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Protective Effect of Curcunan® on Human Lung Epithelial Cells Exposed to Fine Particulate Matter and Response to Allergens

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Abstract

India is the third most polluted nation after Bangladesh and Pakistan in PM2.5 concentration. The average 24-hour PM2.5 concentration in India is 51.90 µg/m3, indicating'very bad' air quality. Air pollution is mostly caused by fine particulate matter (FPM). FPM exposure increases chronic and allergic rhinitis. The anti-inflammatory and antioxidative effects of curcumin protect organs against harmful substances that alter homeostasis. Curcumin protects against PM-induced lung inflammation, however its mechanism has not been extensively investigated. In this study, we investigated different formulations of curcumin (Curcumin 17% powder, Curcumin 17% formulation & Curcumin 95%) on cell viability in preliminary cytotoxicity study using 'A549' lung epithelial cell line. The most effective formulation was further evaluated for its anti-oxidant, anti-inflammatory and anti-allergic activity in human U266B1 multiple myeloma cells against FPM treatment. A significant reduction by Curcumin 95% in the IgE in human U266B1 multiple myeloma cells was demonstrated, and a significant reduction in the expression of the pro-inflammatory cytokines (IL-6 and IL-8) in A549 cells was observed. Finally, malonaldehyde, a clinically significant oxidative stress biomarker, was also potently decreased. The treatment with Curcunan® protected the cells from FPM-induced cytotoxicity and helped revert the cell morphology. The treatment even helped lessen the levels of oxidative stress and inflammatory markers (IL-6 and IL-8). The treatment showed anti-allergic activity, evident by the decreasing secretion of IgE levels. These results suggest that Curcunan® can be an excellent therapeutic agent for preventing pulmonary disorders caused by air pollution.

Keywords: *Air Pollution, Anti-Allergic Activity, Curcumin; Curcunan®, Cytokines, Cytotoxicity; Fine Particulate Matter (FPM); Oxidative Stress; Pro-Inflammatory Mediators; Respiratory Diseases.*

Introduction

Air pollution is a significant environmental issue that living organisms are currently facing. The etiological factors for this issue include extensive industrialization and a sedentary lifestyle, which are made worse by the growing use of cars as a mode of transportation compared to other modes [1]. The increased generation of smoke that contains ambient particulate materials, including coal, asbestos, and combustion

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particles, is strongly correlated with increased automobile use and the placement of industrial facilities close to metropolitan areas. Several epidemiological studies have linked air pollution exposure and poor health outcomes. According to a recent WHO research, indoor and outdoor air pollution exposure may be responsible for 6.5 million deaths worldwide (or around 12% of all fatalities). One of the primary components of air pollution is fine particulate matter (FPM). It has been calculated that 92% of the world's population resides in regions where FPM size is in the range of 1-10 μm levels [20]. This is higher than the WHO-recommended annual mean concentration limit of 10 g/m^3 [2]. Although inhaling these nanoparticles in the short term has no harmful side effects, long-term exposure can cause these particles to settle in the respiratory system and cause major chronic pulmonary diseases like lung cancer, chronic bronchitis, and asthma [3-5]. There has been a shift in research focus towards finding treatments (both allopathic and herbal) since these fine particles can lead to deadly, chronic lung diseases.

Most experts regarded that one of the main ways that FPM ($\leq$ 2.5 μm) causes respiratory damage is through oxidative stress. It is widely known that respiratory deposition of this FPM can activate pulmonary and systemic oxidative stress by causing reactive oxygen species (ROS) in the lung due to a redox interaction between its constituents and lung antioxidants [6]. To prevent the lung damage caused by these FPM, findings from population studies [7] and animal models [8] suggested that the antioxidant system should be upregulated. In addition, immune or non-immune cells produce and secrete cytokines in response to a stimulus, which controls gene expression, inflammation, and other cellular processes. A group of these cytokines known as Interleukins (ILs) have been associated with FPM-induced lung inflammation [9].

