Water Soluble and Insoluble Components of PM2.5 in Nanchang City and Their Cytotoxic Research on Epithelial Cells (A549)

Xiaozhen Liu (liuxiaozhen@ncu.edu.cn)
Nanchang University https://orcid.org/0000-0002-9564-5957
Lan Wang
Nanchang University
Yufeng Ren
Nanchang University

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Abstract

To investigate the toxic effects of $PM_{2.5}$ on human lung epithelial cells A549 and the potential mechanism of action, this research intends to prepare water-soluble (WS-$PM_{2.5}$) and insoluble components (WIS-$PM_{2.5}$) of $PM_{2.5}$ by collecting atmospheric fine particles. The samples of atmospheric $PM_{2.5}$ at the Qianhu Campus of Nanchang University in Nanchang city were collected on this subject. The desiccated $PM_{2.5}$ was prepared into water-soluble and insoluble components, The cell toxicity of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ was assessed by the MTT method measuring the cell survival rate. According to the results of the MTT test, the exposure time was selected 24 h for all subsequent tests. The lactate dehydrogenase (LDH) and intracellular superoxide dismutase (SOD) in the cell supernatant fluid were measured by the trace enzyme method. Cell reactive oxygen species (ROS) were measured by flow cytometry, and the expression of cell inflammatory factors IL-6 and TNF-α were measured by ELISA and RT-QPCR. The results showed that WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ can inhibit the activity of A549 cells, destroy the integrity of cell membranes, promote oxidative stress in cells, and induce inflammatory reactions in cells, thereby producing toxic effects on A549 cells. However, their toxic mechanism is different, and the toxicity of the cells is significantly different. The toxic effect of WIS-$PM_{2.5}$ is significantly stronger than that of WS-$PM_{2.5}$ in inhibiting cell viability, cell membrane damage, and oxidative stress, while significantly lower than that of WS-$PM_{2.5}$ in causing inflammatory damage.

Highlights

1. Toxic effects of different components of atmospheric $PM_{2.5}$ on A549 cells in vitro exposure experiments.
2. Toxic effects of different components of $PM_{2.5}$ in terms of both oxidative stress and inflammatory mediator mechanisms.
3. Significant differences in the toxic effects of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ on A549 cells.

1 Introduction

With the accelerated development of urban industry and transportation, hazy weather caused by $PM_{2.5}$ has occurred frequently in recent years and has become one of the environmental factors that seriously affect the atmospheric environment and endanger public health(Feigin et al. 2016; Liu et al. 2019; Wang et al. 2022), Particles with a diameter less than or equal to 2.5 μm are probably the most harmful environmental risk factors for human health(Huang et al. 2022; Schraufnagel, 2020). Particles with smaller diameters have larger specific surface areas and can absorb large amounts of toxic and hazardous substances(Lin et al. 2018). For example, polycyclic aromatic hydrocarbons (PAHs), nitro PAHs and transition metals (Fe, Zn, Ni, Cu). Studies have shown that $PM_{2.5-10}$ can only reach the upper respiratory tract by respiratory action(Schraufnagel, 2020; Akhtar et al. 2014), but $PM_{2.5}$ can enter the fine bronchi and reach deep into the alveoli to form deposits and even participate in blood circulation, causing

health hazards in the respiratory, immune, and cardiovascular systems. The lungs are the main site of particulate action, and air pollutants can be released on the lung surface and adsorbed on the lung epithelium, so the impact of $PM_{2.5}$ on human lung diseases causes great health problems. Results of epidemiological studies have shown that short-term high levels of $PM_{2.5}$ exposure are associated with increased hospitalization rates for cardiopulmonary diseases(Tasmin et al. 2016; Leoni et al. 2018; Coker et al. 2021). One of the largest epidemiological studies in China provides strong evidence that increased mortality from various lung diseases is associated with short-term $PM_{2.5}$ exposure(Chen et al. 2017).

