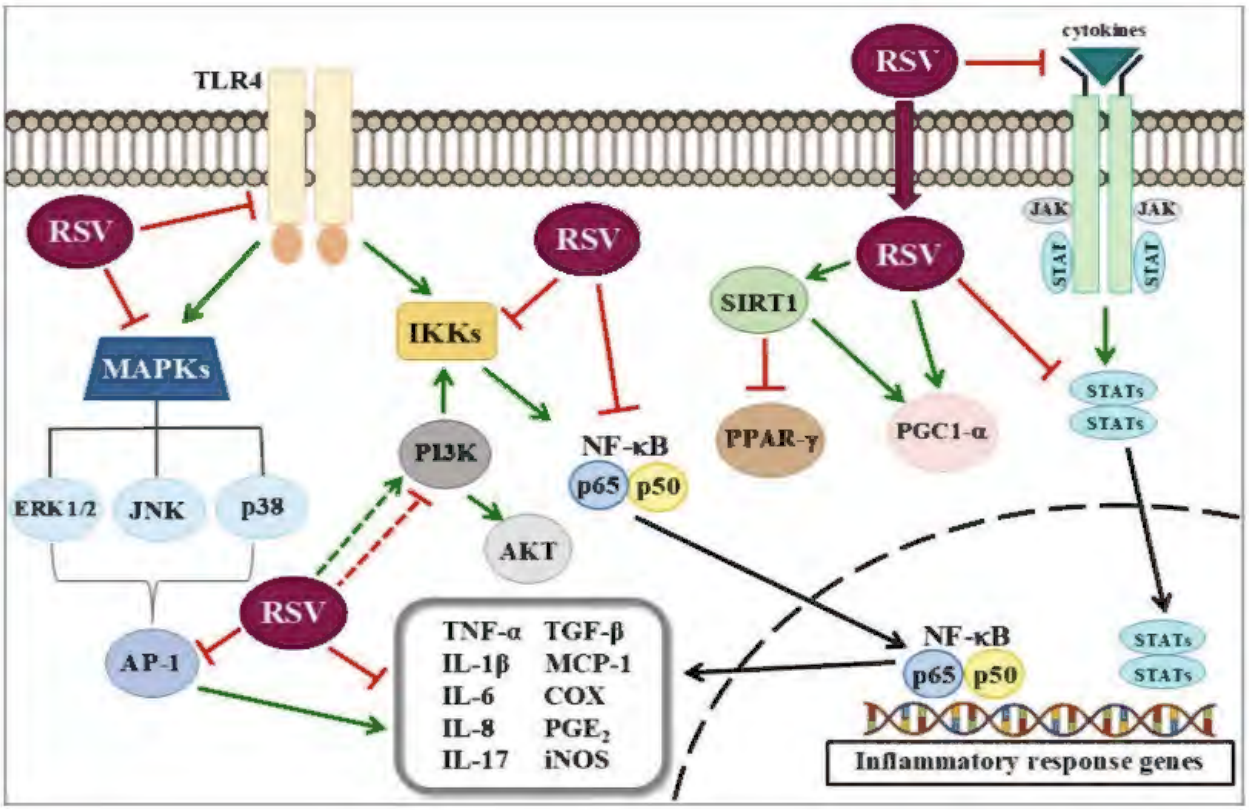

Figure 2. When an inflammatory response is activated there are multiple cell signaling pathways that resveratrol can use to counteract the inflammation. Some of the resveratrol anti-inflammatory mechanisms are described above. Green arrows with a point mean activation. Red arrows with a flat tip indicate inhibition. Arrows that are dashed are mechanisms that are not fully understood. (Figure from De Sá Coutinho et al., 2018).

Similarly, epigallocatechin gallate (EGCG) is known to have anti-inflammatory properties. EGCG is a tea flavonoid found in green tea EGCG is known to downregulate NF-kB expression, which, stated above, also downregulates the expression of the pro-inflammatory cytokine COX-2 (Figure 3). This tea flavonoid is also known to increase the expression of NF-E2-related factor 2, which controls the expression of antioxidant genes such as glutathione S transferase (Wang et al., 2021).

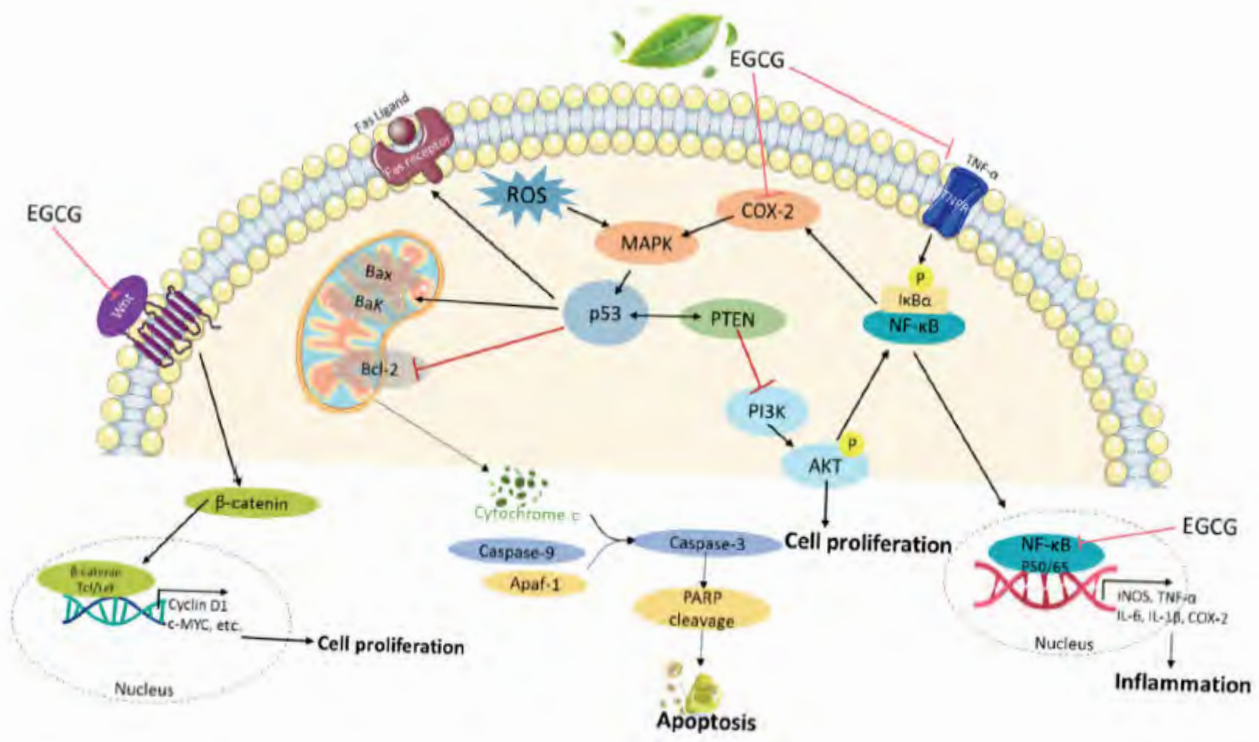

Figure 3. Epigallocatechin gallate mechanism of inhibiting inflammation as described above along with proliferation and apoptosis which are not relevant to this study. Arrows with a point mean activation. Arrows with a flat tip indicate inhibition. (Figure from Huang et al., 2020).