Scientists across the world are trying to findout solutions to manage the impact of pollution on human body. Many medicinal plants have been reported to be useful in combating different diseases caused by air pollution. *Curcuma longa* known as haldi, haridra, manjal, pasupu, etc. in various Indian languages is one of the potential candidates. It contains *curcuminoids* as active phytoconstituent in which *Curcumin* plays a major role [18]. *Curcumin* is a phenolic compound found in turmeric, and this turmeric is a traditional food additive frequently seen in Asian cuisine [10]. By downregulating pro-inflammatory cytokines, *curcumin* has anti-proliferative and anti-inflammatory actions [11-12]. The oxidative stress, in turn, is in charge of lung cancer's early onset and growth [13]. *Curcumin* accelerates gene transcription that promotes the expression of the anti-oxidant system by lowering reactive oxygen species (ROS) and boosting the anti-oxidant defense system [14]. Therefore, this study was conducted to examine the mechanism of different formulations of *curcumin* in lung epithelial cells *in vitro*.

Materials and Methods

To obtain the maximum efficacy of *curcumin* against the human diseases especially on FPM induced inflammation, different formulations of *curcumin* were preliminary tested. It was decided that the best performing formulation would further be examined for its anti-inflammatory and anti-allergic activity. To determine the final formulation's efficacy on FPM induced inflammation, we aimed to test it on inflammatory markers released by human lung epithelial cells and the IgE antibody released from U266B1 after exposure to FPM that resembles the lung toxicity and allergic response induced by FPM in humans.

Chemicals and Kits

Fine Particulate matter (Cat. No. NIST278), Alamar blue was obtained from Sigma Aldrich, India. Human 'A549' Lung Epithelial cells and human U266B1 multiple myeloma cells were procured from National Centre for Cell Science (NCCS), Pune. Enzyme-linked immunosorbent assay (ELISA) kits (ab46042, ab46032, ab195216) were obtained from Abcam, India. This study used different *curcumin* extracts obtained from *Curcuma longa*. This included *curcumin* extract with 95% curcuminoids and tetrahydrocurcuminoids (Cur95), water-soluble *curcumin* formulation containing 17% *curcumin* (WSCur17), and dry powder of *curcumin* 17% (PCur17).

Experimental Groups

This experimental work was conducted with various groups: (i) Control; (ii) FPM; (iii) FPM + WSCur17; (iv) FPM + PCur17 (v) FPM + Cur95.

After careful consideration, it was determined that the sample with the highest performance would be selected for further evaluation.

Cell Culturing

A549 and U266B1 cells were grown under standard conditions of 5% CO_2 at 37°C in a controlled humidified incubator. Cells were cultured in F12K medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (GIBCO, USA). The cells were routinely passaged twice in a week.

Cytotoxicity

Cytotoxicity assay estimates the effect of coumpounds on the cell viability of cells. Briefly, with the help of the Alamar blue assay, we determined the IC_{50} value of FPM in the A549 cells. The cells were exposed to FPM for 3 days at various concentrations. Later, the cells were treated with different *curcumin* formulations for next 3 days. As the 17% *Curcumin* is water soluble, its *curcumin* solution was prepared in PBS whereas Cur95 was prepared in DMSO. After 3 days of FPM and *curcumin* treatments, 10 µg of Alamar blue was added to each well plate. The plate was wrapped in aluminum foil and incubated at 37 °C for 4 h. Fluorescence was measured at a dual wavelength of 560 nm excitation and 590 nm emission using a multi-mode reader.

Treatment with FPMs

To optimize the IC_{50} of FPM, A549 cells around 10^4 were seeded in 96 well plates, and on the next day, cells were treated with different concentrations (0, 0.01, 0.05, 0.1, 0.2, and 0.25 mg/mL) of FPMs for 3 days. The cytotoxicity was evaluated using an Alamar assay. Accordingly, the appropriate concentration of FPM obtained was used further in the analysis.

Treatment with WSCur17

To know the IC_{50} of WSCur17, different dilutions were taken and checked for cytotoxicity. A549 cells, around 10^4 were seeded in 96 well plates, and on the next day, cells were treated with different dilutions (10^3, 5×10^3, 10×10^3, 20×10^3, 25×10^3, 50×10^3, 75×10^3, and 100×10^3) of formulation for 24 h. The cytotoxicity was evaluated using an Alamar assay. All the dilutions less than 20×10^3 showed 100% cell death. So, dilutions of more than 20×10^3 (i.e., 20×10^3 and 25×10^3) were used for further studies to understand the protective effect.

Treatment with PCur17

To know the IC_{50} of PCur17, A549 cells, around $10^{4,}$ were seeded in 96 well plates, and the next day, cells were treated with different concentrations of Cu17 (25-1000 µM) for 24 h. The cytotoxicity was evaluated using an Alamar assay.

