Research Square

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A traditional Chinese medicine formula derived from Siraitia grosvenorii (Monk fruit) allieviates PM2.5 induced airway epithelial damage

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Abstract

PM2.5 is the main component of air pollution and poses a major health hazard to exposed individuals. Due to its small particle size, PM2.5 can carry toxic substances into the alveoli, promote the generation of reactive oxygen species (ROS), and damage respiratory epithelial cells (AECs) and their tight junctions (TJs) and adhesion junctions, causing damage to the epithelial barrier. This study investigated the protective effect of a traditional Chinese medicine (TCM) formula (Siraitia grosvenorii granule, or SGG) consisting mainly of Siraitia grosvenorii (SG) on PM2.5 induced insult on a respiratory epithelial cell line (Calu-3). We found that SGG was able to reverse the change of various markers of oxidative stress, cytotoxicity, and apoptosis induced by PM2.5. Of note, SGG enhanced epithelial barrier function, as demonstrated by the expression of tight (TJP-1, Zo-1) and aderence (CDH-1) junction proteins and the functional assays of the epithelial barrier function. As such, the result suggests that TCM medication may be an effective countermeasure to PM2.5 through protecting epithelial barrier function.

1 Introduction

Smog is a significant form of air pollution and has increaslingly become a major, global threat to human health. PM2.5, that is, particulate matter with a diameter of no more than 2.5 microns (also known as fine particulate matter), is a key component of smog. Due to its small size, PM2.5 can travel to deeper parts of the lung. In addition, the small size of PM2.5 is associated with relatively large surface area, hence results in more damage to the respiratory tract than larger particles. Furthermore, the small size of PM2.5 allows it to permeate into various transit channels, such as blood and nerve, and negatively affect a number of distant organs and systems, such as the brain(Maher et al., 2016; Qi et al., 2022) and cardiovascular system(Bai et al., 2024). The Global Burden of disease (GBD) study estimates that exposure to ambient PM2.5 in 2019 was the fourth leading risk factor for premature death among residents worldwide(Kang et al., 2023).

The Airway Epithelial Cells (AECs) act as a physical barrier between the airway and the external environment, which prevents deleterous agents, such as pathogens and xenobiotics, from entering the bloodstream. In addition to the AECs, intercellular apical junction complexes (AJCs), which include tight junctions (TJs) and adherens junctions (AJs)(Niessen, 2007), play a critical role in connecting and interacting with adjacent cells (Gao and Rezaee, 2022). The barrier function provided by AECs in conjunction with AJCs, form a dynamic defense system(Hiemstra et al., 2015). Studies using *in vitro* and *ex vivo* models have shown that PM2.5 are cytotoxic to various types of AECs(Jeong et al., 2017; Zhang et al., 2018) and disruptive to the barrier function, including reducing the expression of of TJ and AJ proteins (Goksel et al., 2024; Zhao et al., 2020). Therefore, it is crucial to identify interventions that protect against the impairment of barrier function caused by exposure to PM2.5.

When PM2.5 enters the cell, the toxic substances, such as ozone, attached to its surface promote Reactive Oxygen Species (ROS) production, depletes intracellular antioxidant (Gangwar et al., 2020; Zhang et al., 2021). The resulting oxidative stress may be the initial insult happening to the AEC induced

by PM2.5, which subsequently lead to the disruption of epithelial barrier. As such, we hypothesize that an antioxidant approach could potentially protect the barrier function.

Momordica grosvenori (Siraitia grosvenorii, SG) is a climbing herb of the genus Siraitia grosvenorii in Cucurbitaceae. It is considered as a “food medicine” in China and is often used as a natural antitussive and expectorant. Siraitia grosvenorii Granule (SGG) is primarily a blend of traditional Chinese medicine (TCM) herbs comprised of SG, lily, chrysanthemum, honeysuckle, and loquat leaf. Of note, many of the ingredients, inclduing SG, have been shown to have antioxidant effect(Guo et al., 2024; Hao et al., 2022; Li et al., 2024). In addition, chemical analysis and network pharmacology have shown that SGG may activate NRF2-mediated antioxidant signaling pathway(Shao et al., 2025). The aim of this experiment is to determine whether SGG alleviates PM2.5 damage on AEC, AJC, and its effect on barrier function and antioxidant defense.

Table 1
Latin names for all ingredients of SGG

Traditional Chinese medicine name	Latin name
Momordica grosvenori	Siraitia grosvenorii (Swingle) C. Jeffrey ex Lu et Z. Y.
Lily	Lilium brownii var. viridulum Baker
chrysanthemum	Chrysanthemum × morifolium Ramat
honeysuckle	Lonicera japonica Thunb.
loquat leaf	Eriobotrya japonica Thunb.