This research aimed to investigate the ability of biomass smoke, especially woodsmoke, to induce an inflammatory response. Resveratrol and EGCG were then studied for their anti-inflammatory effects. This research was carried out in human lung epithelial cells. The inflammatory response was measured using cytokine (TNF-α, COX-2, and IL-8) secretion and gene expression (HO-1, COX-2, and IL-8).

Materials and Methods

Cell Culture

Adenocarcinomic human alveolar basal epithelial cells (A549) were grown in Dulbecco's Modified Eagle Medium (DMEM) at a constant temperature of 37 °C with five percent CO^2.

Cell treatment

To investigate the impact of woodsmoke on A549 cells, increasing concentrations of woodsmoke were added: 20 µg/mL - 100 µg/mL. For experiments examining the anti-inflammatory effects of resveratrol, 80 µg/mL of woodsmoke were added to each well. Resveratrol was added at increasing concentrations, from 2 pg/mL to 65 pg/mL. In experiments investigating the anti-inflammatory effects of Epigallocatechin gallate (EGCG)s, the following concentration range was used: 1 pg/mL- 20 pg/mL. The following controls were used in all experiments: a negative control with only cell culture media, 1 µg/mL Lipopolysaccharide (LPS), and 10 µg/mL zymosan. Both LPS and Zymosan are Toll-like receptor agonists and serve as positive controls. Treated cells were incubated overnight at 37^{o}C.

Cell harvest

After treatment, supernatants were collected from each well and placed into separate Eppendorf tubes. Supernatants were later used to assess cytokine secretion via the ELISA. Prior to harvesting, cells were washed with PBS. A549 cells were lysed and these lysates were subsequently used for RNA isolation.

Measurement of Cytotoxicity

The CyQUANT™ LDH Cytotoxicity Assay purchased from ThermoFisher Scientific was used. The collected supernatants were placed into a 96-well plate, and the directions from ThermoFisher Scientific were followed. This assay measured the percentage of Lactate dehydrogenase (LDH) present in the supernatants. LDH is found in almost every living cell, and is released upon cell death. A SpectraMax® Plus 384 Absorbance Plate Reader set to 490 UV, and 680 UV was used to quantify the results.

Assessment of the inflammatory response

After harvesting cells, RNA was isolated using the Purelink RNA isolation kit purchased from Invitrogen. After RNA isolation, the concentration was measured using a Nanodrop 2000 UV-Vis spectrophotometer. The isolated RNA was subsequently used to synthesize complementary DNA (cDNA). Once the cDNA was synthesized, PCR and gel electrophoresis were used to assess the expression of pro-inflammatory and antioxidant genes. The primers used were for: Heme oxygenase-1 (HO-1), and cyclooxygenase- 2 (COX-2). Gel electrophoresis was performed to visualize and measure the expression of pro-inflammatory genes. A ChemiDoc Imaging Systems purchased from Bio-Rad was used to take pictures of the gels to be analyzed.

The second method used to investigate the inflammatory response in A549 cells was the enzyme-linked immunosorbent assay (ELISA) kit purchased from R&D Systems. Cytokine release by the A549 cells was measured in the supernatants per instructions provided by R&D Systems. TNF-α, IL-6, and IL-6 human ELISA kits were used because these three cytokines are known to be produced by A549 cells during an inflammatory response. A SpectraMax® Plus 384 Absorbance Plate Reader set to 450 UV, and 540 UV was used to retrieve the raw data prior to analysis.

Both PCR and ELISA were performed for experiments investigating the anti-inflammatory properties of resveratrol and EGCG.

Results

The lactate dehydrogenase assay was used to evaluate the cytotoxicity of increasing concentrations of woodsmoke. We found that concentrations above 100 μg/mL have over 30 percent cytotoxicity (Figure 3). Based on these data we concluded that 80 ug/mL would be the optimal concentration to use to induce an inflammatory response without inducing significant cytotoxicity. When A549 cells were exposed to Resveratrol/EGCG in addition to the

woodsmoke, there was no significant increase in cell death (Figure 4). While the concentrations of resveratrol used had less cytotoxicity compared to the woodsmoke group EGCG concentrations had zero percent cytotoxicity (Figures 4 and 5).

Three cytokines were measured by the ELISA in each experiment: TNF-α, IL-6, and IL-8. When the A549 cells were exposed to increasing concentrations of woodsmoke, the expression of the pro-inflammatory cytokines increased (Figures 6-8). Co-treatment with the antioxidants, resveratrol and EGCG, resulted in decreased secretion of the pro-inflammatory cytokines (Figures 9-14).

To assess changes in gene expression, cell lysates were collected and used for RNA isolation and RT-PCR. Two pro-inflammatory and oxidant-related genes were selected for these studies which include, COX-2 and HO-1. To ensure there was no contamination glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used. For all samples, excluding the water control, bands of equal intensity appeared, demonstrating no contamination (Figures 15, 18, and 21). Our data suggest that COX-2 expression was equally induced with all concentrations of woodsmoke that were tested. (Figure 16). There was a dose-dependent increase in HO-1 expression, with increasing concentrations of woodsmoke (Figure 17). The resveratrol co-treatment resulted, in decreased expression of COX-2 (Figure 19). HO-1 expression does not appear to be impacted by resveratrol co-treatment. When investigating the impact of co-treatment with EGCG, a dose-dependent decrease in COX-2 expression was observed in gel electrophoresis (Figures 22). In contrast the expression of HO-1 was not impacted by co-treatment with EGCG (Figure 23)

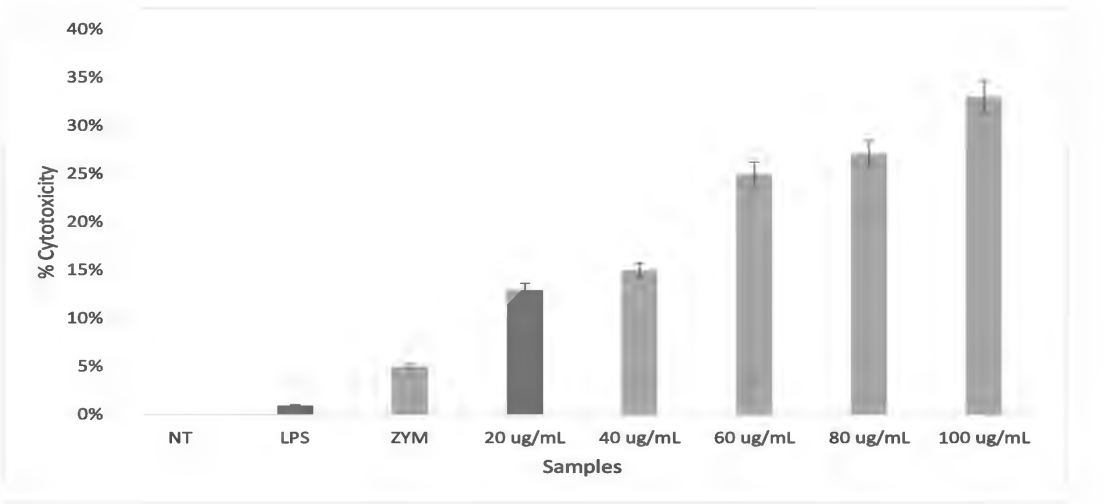

Figure 3. Lactate dehydrogenase release in woodsmoke treated A549 cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with increasing concentrations of solubilized woodsmoke for 24 hours. Supernatants were collected and an LDH assay was performed. Significant cell death was observed in cells treated with 100 ug/mL of woodsmoke compared to the no treatment sample (NT).