Treatment with Cur95

To know the IC_{50} of Cur95, A549 cells, around $10^{4,}$ were seeded in 96 well plates, and

the next day, cells were treated with different concentrations of Cur95 (2-10 µg/mL) for 24 h. The cytotoxicity was evaluated using an Alamar assay.

Effect of WSCur17 and PCur17 on FPMs Induced Cell Death

To evaluate the protective effect of PCur17 and WSCur17 on FPMs induced cell death, A549 cells around 10^4 were seeded in 96 well plates. On the next day, cells were treated with 250 µg/mL concentration of FPMs accordingly with PCur17 (25 and 50 µM) and WSCur17 (20×10^3 and 25×10^3) for 3 days. The cytotoxicity was evaluated using an Alamar assay.

Treatment with Cur95 in Combination with FPM on A549 Cells

To evaluate the protective effect of Cur95, A549 cells around 10^4 were seeded in 96 well plates, and on the following day, cells were treated with FPM (250 µg/mL) and Cur95 (2, 3, and 4 µg/mL) and in combination for 3 days. The cytotoxicity was evaluated using Alamar blue assay. As the results showed by Cur95 was better than the other formulations, it was used for further evaluation.

Anti-Inflmmatory Activity of Cur95 on FPM Induced Inflammation in Lung Cells Using ELISA

A549 cells were exposed to FPM (250 µg), and was treated with Cur95 (2 and 4 µg/mL). After the treatment to detect the released inflammatory markers, supernatants were collected from the treatment media. IL-6 and IL-8 levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Abcam as per the manufacturers instruction.

Oxidative Stress Study using Malondialdehyde Assay

A549 cells were exposed to FPM (250 µg) and was treated with Cur95 (2 and 4 µg/mL). After the treatment to detect the oxidative stress, cells were lysed in the extraction buffer and cell lysates were analysed for MDA content.

Morphology of A549 Cells After Treatment with Cur95 and FPM

Cell morphology after Cur95 and FPM exposure was evaluated with a light microscope (Lecia, India). Lung epithelial cells were cultured on coverslips and treated accordingly with FPM and *curcumin*. After treatment the cells were fixed (4% PFA) and washed (1X PBS) in all the groups and were stained with Haematoxylin and Eosin for examination under the microscope.

Anti-Allergic Activity of Cur95 on FPM Induced Allergy using IgE ELISA

Human U266B1 multiple myeloma cells were cultured at 37 ºC under a 5% CO_2 incubator. RPMI 1640 medium supplemented with 10% FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U penicillin, and 50 µg/mL streptomycin was used as culture medium. Cells (1×10^6 cells/well) were stimulated with FPM (250 µg/mL to attain maximum IgE secretion) and treated with Cur95 (2 and 4 µg/mL) for 72 h. The supernatants were harvested for IgE estimation using Sandwich ELISA (Abcam, USA).

Statistical Analysis

Data expressed as the Mean ± SEM. Experimental groups were compared using ANOVA. Statistical analysis was performed using GraphPad Prism (v.8.0.2) with $\alpha = 0.05$ as the minimal significance level.

Results

Cytotoxicity

The Alamar blue assay measured the IC_{50} value of FPM after cells were exposed to various FPM concentrations for 72 h. In a concentration-dependent way, FPM decreased the viability of A549 cells compared to unexposed control cells (Figure 1A). These results showed that A549 cells obtained significant cell death (50%) by FPM at 250 µg/mL. So, this concentration was used for further experiments as the IC_{50} value of FPM for inducing inflammatory response. Cells were exposed to FPMs at a concentration of 250µg/ml, in line with various treatment groups using *curcumin* (25 and 50µM) and *curcumin* formulation (20×103 and 25×103) for a duration of 3 days. Subsequently, the IC50 values of WSCur17 and PCur17 (Figure 1B-1C) were determined, revealing that they did not demonstrate any protective effect against FPM-induced cell death (Figure 1D). The presence of only 17% *curcumin* in WSCur17 and PCur17 may potentially explain the lack of efficacy in treating FPM-induced cell death.