2 Materials and Methods

2.1 Materials

SGG is provided by BabyCare., Ltd. and comprises a blend of Siraitia grosvenorii Fructus powder(30%), Lilium Bulbus powder (13%), Lonicera japonica Thunb. flos powder (5%), Chrysanthemum morifolium Ramat flos powder (5%), Eriobotrya japonica Thunb. folium piwder (5%), Pyrus pyrifolia fructus powder (5%), and inulin (37%, as excipient). PM2.5 was puchased from Standard Reference Materials (SRM 1648a; Dongguan Baishun Biotechnology Co., LTD., Shenzhen, China).

2.2 Cell culture

The Calu-3 cell, which is an epithelial cell line originally obtained from a human lung adenocarcinoma, was kindly supplied by the National Biomedical Experimental Cell Resource Bank. Calu-3 cells were cultivated in a high-glucose Minimum Essential Medium supplemented with 10% Fetal Bovine Serum. They were kept at an optimal temperature of 37°C in a balanced atmosphere composed of 95% air and 5% carbon dioxide (CO_2).

2.3 Cell viability assay

Cells were seeded into a 96-well plate at a density of 1.8 × 10^4 cells per well and allowed to adhere for 24 hours. For PM2.5 exposure experiments, PM2.5 standard particles were sterilzed by autoclaving and dissolved in the culture medium to achieve concentrations of 300, 350, and 400 μg/ml, and cells were treated with the three concentrations for 24 hours to determine the optimal modeling concentration based on cell viability. For the SGG exposure assay, SGG was added to the culture medium to concentrations of 1000, 750, or 500 μg/ml, and the medium was warmed to 38°C–40°C with constant stirring for 30 minutes to ensure complete dissolution. The cells were then treated with disolved SGG for 24 hours to determine the optimal dosage concentration. Following the exposure to PM2.5 or SGG, 10 μL of CCK-8 reagent (Solarbio, CA1210) was added to each well, and the plate was incubated at 37°C for 1.5 hours. Finally, the absorbance of each well was measured at a wavelength of 490 nm to assess cell viability.

2.4 Flow cytometry

Cell apoptosis assessment was conducted utilizing the Annexin V-FITC Apoptosis Detection Kit (Solarbio, CA1020). Calu-3 cells were collected after exposing to various concentrations of PM2.5 for 24h, rinsed once using Phosphate-Buffered Saline (PBS), and subsequently stained using Annexin V-FITC. Quantification was achieved through flow cytometry analysis employing a BD FACSCalibur device (BD Biosciences, California, USA), with data processed by the FlowJo software suite.

2.5 JC-1 staining

Following the manufacturer's guidelines, the assessment of the mitochondrial membrane potential (MMP) in Calu-3 cells was conducted utilizing the JC-1 Staining Kit (Solarbio, M8650). Calu-3 cells were incubated with JC-1 dye working solution at 37°C for 0.5 h and then rinsed twice with JC-1 buffer solution. JC-1 monomers (as green fluorescence) was determined using a fluorescence microscope (Leica, TCS SP8 STED) at wavelength of 525 nm.

2.6 Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA extraction was carried out employing the FastPure Cell/Tissue Total RNA Isolation Kit (Magen, R4111-03). 500 nanograms of the total RNA was reverse transcribed to complementary DNA (cDNA) via the iScript™ cDNA Synthesis Kit (BIO-RAD, 1708890), which was then amplified using quantitative real-time PCR (Power Realab Green PCR Fast mixture, Applied LabLead) on a QuantStudio 5 platform (Thermo Fisher Scientific, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control. The primer sequences employed throughout this study are detailed in Table 1.

Table 1
The primer sequences employed

Genes	Forward (5'-3')	Reverse (5'-3')
Caspase-3	TGGAACCAAAGATCATACATGGAA	TTCCCTGAGGTTTGCTGCAT
PARP	GAGGTGGATGGAGTGGATGAA	TTGCTGCTTGTTGAAGATGAGT
Bax	GGCCCTTTTGCTTCAGGGGA	TAGAAAAGGGCGACAACCCG
BCL-2	TGGCCTTCTTTGAGTTCGGT	GGGCCGTACAGTTCCACAA
CDH1	TGGAACAGGGACACTTCTGC	CCCCGTGTGTTAGTTCTGCT
TJP1	AGCCATTCCCGAAGGAGTTG	ATCACAGTGTGGTAAGCGCA
GAPDH	TCACCATCTTCCAGGAGCGA	CTTCTCCATGGTGGTGAAGAC

2.7 Fluorescent staining of ROS

Calu-3 cells were gently washed twice with PBS, followed by immersion in a serum-free medium containing 5 μM Dichlorofluorescein diacetate (H2DCFDA, a fluorescent dye of ROS) (Beyotime, S0033S) in the dark at 37°C for half an hour, then washed three times with PBS. Finally, ROS was visualized using an Olympus FV10i laser scanning confocal microscope.