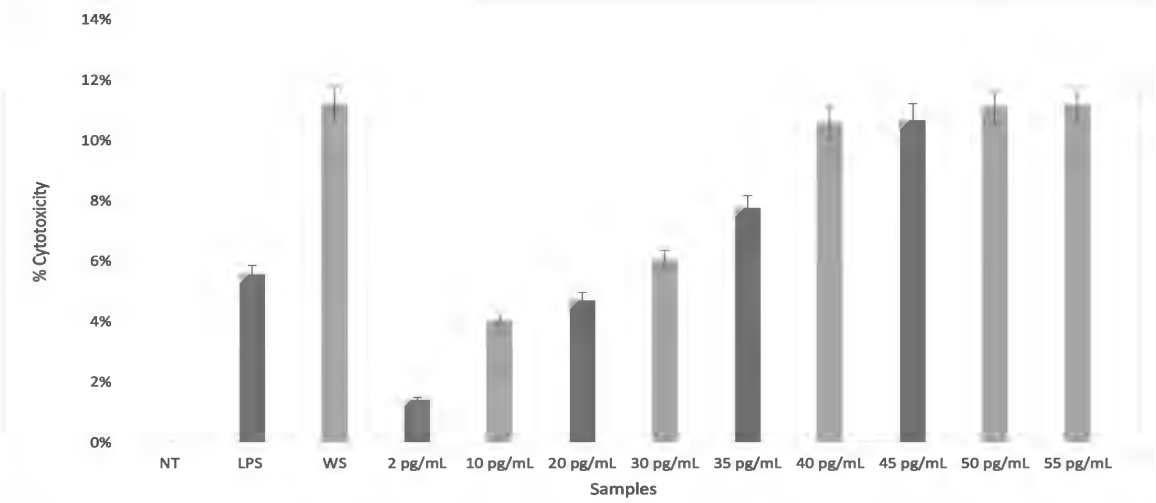

Figure 4. Lactate dehydrogenase release in woodsmoke and resveratrol treated A549 cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with 80 µg/ mL of solubilized woodsmoke and increasing concentrations of resveratrol for 24 hours. Supernatants were collected and an LDH assay was performed. No Significant cell death was observed in cells compared to the no treatment cells (NT).

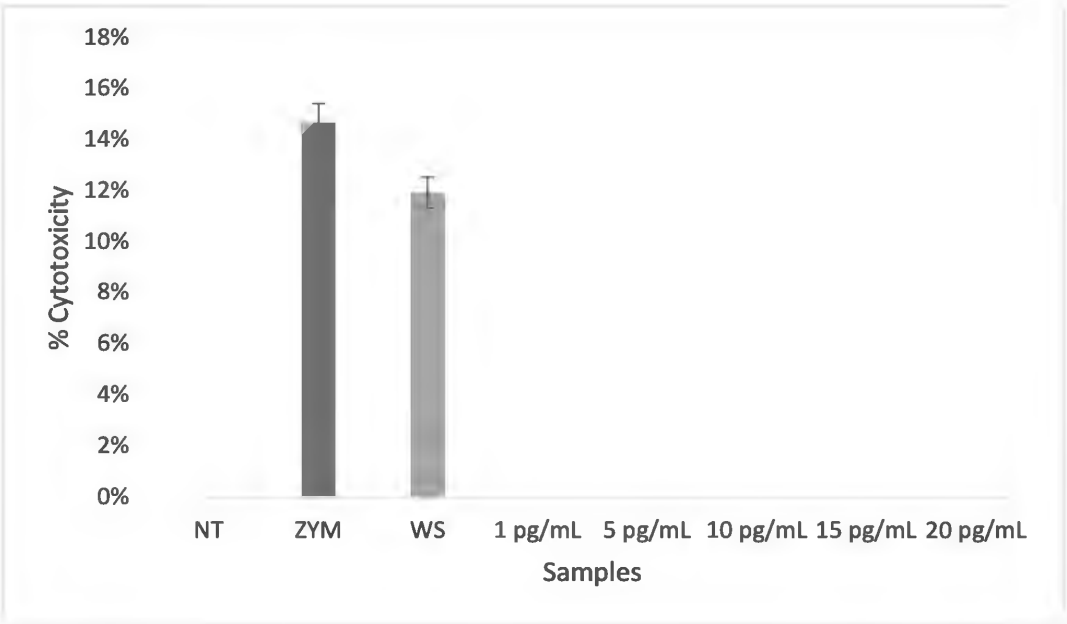

Figure 5. Lactate dehydrogenase release in woodsmoke and Epigallocatechin gallate treated A549 cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with 80 µg/ mL of solubilized wood smoke and increasing concentrations of Epigallocatechin gallate for 24 hours. Supernatants were collected and an LDH assay was performed. No cell death was observed in cells compared to the no treatment cells (NT).