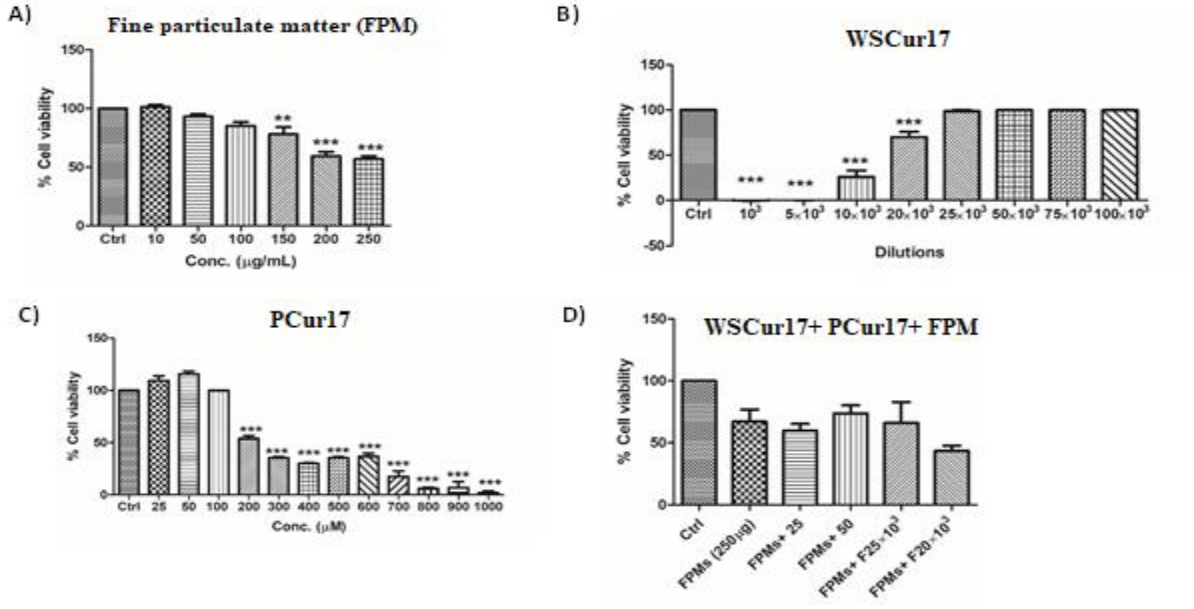

Figure 1. Cytotoxicity Assay Using Alamar Blue in A549 cells. Treatment of A549 Cells with A) FPM, B) WSCur17, C) PCur17, and D) Co-treatment of FPM with WSCur17 and PCur17 for 3 days. Data Represents mean ± SEM (n=3). **p<0.01, ***p<0.001 vs Ctrl.

In light of the absence of the desired protective effect with WSCur17, our attention shifted to standard *curcumin* for further examination. We initiated the study by seeding approximately 104 A549 cells into a 96-well plate. The subsequent day, these cells were subjected to a range of concentrations, encompassing FPM (10, 50, 100, 150, 200, and 250µg/ml), as well as *curcumin*-95% (2, 4, 6, 8, and 10µg/ml), both independently and in conjunction, over a 3-day period. Cytotoxicity profiles were assessed through the Alamar blue assay, with the IC50 value for FPM established at 250µg/ml. Consequently, protective thresholds for *curcumin* were ascertained, with 2 and 4µg/ml identified as effective concentrations, as concentrations surpassing 4µg/ml induced adverse effects in A549 lung epithelial cells (Figure 2A). These determined concentrations of *Curcumin*-95% (Cur95) were subsequently employed to evaluate their protective efficacy against FPM-induced cell death. Noteworthy is the observation that treatment with 4µg/ml of Cur95 exhibited a marked reversal effect on FPM-induced cell death (Figure 2B).