2.8 Biochemical Assay of lactate dehydrogenase (LDH), malondialdehyde (MDA), and superoxide dismutase (SOD) activity

The quantification of LDH (Beyotime, C0016), MDA (Beyotime, S0131S), and SOD (Beyotime, S0109) in Calu-3 cells after 24-hour exposure to PM2.5 was carried out adhering to the manufacturer's guidelines, and the results were determined using spectrophotometry. The assay of SOD activity was based on the dampened generation of chromatic nitro-blue tetrazolium (NBT) formazan in the presence of SOD in a system of xathine and xathine oxidase, and the result was expressed as enzymatic unit and calculated as I%/(1 – I%), wherein the I% is the % of inhibition of light absorbance at 560 nm.

2.9 Transepithelial cell electrical resistance detection (TEER)

Cells were placed onto Matrigel-covered Transwell® inserts within a 12-well plate (Corning, USA), with a seeding density of 2.5×10^5 cells per insert. The electrical resistance across the cell monolayer, known as TEER, was determined utilizing an epithelial Volt/Ohm meter equipped with STX2 “chopstick” style electrodes (EMD Millipore, USA). To ensure accuracy, the background resistance measured from an unseeded (blank) permeable cell culture (PCF) insert was subtracted from every reading. The resulting resistances were adjusted for the surface area of the cell layer, yielding values in ohms times square centimeters (ohms*cm²).

2.10 Fluorescein-5-isothiocyanate (FITC)-dextran permeability assay

Cells were placed onto Matrigel-covered Transwell® inserts within a 12-well plate (Corning, USA), with a seeding density of 2.5×10^5 cells per insert. Afterward, the cells were carefully washed with PBS and then incubated in Hank's balanced salt solution (HBSS) supplemented with 1 mg/mL of FITC-labeled dextran (Yuanye, S19127) for one hour. To evaluate the passage of FITC-dextran, 100 uL of the solution from the bottom chamber of the well were sampled. The fluorescence intensity was quantified employing a microplate reader (Thermo Fisher Scientific, Varioskan™ LUX) at wavelengths of 492 nm for excitation and 520 nm for emission.

2.11 Immunofluorescence staining and confocal analysis

Initially, the cells were rinsed twice with PBS and fixed in 4% paraformaldehyde. Permeabilization of the cells was achieved with a 0.1% Triton X-100 PBS mixture. Subsequently, the cells were blocked with 5% bovine serum albumin (BSA) and then were incubated overnight at 4°C with primary antibodies, rabbit anti-ZO-1 (proteintech, 21773-1-AP, 1:100), rabbit anti-Occludin (proteintech, 27260-1-AP, 1:100), rabbit anti-E-cadherin (proteintech, 20874-1-AP, 1:100). The next day, the cells were tagged with correspondent fluorophore-conjugated secondary antibodies (proteintech, SA00013-2, 1:100). Nuclei were stained with DAPI (Biorigin, BN20923). Fluorescence imaging was performed using a Olympus FV3000 confocal laser scanning microscope.

2.12 Statistical Analysis

The outcomes are presented as mean ± standard error of the mean (SEM). To assess differences among various groups, one-way ANOVA was employed, complemented by Duncan's post hoc test for multiple comparisons. All statistical evaluations were carried out utilizing GraphPad Prism version 9.0 software. A probability level of $p < 0.05$ was deemed statistically significant.

3 Results

3.1 PM2.5 reduced the viability of Calu-3 cells, while SGG is non-toxic to cells

To investigate the cytotoxic impact of PM2.5 on AEC, Calu-3 cells were individually subjected to varying concentrations of PM2.5 for 24h. Cell viability post-exposure was assessed through the CCK-8 assay. Figure 1A illustrates that the exposure to PM2.5 led to a reduction in cell viability in a dose-responsive fashion. Notably, treatment with 350 μg/ml of PM2.5 elicited a moderate effect on cell viability and was therefore selected as the optimal level in the following eperiments that tested SGG’s protective effect.

As depicted in Fig. 1B, exposure to various concentrations of SGG does not induce toxicity to Calu-3 cells.

3.2 SGG alleviated the damage of Calu-3 cells exposed to PM2.5

With co-exposure to SGG at various concentration, the cytotoxicity of PM2.5 to Calu-3 cells was decreased in a dose-responsive manner (Figure. 2A). Among the doses tested, an non-toxic concentration of 750 μg/ml of SGG was selected for drug intervention in the following experiment.

Given the report of PM2.5 on cell apoptosis(Aghaei-Zarch et al., 2023), we determined if PM2.5-induced apoptosis in Calu-3 cells, and if so, if SGG protects cells from such an effect. The findings revealed a substantial increase in the apoptosis rate among cells in the model group. However, following treatment with SGG, there was a significant reduction observed in the apoptosis rate (Figure. 2B). We also assessed the early apoptosis-associated mitochondria damage using the JC-1 assay. As shown in Figure. 2C, the green fluorescence (generated by the monomer released from the disrupted mitochondria) of the model (PM2.5-treated) group significantly intensified compared to the control group. In contrast, the green fluorescence significantly attenuated after co-treatment with SGG, indicating its protective effect.