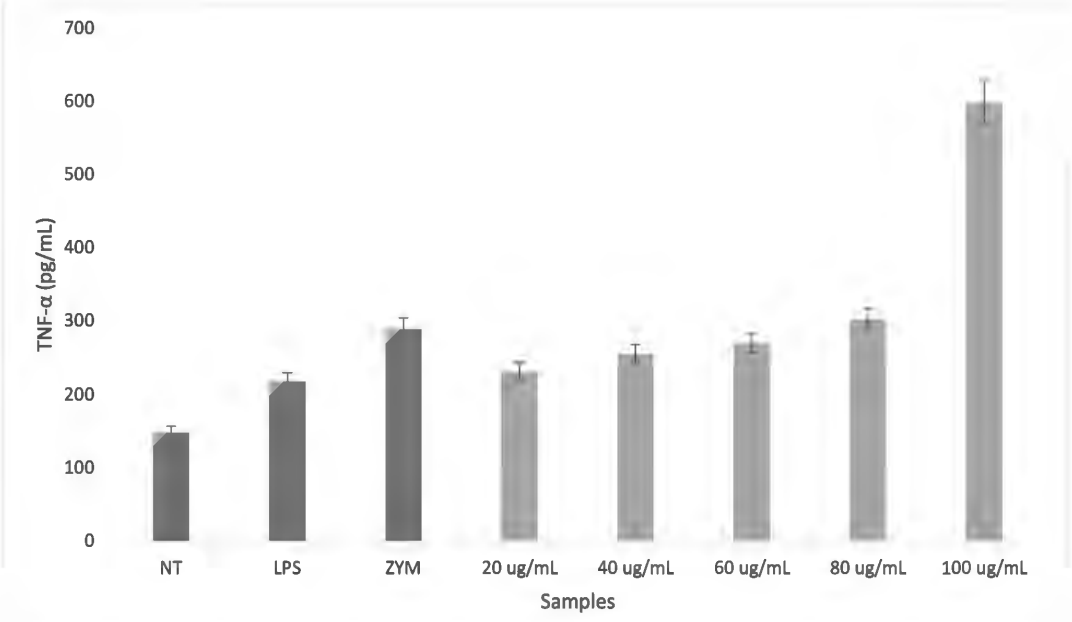

Figure 6. TNF-α secretion in A549 woodsmoke treated cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with increasing concentrations of solubilized woodsmoke for 24 hours. Supernatants were collected and an ELISA was performed. TNF-α secretion increased at a concentration of 100 ug/mL of woodsmoke.

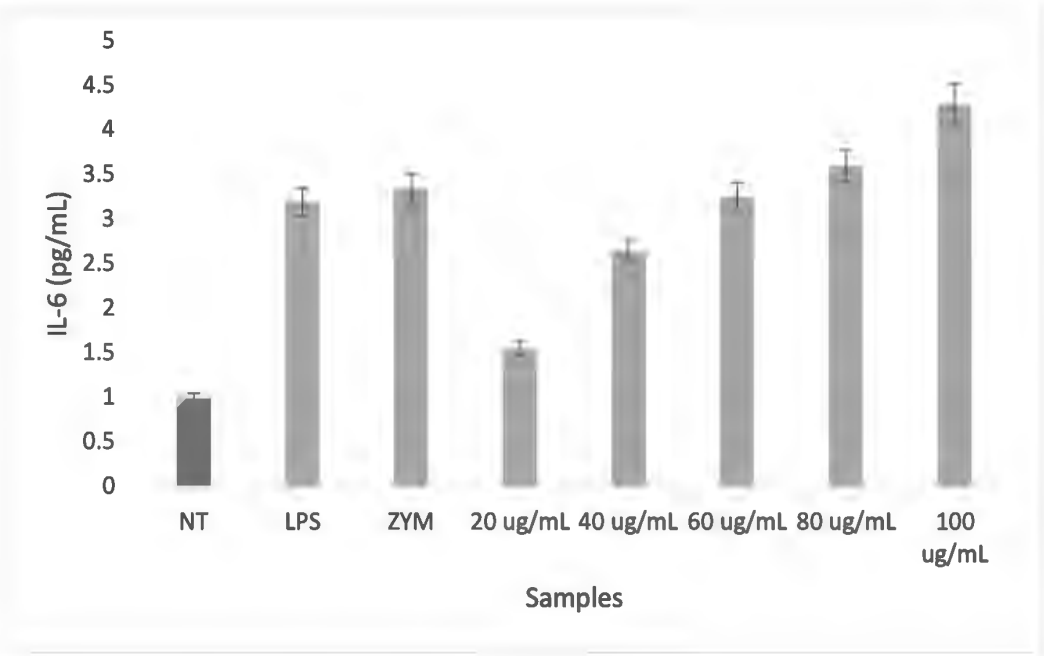

Figure 7. IL-6 secretion in A549 woodsmoke treated cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with increasing concentrations of solubilized woodsmoke for 24 hours. Supernatants were collected and an ELISA was performed. IL-6 secretion increased with increasing concentrations of woodsmoke.

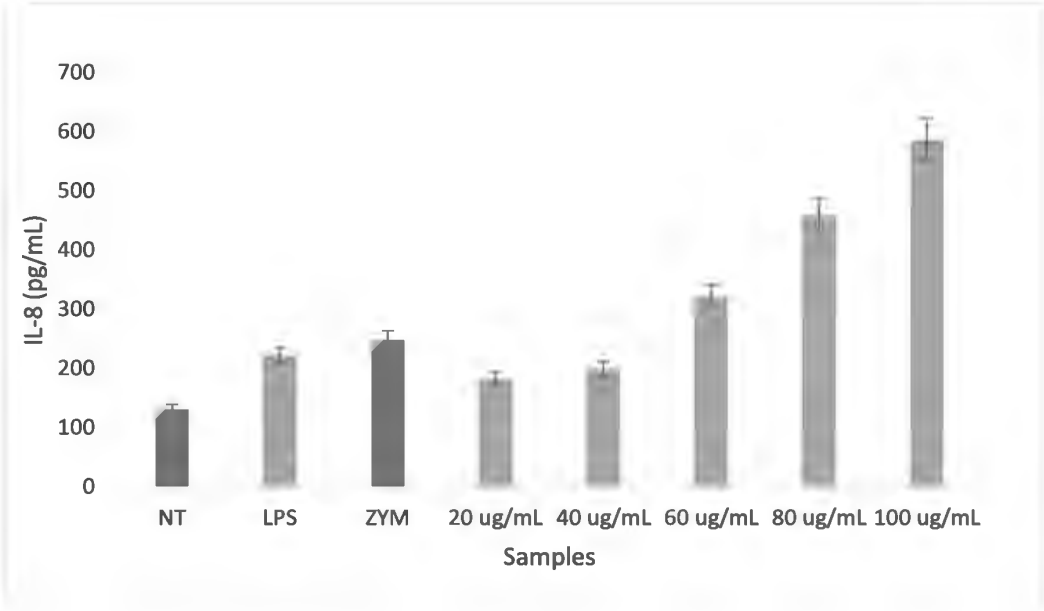

Figure 8. IL-8 secretion in A549 woodsmoke treated cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with increasing concentrations of solubilized woodsmoke for 24 hours. Supernatants were collected and an ELISA was performed. IL-8 secretion increased with increasing concentrations of woodsmoke.