Previously, the presence of PM chemical components has been linked to cell death, production of reactive oxygen species, and cytokines (IL-6, IL-8, TNFa) using human carcinoma cell line A549(Yuan et al., 2019; Goudarzi et al. 2019), Currently, two hypotheses are generally recognized and accepted for the toxicological pathogenesis of $PM_{2.5}$: oxidative stress damage and inflammatory mediators. Oxidative stress is thought to be one of the important mechanisms of pulmonary toxicity from exposure to atmospheric $PM_{2.5}$ particulate matter, where the surrounding $PM_{2.5}$ generates reactive oxygen species leading to oxidative stress (OS) (Li et al. 2008; Soleimani et al. 2015). Aust et alshowed that transition metal components in atmospheric fine particulate matter induce oxidative stress as well as inflammatory mediator production in lung epithelial cells A549(Aust et al. 2002). Jin et al found that PM leads to structural disruption of mitochondria through ROS generation, which in turn leads to respiratory impairment(Jin et al. 2018). Deng et al found that $PM_{2.5}$ -induced Oxidative stress may play a key role in autophagy of A549 cells, which may lead to $PM_{2.5}$-induced lung function impairment(Deng et al. 2013) [18]. Numerous reports have shown that airway inflammation is an important pathological basis for respiratory impairment due to $PM_{2.5}$(Wang et al. 2018; Wu et al. 2016). Inflammation is the most direct airway response to inhaled $PM_{2.5}$ (Li et al. 2020; Ogino et al. 2018; Pan et al. 2021). Zhou et al(Zhou et al. 2021) found that atmospheric $PM_{2.5}$ can induce airway inflammation and mucin secretion and that MUC5B plays a key role in controlling the $PM_{2.5}$-induced inflammatory response, Li et al (Li et al. 2021) treated rats and human bronchial epithelial cells (BEAS-2B) with different doses of $PM_{2.5}$ for 24 h and then performed bioassays at the end of the exposure. The results showed that acute exposure to different doses of $PM_{2.5}$ triggered inflammatory responses and apoptosis.

$PM_{2.5}$ is a complex mixture of components, mainly composed of soluble salts, carbonaceous components, biological components, metallic elements, and organic compounds(Cheng et al. 2015).To identify the key toxic components of $PM_{2.5}$ particles, several researchers have classified collected $PM_{2.5}$ into various submixes (e.g., organic and water-soluble or water-soluble and insoluble components) and compared their different adverse outcomes(Verma et al. 2012; Zhang et al. 2018). Qi Z et alused neonatal rat cardiomyocytes (NRCMs) to assess the toxic effects of $PM_{2.5}$ exposure to different components. The results showed that Total-$PM_{2.5}$ or WIS-$PM_{2.5}$ exposure significantly reduced cell viability(Qi et al. 2019), induced cell membrane damage, and increased ROS levels at concentrations above 50 μg/mL. Zou Y et al(Zou et al. 2016) found different cytotoxic mechanisms for WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ by studying the cytotoxic effects on A549, the water-soluble and insoluble component of urban $PM_{2.5}$, WS-$PM_{2.5}$ induces

early responses to ROS production, mitochondrial and multilayer membrane vesicle proliferation in A549 cells, which may cause cellular damage through oxidative stress Meanwhile, WIS-$PM_{2.5}$ was mainly associated with the disruption of cell membranes, which may lead to cellular damage through cell-particle interactions.

Most of the existing studies have focused on the toxic effects of soluble components and organic matter of $PM_{2.5}$ on cells, while there are few reports on the cytotoxicity studies of insoluble components. The effects of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ on the inflammatory response of A549 have also been less studied. In this study, we collected $PM_{2.5}$ samples of atmospheric particulate matter from Nanchang and cultured human lung epithelial cells A549 in vitro to investigate the effects of different components of $PM_{2.5}$ on cellular activity, cell membrane disruption, oxidative stress, and the degree of inflammatory damage in A549 cells, and to explore the toxic effects of $PM_{2.5}$, to provide a scientific basis for regional risk assessment and population health protection.

2 Materials And Methods

2.1 Materials

The sampling site was located at the Qianhu campus of Nanchang University, with open surroundings and no tall buildings in the way. The sampling membranes were a 90 mm diameter glass fiber membrane and a 47 mm diameter quartz membrane. The membranes were baked in a muffle furnace at 400 ℃ for 5 h before sampling to remove possible organic substances and then cooled and equilibrated in a desiccator for 24 h. After sampling, the membranes were wrapped in aluminum foil and stored in a refrigerator at -20 ℃ to prepare the particulate matter samples.