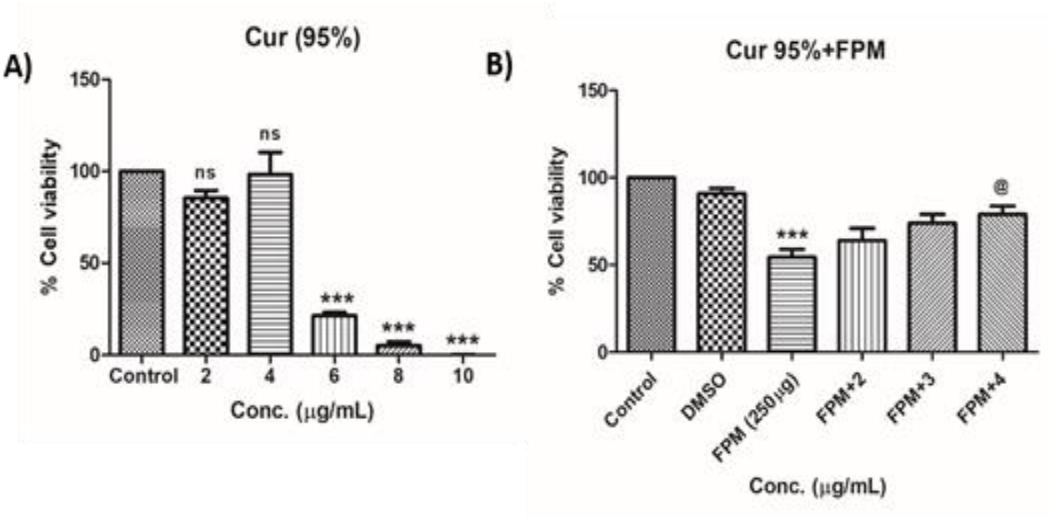

Figure 2. Protective Effect of Cur95 on FPM Induced Cell Death. A) Determination of IC_{50} value of Cur95, B) Cur95 Treatment in FPM Stimulated cells. Data Represents mean ± SEM (n=3). **p<0.01, ***p<0.001 vs ctrl, $^{@}$p<0.05 vs FPM, ns vs ctrl.

Effect on the Restoration of Morphology of Human Lung Epithelial Cell

A549 cells were exposed to various treatments (Control, DMSO, FPM, FPM + Cur95 2 µg/ml, and FPM + Cur95 4 µg/ml) according to experimental plan before being scanned by a light microscope to see whether FPM could change the morphology of cells. The ciliated cell membrane of A549 cells was strongly adhered to FPM. It was observed that A549 cells in exposure to FPM altered their morphology. Whereas the treatment with Cur95 could reverse the morphological changes induced by FPM (Figure 3).

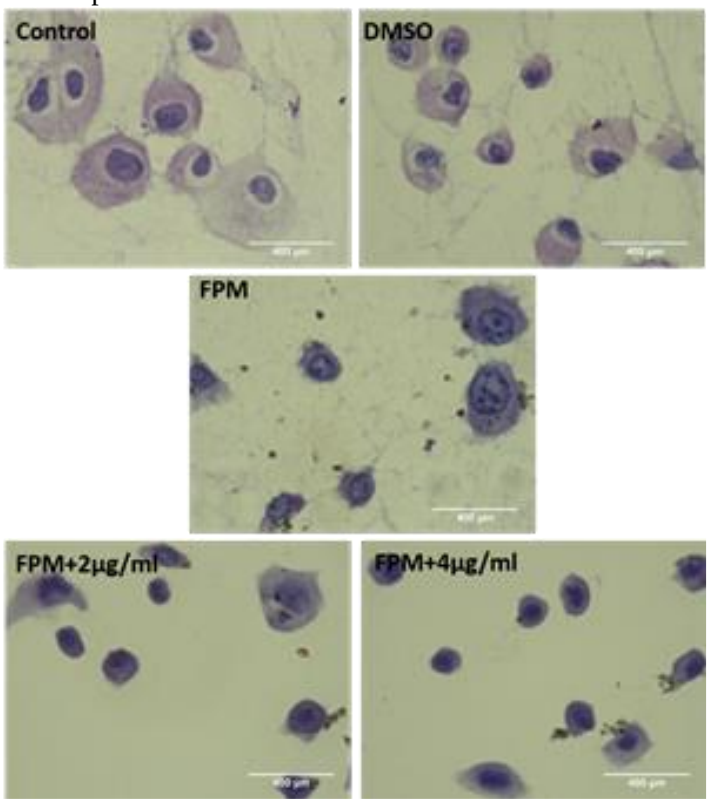

Figure 3. Cell Morphology of FPM Stimulated Cells with Cur95 Treatment

Anti-Oxidant Effect of Cur95 Against FPM Induced Oxidative Stress

Intracellular levels of malondialdehyde (biomarker in oxidative stress) were measured using an MDA assay after A549 cells were exposed to FPM (250 µg) and Cur95 (2 and 4 µg/ml). After the treatment with FPM, there was a significant increase in intracellular MDA levels (Figure 4). Treating the FPM exposed A549 cells with Cur95, showed a protective effect of Cur95 that was evident by a decrease in the MDA levels induced by FPM, but not to a significant level. These results suggest the anti-oxidant effect of Cur95 against the FPM induced oxidative stress.