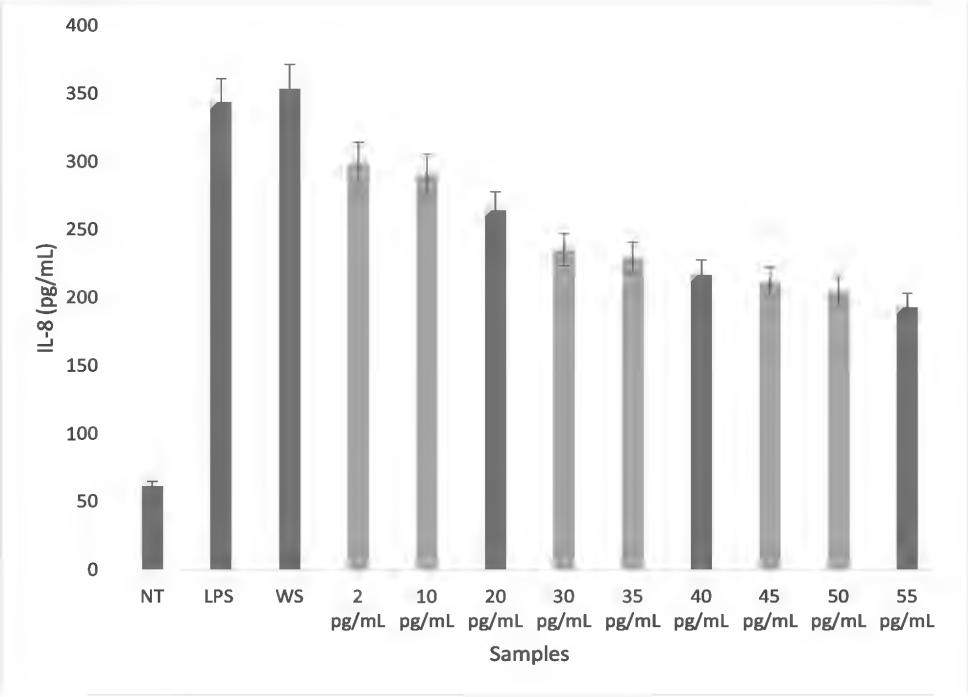

Figure 9. IL-8 secretion in A549 woodsmoke and resveratrol treated cells. 6 X 10^6 cells were cultured in a six-well dish. Cells were incubated with 80 µg/ mL of woodsmoke and increasing concentrations of resveratrol for 24 hours. Supernatants were collected and an ELISA was performed.

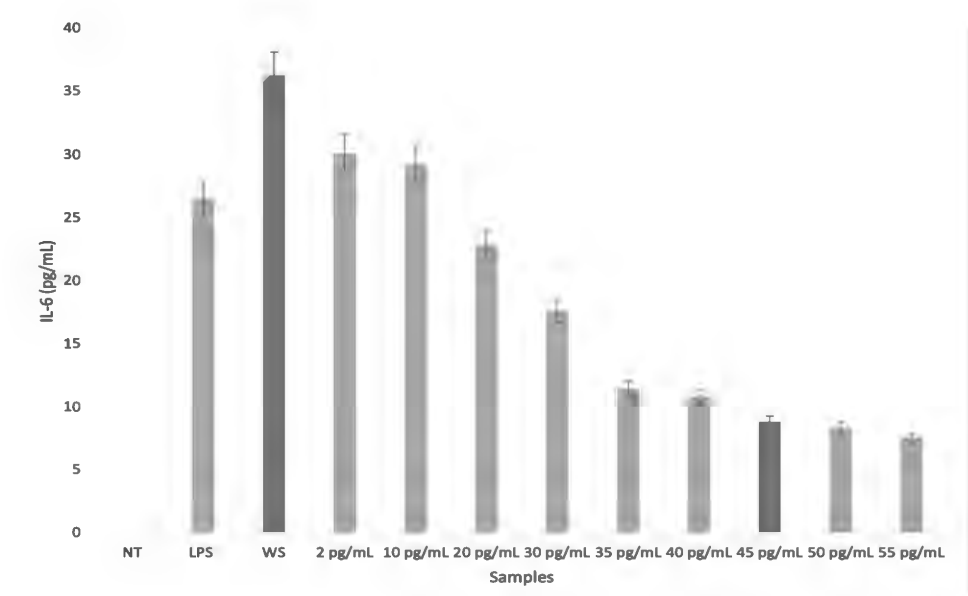

Figure 10. IL-6 secretion in A549 woodsmoke and resveratrol treated cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with 80 µg/ mL of woodsmoke and increasing concentrations of resveratrol for 24 hours. Supernatants were collected and an ELISA was performed. IL-6 secretion decreased with increasing concentration of resveratrol.

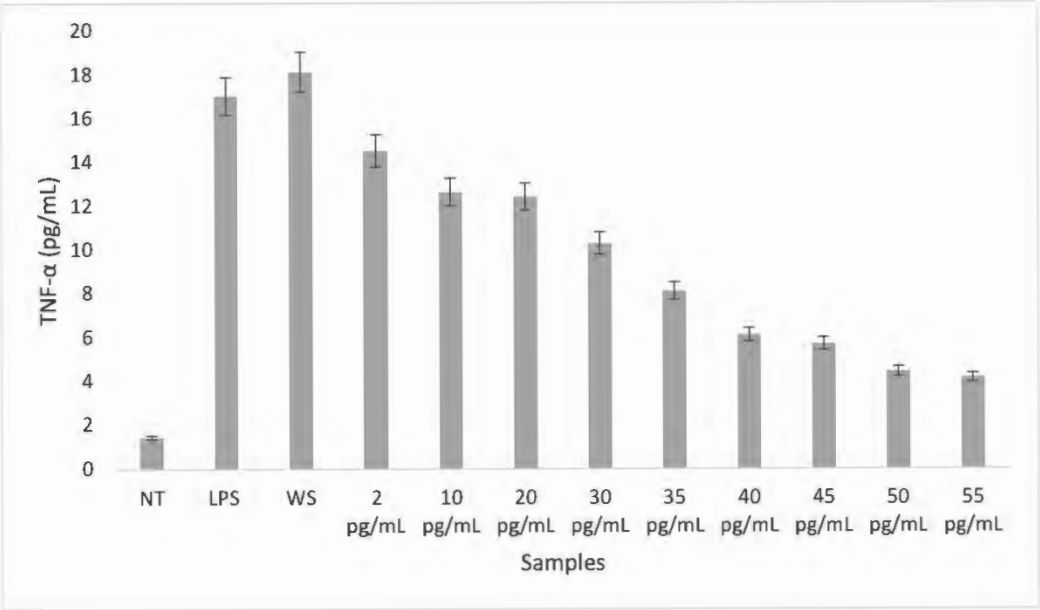

Figure 11. TNF-α secretion in A549 woodsmoke and resveratrol treated cells. 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with 80 µg/mL of woodsmoke and increasing concentrations of resveratrol for 24 hours. Supernatants were collected and an ELISA was performed. TNF-α secretion decreased with increasing concentrations of resveratrol.