2.2 $PM_{2.5}$ sampling

The filter membrane with fine particles was cut into pieces of about 1 cm2, immersed in ultrapure water, extracted by ultrasonication for 30 min, and repeated four times, adding ice bags during the sonication process and replacing them in time to control the water temperature below 20℃. The suspension was filtered with 6 layers of sterile gauze, transferred to a 100 mL glass vial, frozen at -20℃ for one night, and then freeze-dried under vacuum to obtain gray flocculent, i.e. $PM_{2.5}$ complete particulate matter, which was stored in a low-temperature refrigerator for backup.

2.3 Cell culture

A549 cells were provided by the Stem Cell Bank of the Chinese Academy of Sciences, and the cell lyophilization solution was 95% complete culture medium and 5% DMSO, and the complete culture medium was F-12K medium with 10% fetal bovine serum and 1% double antibodies and incubated in a 5% CO_2 thermostat at 37 ℃. A549 cells were cultured by the monolayer apposition method. Cells were grown at a density of fusion to 80%-90% and digested using 1–2 mL of 0.25% trypsin. The cells were passaged 2–3 times per week.

2.4 Preparation of poisoning solution

A certain amount of $PM_{2.5}$ complete particulate matter was weighed and prepared as 4 mg/mL of the dye masterbatch, diluted with 0.9% physiological saline to make the final concentrations of 500 μg/mL, 1000 μg/mL, 2000 μg/mL, and 4000 μg/mL mixed thoroughly, left overnight at 4°C, and then centrifuged at 13000 g/min for 20 min to extract the supernatant was extracted as the WS-$PM_{2.5}$ of the concentration; the precipitate after centrifugation was resuspended with the same volume of 0.9% physiological saline as the WIS-$PM_{2.5}$ of the concentration. The concentrations were diluted 10 times for the final concentration of 50, 100, 200, and 400 μg/mL.

2.5 MTT assay

The MTT solution was prepared with PBS at a 5 mg/mL concentration, filtered and de-bacterized by a 0.22 μm filter membrane, and stored at 4°C away from light. A549 cells at the logarithmic growth stage were digested, counted, and diluted into 2×10^4/mL cell suspension, and the cell suspension was inoculated into 96-well plates at 100 μL/well, incubated at 37 ℃ in a 5% CO_2 incubator, and the supernatant was discarded after the cells were walled for 24 h. Each staining sample was diluted with F-12K medium at a volume ratio of 1:9 to 50, 100, 200, and 400 μg/mL of $PM_{2.5}$ staining solution. After the cells were incubated for 6h, 18h, 24h, 48h, and 72h, the supernatant was discarded, the cells were washed three times with PBS, 20 μL of MTT reagent was added to each well, and the culture medium was removed after 4h of incubation. The optical density (OD) values were measured at 490 nm with an enzyme marker, and the OD values of each treatment group were representative of the cell activity. The cell survival rate was calculated as $(OD_{\text{experimental group}}\text{-}OD_{\text{blank group}})/ (OD_{\text{control group}}\text{-}OD_{\text{blank group}}) \times 100\%$.

2.6 LDH assay

A549 cells were cultured under the same conditions as above. After digestion, the density of cell suspension was adjusted to 1×10^6/well and inoculated in 6-well plates at 1 mL per well. $PM_{2.5}$ staining solution at final concentrations of 50, 100, 200, and 400 μg/mL was added to the 6-well plates at 5 mL/well for 24 h. Three parallel wells were set for each treatment group, and a blank control group without a sample was sent. The supernatant was transferred to a 1.5 ml centrifuge tube after 24 h of cell transfection and centrifuged at 3000 r/min for 20 min at 4°C. After centrifugation, the supernatant was carefully collected. The absorbance value of each well was measured at 450 nm with an enzyme marker, and then the standard curve was plotted according to the absorbance value of the standard, and the number of active units of LDH in the cell culture supernatant was calculated.