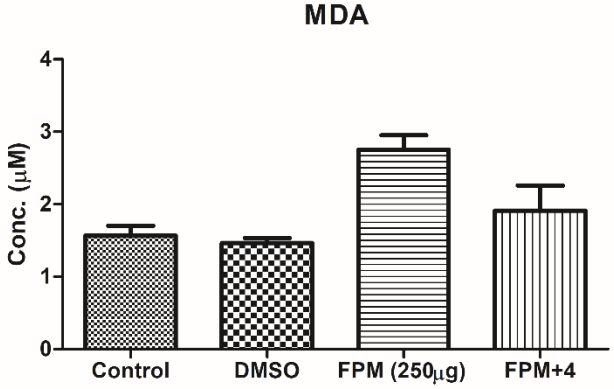

Figure 4. Anti-Oxidant Potential of Cur95 against FPM Induced Oxidative Stress. Data represents mean ± SEM (n=3).

Anti-Inflammatory Effect of Cur95 Against FPM Induced Inflammatory Response

FPM was found to trigger the release of inflammatory cytokines (IL-6 and IL-8) indicating the induction of inflammatory response. To study how FPM affects the production of inflammatory cytokines in A549 cells, the Cur95 cells were exposed to FPM. We found there is a significant increase in the protein levels of IL-6 and IL-8 inflammatory cytokines when evaluated with ELISA in the FPM treated group in comparison with untreated or control cells. These results show that inflammatory cytokines were expressed in A549 cells due to FPM exposure indicating the inflammatory condition. The Cur95 treatment in the FPM stimulated cells significantly decreased the protein levels of IL-6 and IL-8, signifying the anti-inflammatory potential of Cur95 against FPM induced inflammation (Figure 5A-B).

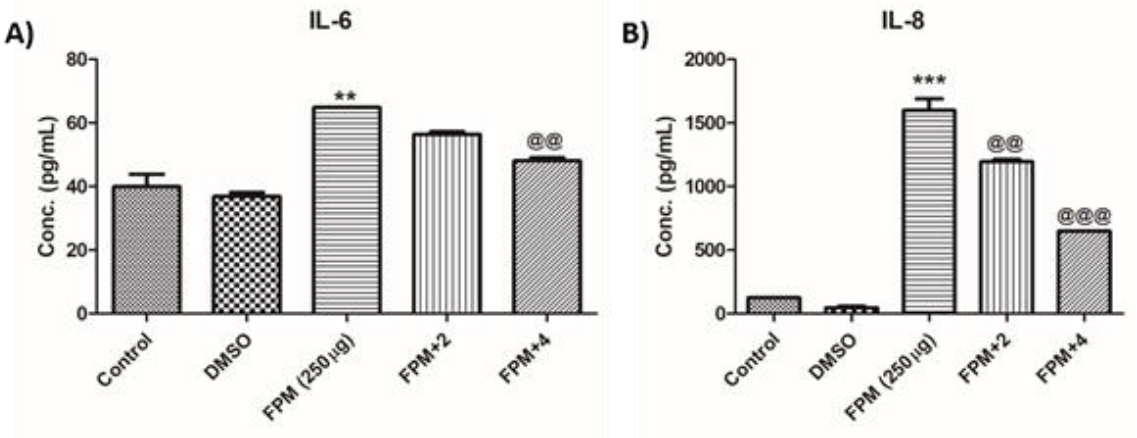

Figure 5. Anti-Inflammatory Potential of Cur95 Against FPM Induced Inflammation. **p<0.01, ***p<0.001 vs ctrl, $^{@@}$p<0.01, $^{@@@}$p<0.001 vs FPM.

Anti-Allergic Effect of Cur95 Against FPM Induced Allergy

Release of IgE from B-cells is an indicative of an allergic response. U266B1 is a B-lymphocyte which secretes IgE antibodies during an allergic response. FPM are the constituents of air pollution which are known to induce allergic reponse in lungs. When FPM at a concentration of 250 µg/mL was exposed to A549 cells, we detected the secretion levels of IgE antibodies from B cells after exposure using IgE ELISA assay. A significant increase in IgE release in U266B1 cells was observed after exposure to FPM, which was significantly decreased after treatment with Cur95 in FPM-induced inflammatory condition (Figure 6).