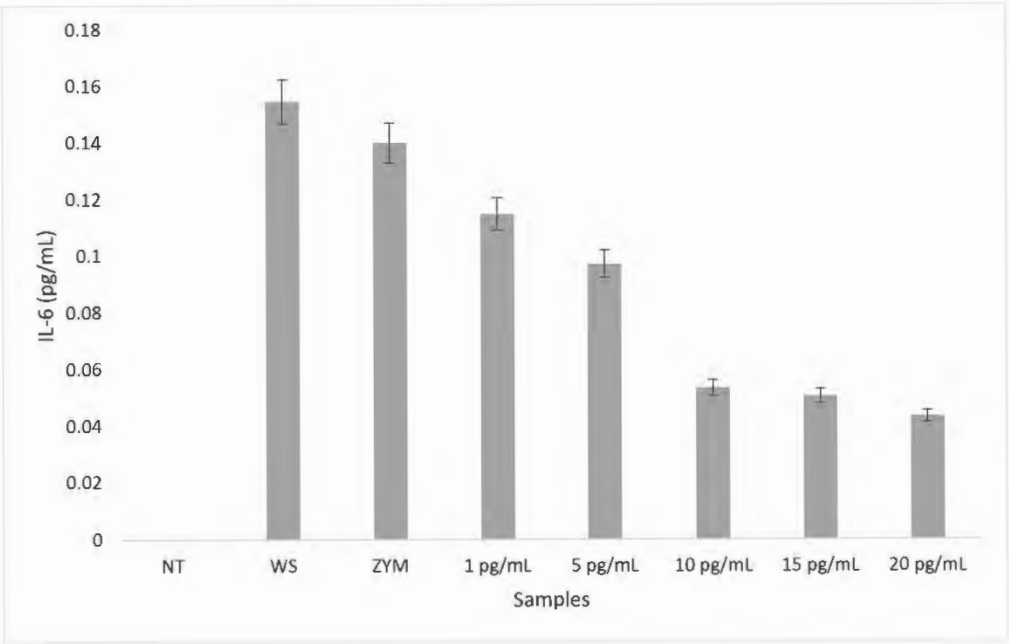

Figure 12. IL-6 secretion in A549 woodsmoke and Epigallocatechin gallate treated cells . 6 X 10^6 cells/mL media were cultured in a six well dish. Cells were incubated with 80 µg/ mL of woodsmoke and increasing concentrations of Epigallocatechin gallate for 24 hours. Supernatants were collected and an ELISA was performed. IL-6 secretion decreased with increasing concentrations of Epigallocatechin gallate.

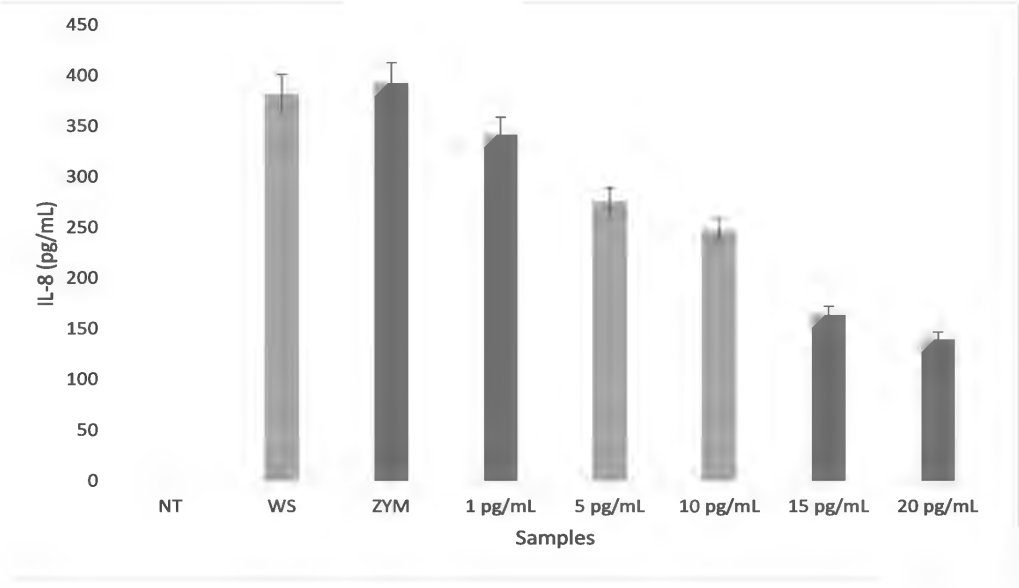

Figure 13. IL-8 secretion in A549 woodsmoke and Epigallocatechin gallate treated cells . 6 X 10^6 cells were cultured in a six-well dish. Cells were incubated with 80 µg/ mL of woodsmoke and increasing concentrations of Epigallocatechin gallate for 24 hours. Supernatants were collected and an ELISA was performed. IL-8 secretion decreased with increasing concentrations of Epigallocatechin gallate.

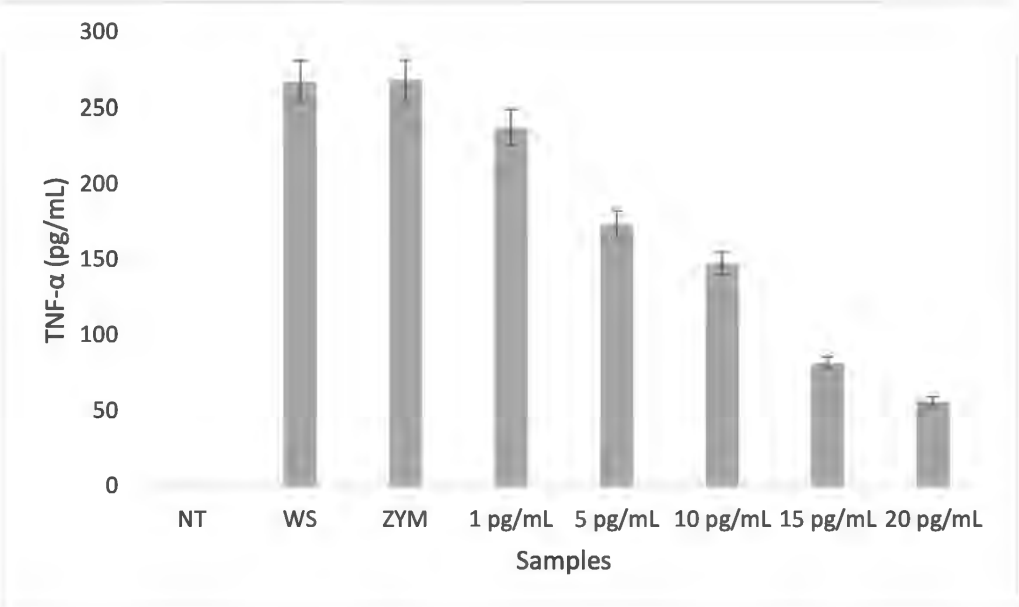

Figure 14. TNF-α secretion in A549 woodsmoke and Epigallocatechin gallate treated cells . 6 X 10^6 cells were cultured in a six well dish. Cells were incubated with 80 µg/ mL of woodsmoke and increasing concentrations of Epigallocatechin gallate for 24 hours. Supernatants were collected and an ELISA was performed. TNF-α secretion decreased with increasing concentrations of Epigallocatechin gallate.