Next, we measured the mRNA expression levels of apoptosis related genes. In consistence with the PI-annexin IV finding, the expression of pro-apoptotic genes (Caspase3, PARP, and Bax) in the model group increased, while the expression of anti-apoptotic gene (BCL-2) decreased (Figure. 2D). Such changes were reversed by SGG. In addition, SGG reduced PM2.5-induced release of LDH into the medium, a marker of cell damage (Figure. 2E).

Overall, these data indicate that exposure to PM2.5 standard particles induces epithelial cell damage, and SGG can effectively reduce the degree of this damage.

3.3 SGG reduced oxidative damage in Calu-3 cells exposed to PM2.5

As shown in Fig. 3A, the model (PM2.5-treated) group exhibited a substantial rise in ROS staining when contrasted with the control group, whereas this intensity significantly declined in the presence of SGG. In addition, we examined the level of lipid peroxidation (using the marker MDA, Fig. 3B) and antioxidant enzyme (using SOD as a marker, Fig. 3C) under various conditions. PM2.5 markedly increased MDA in the model group but not in the combined (SGG + PM2.5) group. We did not observe a significant change of SOD acticity in either model or SGG group, likely due to the complex impact of PM2.5 on antioxidant enzymes, as ROS generated by PM2.5 may lead to induction of these enzymes as well as lower expression due to cell damage.

3.4 SGG alleviated epithelial barrier damage in Calu-3 cells exposed to PM2.5.

PM2.5 increased dextran permeability across the cultured Calu-3 cells (Fig. 4A) and decreased cell trans-epithelial membrane resistance (TEER, Fig. 4B), markers of epithelial barrier impairment. Conversely, SGG effectively reversed both alterations.

We also employed RT-PCR to determine the gene expression of epithelial barrier-associated proteins. We observed a decline in mRNA of adherens (CDH-1, Fig. 4C) and tight (TJP1, Fig. 4D) junction gene with the model group, which was reversed by SGG. Such a change in gene expression was also investigated at the protein level using immunofluorescent staining (Fig. 4E). We found that SGG significantly improved the abundance of ZO-1 (encoded by gene TJP1) protein; and a similar pattern, although not statistically significant, of change was observed in occludin (encoded by gene OCLN) and E-cadherin (encoded by gene CDH-1).

In sum, these data demonstrated SGG helps to maintain barrier function in the presence of PM2.5, which may be explained by the decrease of AEC death and increased expression of AJC proteins.

4 Discussion

PM2.5 contributes to the development and acute episode of numerous respiratory illnesses, such as chronic inflammatory lung conditions and athema(Chen et al., 2022; Li et al., 2020; Zhao et al., 2020). This study investigated the protective effect of a TCM herbal blend (SGG) against PM2.5-induced airway epithelial damage. Using Calu-3 cell as a model, we observed that SGG ameliorated the oxidative stress and cytotoxicity of AEC after exposure to PM2.5. It is of note that SGG not only maintained cell viability, but it specifically protected the cell against apoptosis, an established form of cell death induced by PM2.5(Aghaei-Zarch et al., 2023). Furthermore, we found that SGG restored the epithelial barrier that was a critical property of the epithelium against further invasion and subsequent damage by PM2.5 at the respiratory tract as well as other organs and systems.

Oxidative stress is a key mechanism underlying the harmful effects of particulate matter(K et al., 2016). It may result from two major sources of ROS: first, through the inhalation of particles that are already bound with ROS (particle-bound ROS, such as oxygenated organic aerosol), and second, through ROS that is generated after the inhalation (oxidative potential, OP, such as ozone). Regardless of the source, ROS exert oxidative damage to lipids, proteins, and DNA, leading to cellular dysfunction, cell death, and sbusequently inflammation. As such, enhancing antioxidant defense may provide the first line of protection against PM2.5 induced damage. In fact, our unpublished data, together with other reports(Lee et al., 2023), indicate that TCM herbs (such as Lonicera Japonica) are able to elicit antioxidant effect through activing NRF2 and protect mice from PM2.5 induced pulmonary oxidative stress and tissue damage.

In consistence with the reports using *in vitro* and *in vivo* AEC models studying PM2.5 (Goksel et al., 2024; Zhao et al., 2020), we have found PM2.5 disrupt AEC barrier function. The barrier function of AEC consists of two components: the epicethial cells (AEC) and the intercellular adhesive junctions (AJC, including tight junction and adherens junction). Therefore, impairment of either AEC (such as apoptosis)

or AJC allows PM2.5 to penetrate the airway. In fact, we have observed that both effects had occurred in our model. In particular, our study indicates that PM2.5 decreases the gene expression of tight junction proteins such as E-cadherin, ZO-1 and Occludin. E-cadherin facilitates calcium-dependent adhesion in epithelial cells as a transmembrane component. Occludin is a key transmembrane proteins for tight junctions, while ZO-1 is an intracellular protein linking tight junctions to cytoskeleton such as actin (Wan et al., 2001). These proteins form a complex network that regulates the paracellular permeability of the epithelial layer, ensuring that only specific molecules can pass through while blocking others. Disruption of AJCs, therefore, can lead to increased permeability of the epithelial barrier, allowing potentially harmful substances to enter the underlying tissue and circulation, which can result in inflammation and disease(Herrero et al., 2019). Of note, as PM2.5 reduces the viability of AEC via ROS, it may also deplete AJC proteins through oxidative stress. This postulate is supported by other studies that report decreased AJC protein expression due to H2O2 treatment (Shen et al., 2024)d acetyl-cysteine (an antioxidant) attenuated the effect of PM2.5 on AJC proteins (Zhao et al., 2018). Nonetheless, it remains to be determined if the effect of SGG on junction proteins is due to a direct, regulatory effect on their expression or indirectly through maintaining the viability of cell.