2.7 The secretion of inflammatory cytokines IL-6 and TNF-α

A sterile 6-well plate was taken, and cells in the logarithmic growth phase were digested with 0.25% trypsin digestion solution, then cells were counted under a microscope through a cell counting plate, and the cell density was adjusted to 1×10^6/well, and cells were inoculated. Each well was added with 1 mL of

cell suspension, incubated at 37℃ and 5% CO_2 incubator for 24h, and the supernatant was aspirated and discarded. The infected samples were diluted with F-12K medium at a volume ratio of 1:4 to a final concentration of 50, 100, 200, and 400 μg/mL of $PM_{2.5}$, respectively, and incubated in 6-well cell culture plates at 5 mL/well for 24h. A control group was set up without the toxic sample. The supernatant was carefully transferred to a 1.5 mL centrifuge tube and centrifuged at 3000 r/min for 20 min, and the supernatant was carefully collected. The absorbance of each well was measured at 450 nm with an enzyme marker, and then a standard curve was plotted according to the absorbance of the standards to calculate the concentrations of IL-6 and TNF-α in the culture supernatant of each treatment group.

2.8 IL-6、TNF-α Detection of mRNA expression

RT-qPCR (SYBR Green Ⅰ chimeric fluorescence method) was used to detect the expression of cytokine mRNA in infected cells. Genomic DNA removal. The reaction mixture was prepared in an RNASE-free centrifuge tube, gently blown and mixed with a pipettor, and incubated at 42℃ for 2 min. cDNA was synthesized by reverse transcription. Incubated at 50℃ for 15 min and 85℃ for 5 s according to Table 2–8.

The mRNA expression was detected by fluorescence real-time quantitative PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the internal reference, and the primers were synthesized by Suzhou Hongxunbiotechnology Co., Ltd. The upstream sequence of GAPDH primer was CCATGGAGAAGGCTGGG, and the downstream sequence was CaAAGTTGTCATGGATGACC, with a length of 194bp. Upstream of IL-6 primer sequence: GAGTAACATGTGTGAAAGCAGC, Downstream: CCAGGCAAGTCTCCTCATTGAATCC, length of 116 bp; TNF-α primer sequence upstream: ACCACGCTCTTCTGCCTGCTG, downstream: GAGGGTTTGCTACAACATGG, length of 157bp. The reaction mixture was prepared in the PCR tube, and three parallel holes were set for each treatment. The PCR reaction program was carried out on a fluorescence quantitative PCR instrument. The amplification conditions were as follows: pre-amplification at 95℃ for the 30s; Then 95℃ 10s, 60℃ 30s, 40 cycles, each cycle detected a fluorescence change; Fusion curves were performed at 95℃ for 15s, 60℃, 60s, and 95℃ for 15s. Fluorescence changes were detected continuously during the whole process, and the fusion curves could be used to verify whether the amplification had specificity. Based on the internal reference Ct value, the expression level of the target gene in each treatment group was calculated by formula $2^{-\triangle\triangle Ct}$ as the multiple of the control group.

2.9 Statistical analysis

Excel 2010 was used to draw the standard curve and organize the experimental data, OriginPro 8.0 software was used to draw the chart, IBM SPSS Statistics 24.0 software was used for statistical analysis and processing, and the experimental data was represented as "$\bar{x} \pm s$". Factorial design analysis of variance and one-way ANOVA were used. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely significant.

3 Results

3.1 Effects of different $PM_{2.5}$ components on A549 cell activity

As shown in Figure 3-1, the cellular activity of WS-$PM_{2.5}$ showed an overall trend of increasing and then decreasing with the extension of the dyeing time, and the cellular activity of WIS-$PM_{2.5}$ showed an overall trend of decreasing. The low concentration dose group (50 and 100 μg/mL) showed the inhibitory effect on cell growth at 6 h of WS-$PM_{2.5}$, and the cell activity was significantly lower than that of the control group (P 0.01), and the low concentration dose group (50 μg/mL) did not show the inhibitory effect on cell growth at 48 h of contamination, while the higher concentration dose group (200 and 400 μg/mL) always showed relatively strong inhibition of cell growth. The higher dose groups (200 and 400 μg/mL) always showed a stronger inhibitory effect on cell growth. The cell survival rate was 35.6% at the lowest concentration of 50 μg/mL of atmospheric WS-$PM_{2.5}$ at 6 h. WIS-$PM_{2.5}$ always showed an inhibitory effect on cell growth in all contaminated concentration groups, and only the contaminated concentration of 50 μg/mL at 18 h showed a promotive effect on cell growth. The cell survival rate decreased to different degrees with the increase of the concentration at the same time, and there was a dose-effect relationship. Under the interaction effect of concentration and time, the highest concentration (400 μg/mL) and the longest duration (72 h) showed the greatest cytotoxic effect with a cell survival rate of 41.3%.