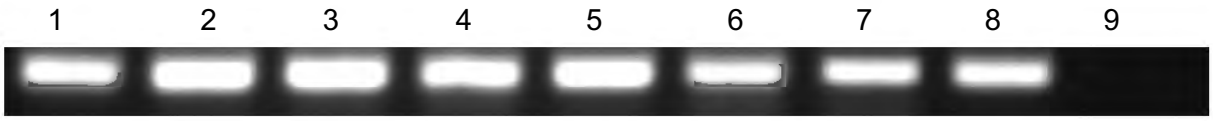

Figure 15. GAPDH expression in woodsmoke-challenged A549 cells 6 X 10^6 cells were treated from left to right as follows: (1) No Treatment, (2) LPS 1 µg/mL , (3) zymosan 10 ug/mL, (4) WS 20 µg/mL, (5) WS 40 µg/mL, (6) WS 60 µg/mL, (7) WS 80 µg/mL, (8) WS 100 ug/mL, (9) Water. No significant changes in expression were observed.

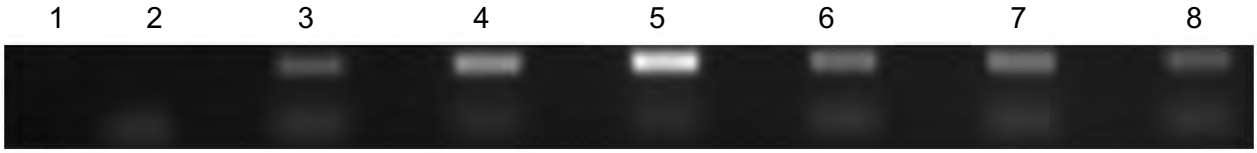

Figure 16. COX- 2 expression in woodsmoke-challenged A549 cells 6 X 10^6 cells were treated from left to right as follows: (1) Water, (2) No Treatment, (3) LPS 1 µg/mL, (4) WS 20 µg/mL, (5) WS 40 µg/mL, (6) WS 60 µg/mL, (7) WS 80 µg/mL, (8) WS 100 µg/mL. Cells treated with woodsmoke expressed COX-2 compared to the no treatment cells.

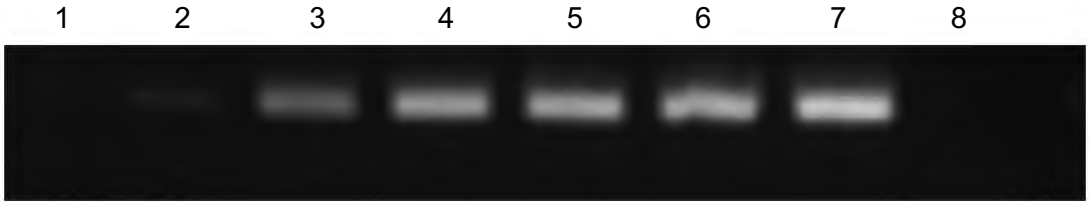

Figure 17. HO-1 expression in woodsmoke-challenged . 6 X 10^6 cells were treated from left to right as follows: (1) No Treatment, (2) LPS 1 µg/mL , (3) WS 20 µg/mL, (4) WS 40 µg/mL, (5) WS 60 µg/mL, (6) WS 80 µg/mL, (7) WS 100 µg/mL, (8) Water. As the concentration of woodsmoke increased so did the expression of HO-1. LPS, the control, did not induce HO-1 anti-oxidant expression which is why others experiments used zymosan instead.

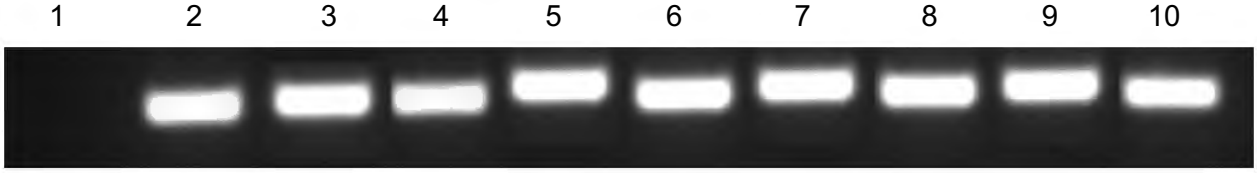

Figure 18. GAPDH expression in cells co-treated with resveratrol. 6 X 10^6 cells were treated from left to right as follows: (1) water (2) zymosan 10 ug/mL, (3) WS 80 µg/mL, (4) resveratrol 2 pg/mL, (5) resveratrol 10 pg/mL, (6) resveratrol 20 pg/mL, (7) resveratrol 30 pg/mL, (8) resveratrol 40 pg/mL, (9) resveratrol 50 pg/mL (10) No treatment. No significant treatments were observed.

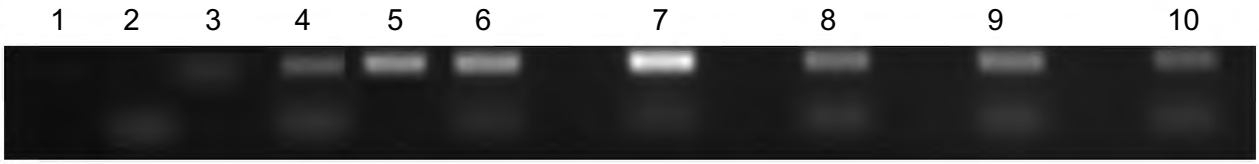

Figure 19. COX-2 expression in A549 cells co-treated with resveratrol. 6 X 10^6 cells were treated from left to right as follows: (1) Water , (2) No treatment (3) zymosan 10 µg/mL, (4) resveratrol 2 pg/mL, (5) resveratrol 10 pg/mL, (6) resveratrol 20 pg/mL, (7) WS 80 µg/mL, (8) resveratrol 30 pg/mL, (9) resveratrol 40 pg/mL, (10) resveratrol 50 pg/mL. The expression of COX-2 decreased with all concentrations of resveratrol that were used, when compared to the woodsmoke only control.

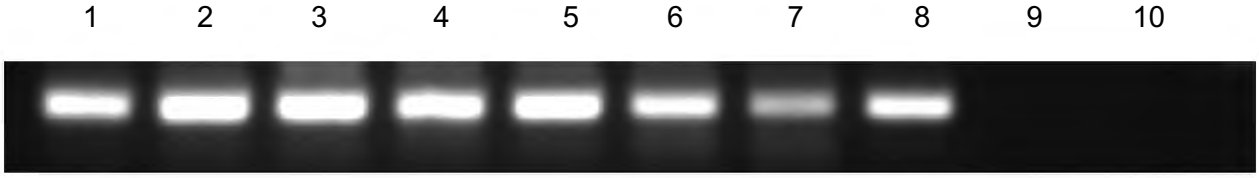

Figure 20. HO-1 expression in A549 cells co-treated with resveratrol. 6 X 10^6 cells were treated from left to right as follows: (1) zymosan 10 ug/mL, (2) WS 80 µg/mL, (3) resveratrol 2 pg/mL, (4) resveratrol 10 pg/mL, (5) resveratrol 20 pg/mL, (6) resveratrol 30 pg/mL, (7) resveratrol 40 pg/mL, (8) resveratrol 50 pg/mL (9) No treatment, (10) Water. No significant changes in HO-1 expression were observed.