In summary, SGG can alleviate oxidative damage to epithelial cells caused by PM2.5, reduce cell apoptosis rate, and provide a protective effect on the epithelial barrier. These findings provide amechanistic insights that may account for the protective effect of SGG on PM2.5-induced airway, and potentially systemic, damage. Meanwhile, the study also illustrates the importance of antioxidant defense and barrier function of AEC in fending against PM2.5 toxicity.

Abbreviations

Airway Epithelial Cells (AECs)

Apical Junction Complexes (AJCs)

Adherens Junctions (AJs)

bovine serum albumin (BSA)

complementary DNA (cDNA)

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Global Burden of disease (GBD)

Hank's balanced salt solution (HBSS)

Lactate dehydrogenase (LDH)

Malondialdehyde (MDA)

Mitochondrial Membrane Potential (MMP)

Nitro-blue tetrazolium (NBT)

Phosphate-Buffered Saline (PBS)

Reactive Oxygen Species (ROS)

standard error of the mean (SEM)

Siraitia grosvenorii (SG)

Superoxide dismutase (SOD)

Traditional Chinese medicine (TCM)

Transepithelial cell electrical resistance (TEER)

Tight Junctions (TJs)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The author Hua Bai is an employee of BabyCare Inc. that manufactures and sells SGG® as a commercial product in China. The authors Junqiang Tian, Haojie Cheng, and Robert Sinnott, are employees of the USANA Health Science, Inc. that is a parent company of BabyCare, Inc.

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

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Authors' contributions

WX and TJ developed project conception, supervision, and manuscript generation. ZY , LC , RZ and CF contributed to the generation of the cell culture and experiments. BH developed project conception. SR guided the conceptualization of the project. CH participated in data processing and analysis.