3.1.1 Effects of different $PM_{2.5}$ components on the damage degree of A549 cell membrane

As shown in Figure 3-2, compared with the control group, different concentrations of both $PM_{2.5}$ components could cause different increases in LDH leakage from A549 cells. Among them, the change of LDH activity in cell culture supernatant after WS-$PM_{2.5}$ staining was not significant, and the difference was statistically significant only in the staining dose 400 μg/mL group (P 0.01); The LDH activity in the cell culture supernatant after WIS-$PM_{2.5}$ staining showed a trend of increasing and then decreasing with the increase of staining concentration, and the difference was statistically significant when compared with the control group (P 0.05), and the LDH leakage was the largest when the staining dose group was 100 μg/mL (P 0.01).

The LDH content induced by WS-$PM_{2.5}$ in A549 cells at 50 and 100 μg/mL in the concentration dose groups was significantly lower than that of WIS-$PM_{2.5}$ (P 0.05). There was no significant difference (P 0.05) in the amount of LDH leakage from the cell supernatant after staining with WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ at high concentrations (200 and 400 μg/mL). This shows that the WIS-$PM_{2.5}$ had a greater effect on the LDH viability of A549 cells compared with the WS-$PM_{2.5}$.

3.2 Cell oxidative stress damage

3.2.1 Effects of $PM_{2.5}$ poisoning samples with different concentrations on ROS of A549 cells

As shown in Figure 3-3, compared with the control group, each concentration gradient of different components of $PM_{2.5}$ caused the enhancement of intracellular ROS fluorescence intensity in A549 cells.

Among them, the intracellular ROS fluorescence intensity was significantly enhanced in all concentration dose groups of $PM_{2.5}$ water-soluble components (P<0.01), and the maximum intracellular ROS fluorescence intensity was 499.77 in the dyed dose group of 100 μg/mL; The intracellular ROS fluorescence intensity of $PM_{2.5}$ non-water soluble components showed a trend of increasing and then decreasing with the increase of the dyeing concentration, and the difference was statistically significant when compared with the control group (P<0.01), and the maximum intracellular ROS fluorescence intensity was 951.30 when the dyeing dose group was 100 μg/mL.

The fluorescence intensity of ROS in WIS-$PM_{2.5}$ A549 cells in the same concentration dose group was significantly higher than that of the WS-$PM_{2.5}$ (P<0.01). This shows that the ability of the WIS-$PM_{2.5}$ to induce ROS production was significantly stronger than that of the WS-$PM_{2.5}$ (P<0.01).

3.2.2 Effects of $PM_{2.5}$ poisoning samples with different concentrations on SOD in A549 cells

As shown in Figures 3-4, the SOD activity of A549 cells treated with WS-$PM_{2.5}$ tended to increase with the increase of $PM_{2.5}$ concentration, while the SOD activity of A549 cells treated with WIS-$PM_{2.5}$ tended to decrease with the increase of $PM_{2.5}$ concentration. The SOD activity of A549 cells was significantly lower than that of the control group at the concentration of 50 μg/mL of WS-$PM_{2.5}$ (P<0.01), and the differences between the rest of the staining concentrations and the control group were not significant (P>0.05); The cellular SOD activity decreased significantly (P<0.05) when the concentrations of WIS-$PM_{2.5}$ were 100 and 200 μg/mL compared with the control group, while there was no significant difference (P>0.05) when the low dose group was 50 μg/mL and the high dose group was 400 μg/mL.

When the concentration of WS-$PM_{2.5}$ was 50 μg/mL, the SOD activity in the supernatant of the A549 cell culture was significantly lower than that of WIS-$PM_{2.5}$ at the same concentration (P<0.01). SOD activity in the supernatant of the A549 cell culture was significantly higher than that of WIS-$PM_{2.5}$ at the same concentration (P<0.01), and there was no significant difference between the other concentrations and WIS-$PM_{2.5}$ at the same concentration (P>0.05).