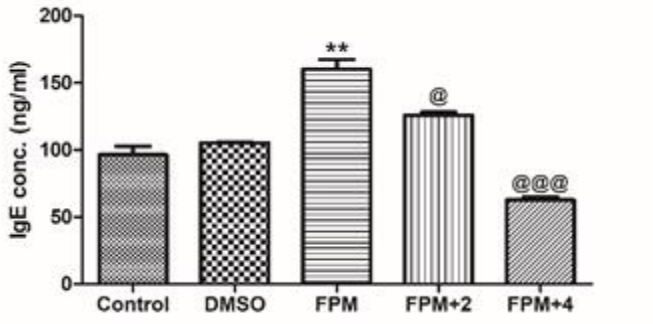

Figure 6. Anti-Allergy Potential of Cur95 Against FPM Induced Allergy. **p<0.01 vs ctrl, $^{@}$p<0.05, $^{@@}$p<0.01 vs FPM

Discussion

There are several epidemiological studies on the impact of FPM on health, particularly those that focus on lung conditions that cause significant morbidity and mortality over time. Scientists are highly concerned about the prevalence of chronic pulmonary disorders, including lung cancer, which develops due to long-term deposits of these FPM in tissues. To prevent PM-induced chronic diseases, many scientists are working to develop various preventative and therapeutic remedies of chemical and herbal origin. The present lifestyle trend inevitably exposes living organisms to inhaling these particles.

Several research has been conducted using a mouse model of FPM-induced airway inflammation to examine the effects of natural herbal extracts or chemicals. Nonetheless, various researches have been carried out to investigate the effects of FPM on reactive oxygen species or pro-inflammatory cytokines utilizing lung epithelial cells [19]. Cell viability assays have been performed to investigate the biological effects of FPM. We observed that exposure to FPM reduced the viability of A549 cells in a concentration-dependent manner. In this study, we showed that FPM changed A549 cells morphology and decreased cell viability. In addition, FPM increased malondialdehyde levels, indicating decreased intracellular antioxidant enzyme activity. FPM significantly increased the expression of inflammatory cytokines such as IL-6, and IL-8. These findings demonstrated that FPM caused oxidative stress and inflammatory response in A549 cells. Our results imply that oxidative stress and inflammatory response may be significant factors in A549 cells proliferation and malfunctioning of the nasal epithelial barrier in response to FPM exposure. Further research revealed that in A549 cells, FPM induced an increase in intracellular malondialdehyde. These findings imply that the dysfunctional nasal epithelial barrier brought on by FPM may be partially mediated by oxidative stress-induced cell proliferation. Further research is necessary to thoroughly understand the underlying molecular mechanisms of FPM and

antioxidant enzyme activity. This oxidative stress might be the reason for cytotoxicity to lung epithelial cells. Treating exposed A549 cells with Cur95 showed a protective effect towards this oxidative stress. As *Curcumin* 95% was the most effective formulation, it was branded later as Curcunan®. The epithelial barrier in the nasal cavity is made up of nasal epithelial cells, which also produce different inflammatory mediators during nasal inflammatory diseases. When there is localized inflammation, airway epithelial cells can produce and release inflammatory cytokines into the surrounding tissues. The critical mediators of the pathophysiology of inflammation are thought to be IL-6 and IL-8. This study found that FPM elevated the expression of IL-6 and IL-8. Our results imply that FPM may cause local inflammation, exacerbate it, and worsen disease by promoting the production of IL-6 and IL-8. However, as anticipated, treatment with Curcunan® reduced these secreted protein levels, and the results were statistically significant. This shows that these proteins' expression was downregulated, preventing the invasion of inflammatory cells and reducing the airway epithelium's thickness.IgE and related cellular reactions cause allergic airway diseases. In allergic asthma, an immune system-driven hypersensitivity reaction is triggered and is mediated by IgE antibodies. Hence, the IgE levels in the U266B1 cells were therefore examined. It was observed that the Curcunan® exhibited a positive effect on suppressing the immune system's overactivation brought on by FPM, and this help in preventing inflammation from getting worse.