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Figures

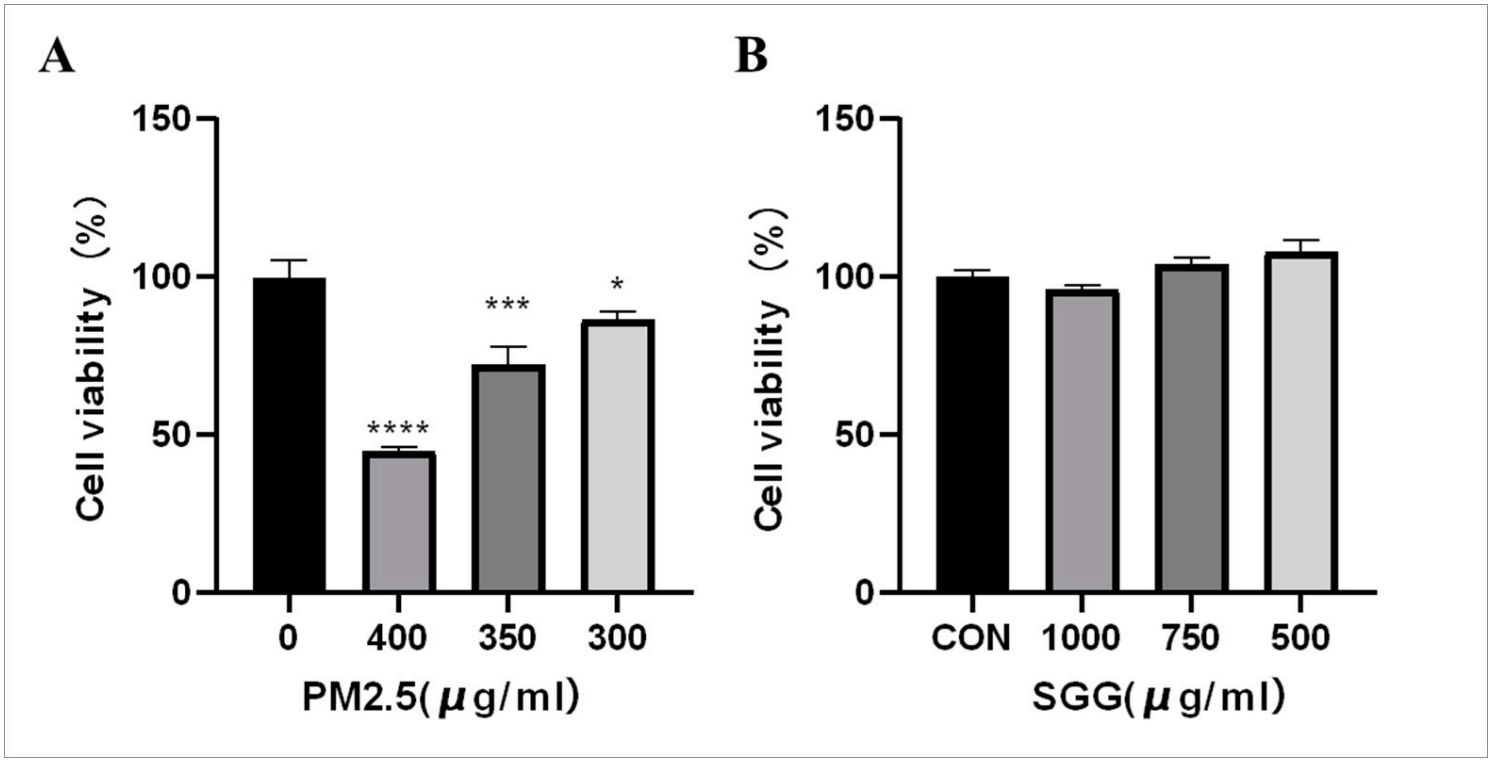


Figure 1

The effects of PM2.5 and SGG on cell viability. Calu-3 cell were incubated with different concentrations of PM2.5 (A) and SGG (B) for 24 hours, and the viability was determined by CKK8 assay. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, vs. the control group.