3.3 Inflammatory damage to cells

3.3.1 Effects of $PM_{2.5}$ poisoning samples with different concentrations on the secretion of inflammatory factors IL-6 and TNF-α in A549 cells

(1) Effects of PM2.5 poisoning samples on IL-6 in A549 cells

When the different components of PM2.5 were contaminated for 24 h, compared with the control group, the IL-6 content in the supernatant of the A549 cell culture was increased in each concentration dose group, and the difference was statistically significant (P<0.01) as the concentration increased first and then decreased. For WS-$PM_{2.5}$, there was no significant change in IL-6 activity in cell culture supernatant when the dose was lower than 50 μg/mL, and the difference was significant (P<0.05) when the

concentration increased compared with the control group, and the change in IL-6 activity in the cell culture supernatant was not significant when the high concentration of 400 μg/mL was reached. For WIS-$PM_{2.5}$, the IL-6 activity in the A549 cell culture supernatant was significantly higher than that in the control group when the dose of staining was ≥100 μg/mL (P＜0.05), and there was no significant difference compared with the control group when the concentration was 50 μg/mL (P＞0.05). In addition, Figures 3-5 show that for the WIS-$PM_{2.5}$ staining there was no significant difference in supernatant IL-6 activity compared to the same concentration of WS-$PM_{2.5}$.

It can be seen from the figure that the secretion of TNF-α in the cell culture supernatant increased significantly with the increase of the staining concentration of both WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ compared with the solvent control group, and there was a dose-effect relationship (P＜0.01). The TNF-α activity in the supernatant of the A549 cell culture was significantly higher (P＜0.01) than that of the WIS-$PM_{2.5}$ at the same concentration of 50 and 200 μg/mL of WS-$PM_{2.5}$, which showed that the ability of WIS-$PM_{2.5}$ n to induce TNF-α was significantly stronger than that of the WS-$PM_{2.5}$.

3.3.2 The relative expression levels of inflammatory cytokines IL-6 and TNF-α mRNA in A549 cells in $PM_{2.5}$ infected samples with different concentrations

(1) Relative expression of inflammatory factor IL-6 mRNA in A549 cells by PM2.5 stained samples

As shown in the figure, compared with the control group, the relative expression of IL-6 mRNA in A549 cells was significantly increased by both WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ at different dose concentrations (P＜0.01). The differences were statistically significant (P＜0.01). The expression level of IL-6 mRNA in A549 cells increased gradually with the increase of WS-$PM_{2.5}$; the expression level of IL-6 mRNA in A549 cells decreased and then increased with the increase of WIS-$PM_{2.5}$.

The relative expression of IL-6 mRNA in the WS-$PM_{2.5}$ was significantly higher than that in the WIS-PM2.5 at the same concentration (P＜0.05).

As shown in the figure, the relative expression of TNF-α mRNA in A549 cells in each concentration dose group of different $PM_{2.5}$ components was significantly higher than that in the solvent control group (P＜0.05). Among them, the maximum TNF-α mRNA expression in the WS-$PM_{2.5}$ dose group was more than 12 times that in the solvent control group at 200 μg/mL. The relative expression of TNF-α mRNA in A549 cells at 200 and 400 μg/mL of WS-$PM_{2.5}$ in the high concentration group was significantly higher than WIS-$PM_{2.5}$ in the same concentration group (P<0.01), while at 50 μg/mL of WS-$PM_{2.5}$ in low concentration group, The relative expression level of TNF-α mRNA in A549 cells was significantly lower than that of WIS-$PM_{2.5}$ at the same concentration (P<0.01), and there was no significant difference between other concentrations of TNF-α and WIS-$PM_{2.5}$ at the same concentration (P>0.05).

4 Discussion

Epidemiological and experimental studies have proven that exposure to atmospheric particulate matter (PM), especially exposure to fine particulate matter with sizes below 2.5 μm ($PM_{2.5}$), is closely associated with population mortality(Neira and Prüss-Ustün, 2016; Sanyal et al. 2018). With small particle size, large specific surface area, long suspension time in the atmosphere, and long transport distance, atmospheric $PM_{2.5}$ can adsorb a large number of pollutants including many heavy metals, organic substances, acidic oxides, etc., which are the main components causing damage to the cardiopulmonary system. Animal models and cellular studies have demonstrated that $PM_{2.5}$ can induce oxidative damage and inflammatory responses in the lung(Orona et al. 2020; Li et al. 2022). In this study, we collected atmospheric fine particulate matter and determined the toxic effects of water-soluble (WS-$PM_{2.5}$) and water-insoluble components (WIS-$PM_{2.5}$) of $PM_{2.5}$ on human lung epithelial cells A549, and explored the effects of each different component of fine particulate matter on A549 cells in terms of cell survival, cell membrane damage, oxidative stress, and inflammatory factors, respectively, in comparison. To assess the possible toxic damage of different components of $PM_{2.5}$ on the respiratory system.