The impact of Fine Particulate Matter (FPM) on human health, particularly its association with chronic lung conditions, has garnered substantial attention in epidemiological studies. Chronic pulmonary disorders, including lung cancer, are of particular concern due to the long-term accumulation of FPM in tissues. As a consequence, numerous efforts are underway to develop preventative and therapeutic solutions, drawing from both chemical and herbal sources. Our study aligns with the growing concern about the prevalence of chronic pulmonary diseases induced by FPM exposure. We conducted experiments to assess the effects of FPM on lung epithelial cells, specifically A549 cells, which serve as a model for airway inflammation. Our findings revealed that exposure to FPM resulted in a concentration-dependent reduction in A549 cell viability, accompanied by morphological changes. Importantly, FPM exposure led to increased malondialdehyde levels, indicative of reduced intracellular antioxidant enzyme activity, and elevated expression of inflammatory cytokines such as IL-6 and IL-8. These results underscore the role of oxidative stress and an inflammatory response in A549 cell proliferation and the impairment of the nasal epithelial barrier following FPM exposure. Furthermore, our investigation uncovered that FPM induced intracellular malondialdehyde, suggesting a potential link between oxidative stress-induced cell proliferation and the dysfunctional nasal epithelial barrier. However, our study also revealed a promising avenue for mitigating these effects. Treatment with Curcunan®, a formulation primarily composed of *Curcumin* 95%, exhibited a protective effect against oxidative stress induced by FPM in A549 cells. Our findings align with the broader scientific literature on the effectiveness of *curcumin* in mitigating the harmful effects of pollution. Several other studies have explored the potential of *curcumin* in countering the oxidative stress and inflammatory damage induced by particulate matter. For instance, research has shown that *curcumin* can reduce oxidative stress and inflammatory responses, potentially protecting vital organs. Additionally, it has been suggested that *curcumin* may act through pathways involving

the HO-1/CO/P38 MAPK pathway to alleviate oxidative stress and inflammation induced by PM2.5 [15]. Moreover, predictive studies have indicated that *curcumin* has the potential to inhibit the hazardous effects of PM2.5 by modulating bio-functions such as airway inflammation, cancerogenesis, and apoptosis, along with pathways like NF-kappaB signaling. These findings emphasize the multifaceted nature of *curcumin's* protective mechanisms against the adverse impacts of pollution [17]. Another study investigating nasal fibroblasts exposed to urban PM (UPM) supports our observations. UPM exposure led to increased ROS production, activation of ERK, and alterations in HO-1 and SOD2 expression, all of which were mitigated by *curcumin* treatment. These results further highlight *curcumin's* anti-oxidative properties and its potential as a therapeutic agent for nasal diseases caused by airborne particulate matter [16]. However, our study contributes to the growing body of evidence suggesting that *curcumin*, particularly in the form of Curcunan®, may offer significant protection against the oxidative stress and inflammatory responses induced by fine particulate matter, thereby potentially mitigating the health risks associated with pollution exposure. Further research is warranted to comprehensively elucidate the underlying molecular mechanisms and optimize *curcumin*-based interventions in combating the adverse effects of pollution on human health.

Conclusion

Different formulations of *curcumin* were preliminary tested for their efficacy on FPM-induced inflammation in ‘A549’ lung epithelial cell line. The best-performing formulation Curcunan® was further evaluated for its anti-inflammatory and anti-allergic activity. It was discovered to have tremendous potential for combating pollution-related respiratory illnesses. This research has demonstrated that exposure to FPM results in oxidative stress and inflammatory response, which may impede cell proliferation and compromise the function of the nasal epithelial barrier. On the other hand, the treatment with Curcunan® showed a protective effect in protecting the cells from FPM-induced cytotoxicity and helped in reverting the cell morphology. The treatment exhibited protective activity against oxidative stress and anti-inflammatory properties of *curcumin*. In addition, the treatment showed anti-allergic activity by decreasing the secretion of IgE levels. Hence, Curcunan® may be demonstrated to be a promising herbal component for preventing FPM-induced airway diseases using a more thorough mechanistic analysis. To further verify these in vitro outcomes in humans, additional clinical research may be taken up.

Conflict of Interest

This study, "Protective Effect of Curcunan® on Human Lung Epithelial Cells Exposed to Fine Particulate Matter and Response to Allergens," involved authors with direct affiliations to Dr. Willmar Schwabe India Pvt. Ltd., the entity responsible for marketing Curcunan®. Given these affiliations, there exists a potential conflict of interest that could be perceived as influencing the study's design, outcomes, and conclusions. Despite this, the authors affirm their commitment to scientific objectivity and the principles of unbiased research. All necessary steps have been taken to ensure the study's integrity and to mitigate any potential influence arising from these affiliations.

Acknowledgement

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Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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