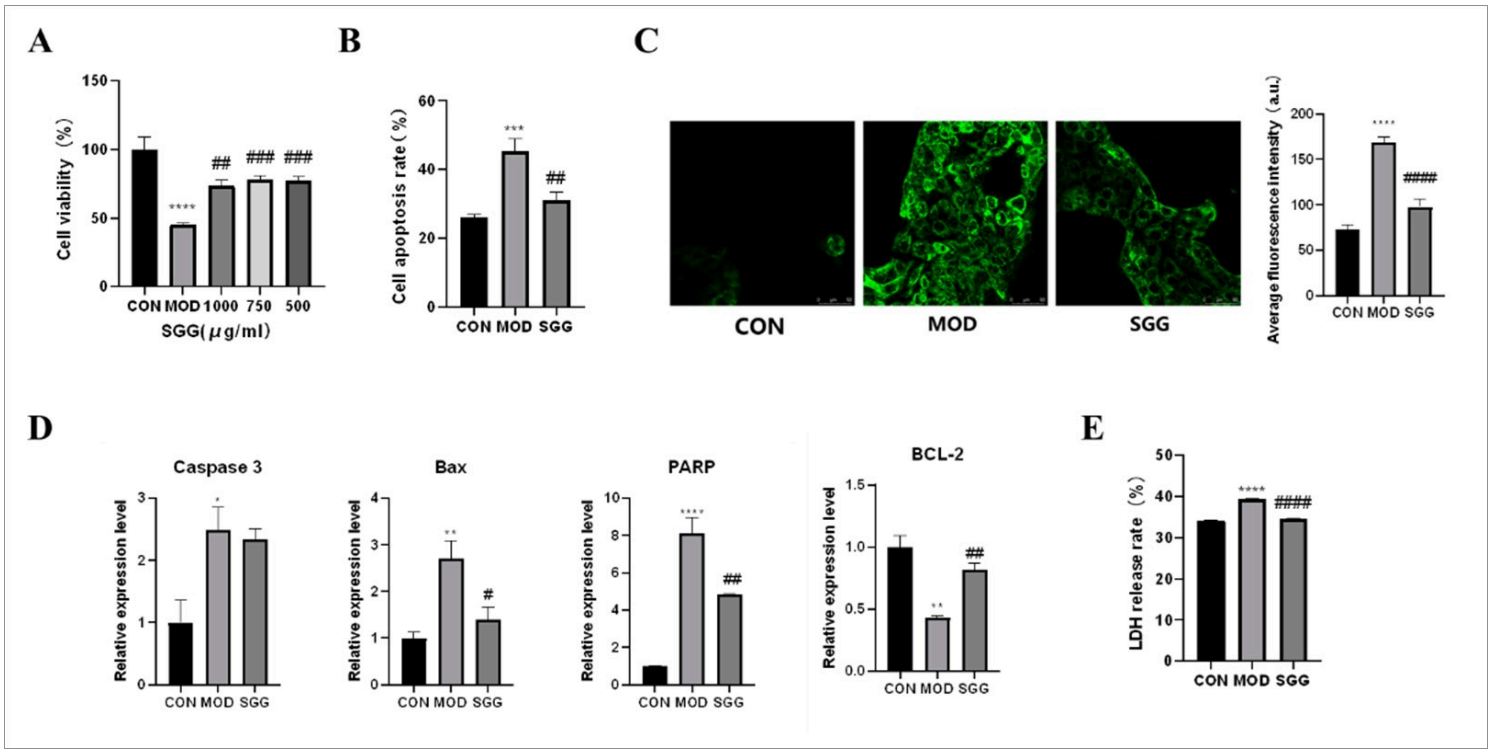


Figure 2

The effects of PM2.5 and SGG on PM2.5-induced cell damage. Calu-3 cells were incubated with PM2.5 in the absence or presence of SGG for 24 hours, and analyzed by (A) CKK8 assay for cell viability; (B) PI-annexin IV Flow cytometry for apoptosis; (C) fluoresence of JC-1 monomers for mitochondria damage; (D) Expression of apoptosis related genes for apoptosis; and (E) release of LDH. CON, control cells treated with PBS; MOD, cells treated with PM2.5 (350 ug/mL); SGG, cells treated with PM2.5 (350 ug/mL) and SGG (750 ug/mL). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, vs. the control group; # $p < 0.05$, ## $p < 0.01$, ## $p < 0.001$, #### $p < 0.0001$, vs. the model group.

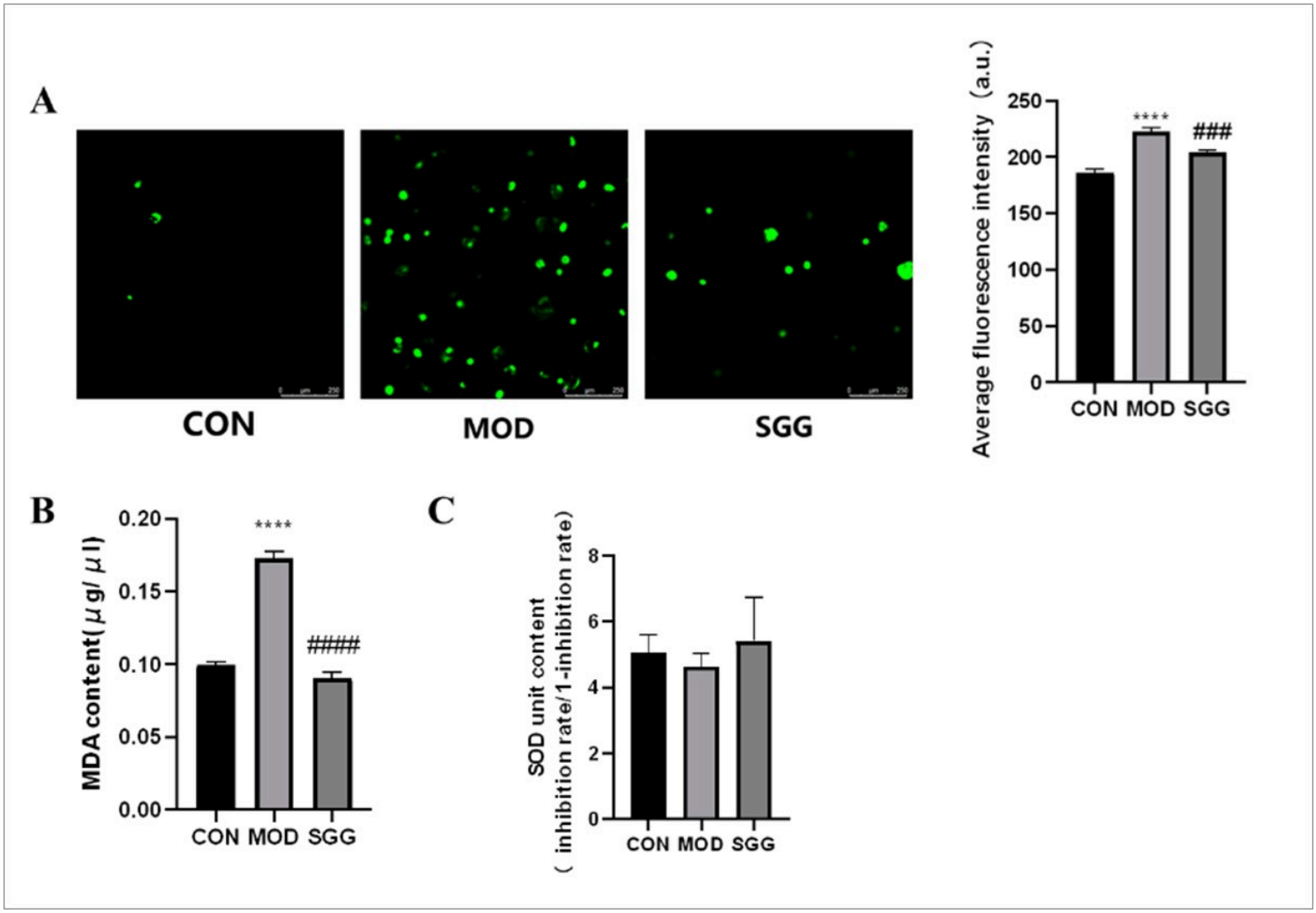


Figure 3

The effects of PM2.5 and SGG on oxidative stress. Calu-3 cells were incubated with PM2.5 in the absence or presence of SGG for 24 hours, and analyzed by (A) ROS fluorescent dye DCFDA; (B) marker of lipid peroxidation MDA; and (C) SOD activity. CON, control cells treated with PBS; MOD, cells treated with PM2.5 (350 ug/mL); SGG, cells treated with PM2.5 (350 ug/mL) and SGG (750 ug/mL). **** $p < 0.0001$, vs. the control group; ### $p < 0.001$, #### $p < 0.0001$, vs. the model group.