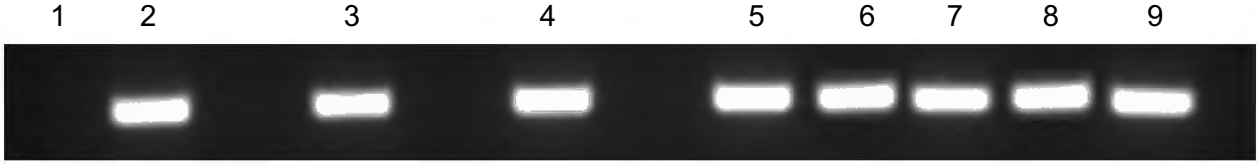

Figure 21. GAPDH expression A549 cells co-treated with EGCG (6 X 10^6 cells were treated from left to right as follows: (1) water, (2) No treatment. (3) zymosan 10 ug/mL, (4) WS 80 µg/mL, (5) Epigallocatechin gallate 1 pg/mL, (6) Epigallocatechin gallate 5 pg/mL, (7) Epigallocatechin gallate 10 pg/mL, (8) Epigallocatechin gallate 15 pg/mL, (9) Epigallocatechin gallate 20 pg/mL. Please note that no samples were loaded into the blank wells.

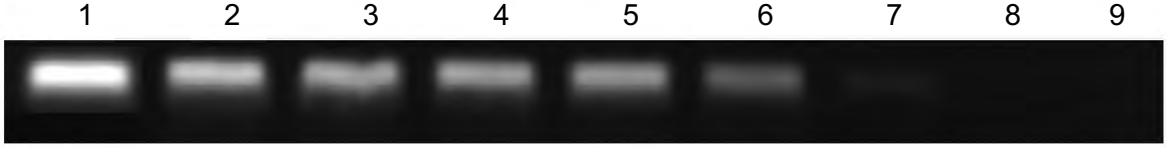

Figure 22. COX-2 gene expression in A549 cells co-treated with EGCG 6 X 10^6 cells were treated from left to right as follows:, (1) WS 80 µg/mL, (2) Epigallocatechin gallate 1 pg/mL, (3) Epigallocatechin gallate 5 pg/mL, (4) Epigallocatechin gallate 10 pg/mL, (5) Epigallocatechin gallate 15 pg/mL, (6) Epigallocatechin gallate 20 pg/mL, (7) zymosan 10 ug/mL, (8) No treatment, (9) water. As the concentration of Epigallocatechin gallate increases the expression of COX-2 decreases.

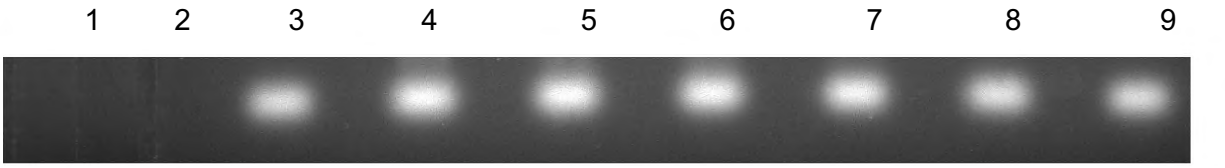

Figure 23. HO-1 gene expression in A549 cells co-treated with EGCG. 6 X 10^6 cells were treated from left to right as follows: (1) water, (2) No treatment. (3) zymosan 10 ug/mL, (4) WS 80 μg/mL, (5) Epigallocatechin gallate 1 pg/mL, (6) Epigallocatechin gallate 5 pg/mL, (7) Epigallocatechin gallate 10 pg/mL, (8) Epigallocatechin gallate 15 pg/mL, (9) Epigallocatechin gallate 20 pg/mL. No significant changes in gene expression were observed.

Discussion and Conclusions

This results of this study support our hypotheses that woodsmoke induces an inflammatory response within human airway epithelial cells. Specifically, our data show that when the lung epithelial cells were challenged with 100 μg/mL of solubilized woodsmoke, the secretion of TNA-alpha, IL-8, and IL-6 increased, when compared to the no treatment control. Our RT-PCR data indicate the upregulation of the pro-inflammatory gene COX-2, as well as increased expression of HO-1. These data may suggest that increasing concentrations of woodsmoke may also induce an oxidative stress response in the lung epithelial cells. These data align with other published studies indicating the particulate matter can induce innate immune system activation.

We also hypothesized that pretreatment with the anti-oxidant molecules, resveratrol and EGCG, would attenuate the pro-inflammatory effects of woodsmoke exposure. This hypothesis was supported by our data indicating decreased secretion of TNF-α, IL-6, and IL-8 after pre-treatment with resveratrol and EGCG. Interestingly, we did not observe a similar trend when examining gene expression by RT-PCR and gel-electrophoresis. Resveratrol and EGCG are both known to inhibit anti-inflammatory and anti-oxidant properties. We expected to observe a dose-dependent decrease in expression in pro-inflammatory and pro-oxidant genes with the resveratrol

and EGCG pre-treatment. We found a subtle decrease in COX-2 expression with all of the concentrations of resveratrol that were administered. There did not appear to be a dose-dependent trend. In contrast, we did observe a dose-dependent decrease in COX-2 expression when cells were treated with increasing concentrations of EGCG. This finding supports previous studies that describe EGCG's ability to inhibit COX-2 expression. When examining HO-1, a prominent anti-oxidant gene, we found that neither EGCG or resveratrol impacted its expression. These preliminary results suggest that resveratrol and EGCG may not exhibit anti-oxidant effects with regards to woodsmoke exposure.

Overall, we concluded that woodsmoke does induce an inflammatory response in the human lung epithelial cells. This is indicated by changes in pro-inflammatory secretion as well as subtle changes in pro-inflammatory gene expression. These findings are preliminary and further investigation is needed to confirm our results. In-depth statistical analysis will also be necessary to confirm that woodsmoke is indeed a pro-inflammatory toxicant. Resveratrol and EGCG may potentially reduce these anti-inflammatory effects. These two natural compounds are readily available and relatively inexpensive to purchase. They could potentially be consumed by those chronically exposed to indoor air pollution to help reduce the negative effects of the particulate matter in the biomass smoke.

In the future, we hope to further elucidate the mechanisms by which woodsmoke induces inflammation of lung epithelial cells. We propose that woodsmoke may be triggering the activation of pattern recognition receptors (such as TLRs). Of course, further molecular studies are require to confirm this hypothesis. We also hope to identify additional naturally occurring compounds that can potentially reduce the pro-inflammatory response initiated by woodsmoke exposure. One compound of interest is capsaicin, which is found in hot peppers. We would like

to determine whether combined compounds will have a more significant anti-inflammatory effect on the woodsmoke-challenged A549 cells, without eliciting a cytotoxic response.

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