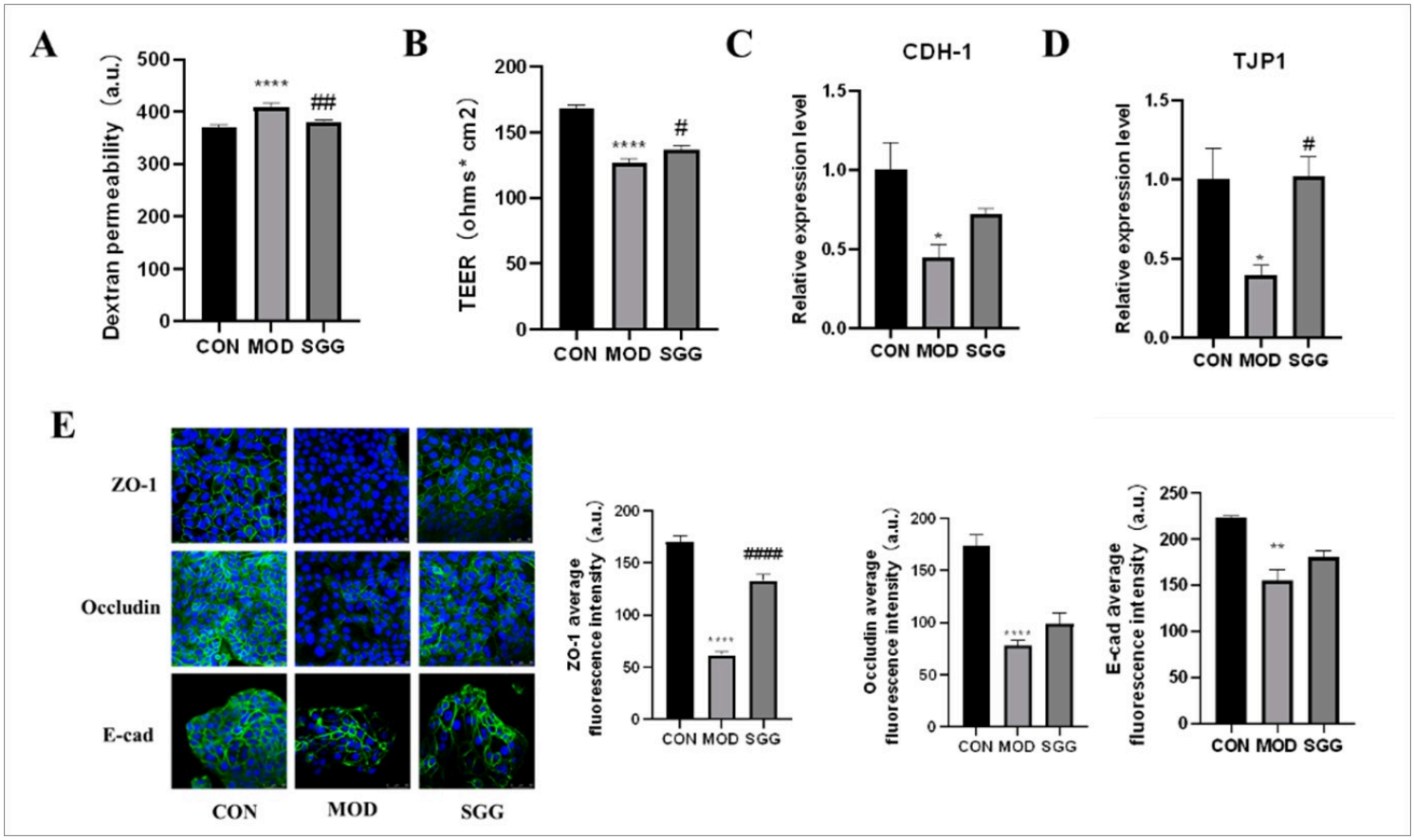


Figure 4

The effects of PM2.5 and SGG on epithelial barrier. Calu-3 cells were incubated with PM2.5 in the absence or presence of SGG for 24 hours, and the barrier function was analyzed by (A), FITC-dextran permeability; (B) TEER; (C) mRNA expression of tight junction genes CDH-1, (D) mRNA expression of adherens junction genes TJP1; and (E) protein expression of junction relevant genes. CON, control cells treated with PBS; MOD, cells treated with PM2.5 (350 ug/mL); SGG, cells treated with PM2.5 (350 ug/mL) and SGG (750 ug/mL); E-cad, E-cadherin. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, vs. the control group; # $p < 0.05$, ## $p < 0.01$, #### $p < 0.0001$, vs. the model group.