The cytotoxic effects of WS-$PM_{2.5}$, and WIS-$PM_{2.5}$ were evaluated with A549 cells in vitro by MTT and LDH assay. It was found that the substances in the WIS-$PM_{2.5}$ exerted their maximum effect only at high doses over a long period, and at low doses in the short term, they had lower effects than the WS-$PM_{2.5}$. This may be due to the different effects of the components on cell membrane permeability and the different number of surviving cells at the end of the staining. In the case of a comparative exposure time of 24 h, the same concentration of WIS-$PM_{2.5}$ inhibited the cellular activity to a greater extent than WS-$PM_{2.5}$. This is in good agreement with the studies of others (Qi et al. 2019; Zou et al. 2016), and it may be that insoluble particles cause physical damage to the cell membrane when they are taken up by cells through active processes(Liu et al. 2013; Tang et al. 2015). In addition, particle agglomeration may lead to the production of so-called phagocytic vesicles within the membrane; thus also causing surface membrane damage (Qi et al. 2019).

$PM_{2.5}$-induced oxidative stress is considered to be an important biological effect associated with cytotoxicity(Miller, 2014). The extent of oxidative damage in cells can be determined by measuring cellular ROS and SOD levels, which increase after cellular damage by stimulation, and SOD, which is an intracellular reactive oxygen species inhibitory enzyme, is negatively correlated with ROS levels, and from the results of the assay, both WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ induced a significant increase in ROS in A549 cells, as well as a decrease in SOD activity, and the WIS-$PM_{2.5}$ had more significant oxidative damage effects than WS-$PM_{2.5}$ (Kim et al. 2018), which is in good agreement with the results reported by others (Rumelhard et al. 2007; Huang et al. 2014). This study also found that the substances in the WIS-$PM_{2.5}$ were most effective only at high doses over a long period, and in the short term at lower doses than the WS-$PM_{2.5}$. It has also been found that WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ have different temporal effects on ROS production in A549 cells (Zou et al. 2016). The early ROS response induced by the soluble chemical components of WS-$PM_{2.5}$ may be an early response in which the soluble components can rapidly enter the cells and induce ROS generation(Gualtieri et al. 2009; Bonetta et al. 2009). A possible explanation for

the late ROS response of WIS-$PM_{2.5}$ is that cells need a longer time to make the uptake of particles, which leads to mitochondrial damage associated with particles.

In addition to the oxidative stress caused by atmospheric $PM_{2.5}$, the inflammatory response is also considered to be one of the important mechanisms that induce damage to the body(Sigaud et al. 2007; Liu et al. 2022), and fine particulate matter induces changes in the biochemical composition of lung tissue and the release of inflammatory factors, mainly through adsorbed toxic substances, triggering inflammatory damage. TNF-α is an important inflammatory transmitter that plays a crucial role in initiating and maintaining increased secretion of TNF-α induces other pro-inflammatory molecules (e.g. IL-6) and amplifies inflammatory signals(Zhu et al. 2019). From the results of the inflammatory factor assay in this study, both WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ components contributed to the secretion of IL-6 and TNF-α proteins, as well as the relative expression of IL-6 and TNF-α mRNA was significantly higher while this effect was characterized, and WS-$PM_{2.5}$ was more significant than WIS-$PM_{2.5}$. This may be related to the high content of metallic elements such as iron, nickel, vanadium, and copper in WS-$PM_{2.5}$, as well as bioactive components(Valavanidis et al. 2005), which are the main determinants of oxidative stress-induced in cells and release inflammatory mediators IL-6, IL-8, and TNF-α, which aggravate the inflammatory response (Rodríguez-Cotto et al. 2013).

Most studies have focused on the toxic effects of $PM_{2.5}$ or WS-$PM_{2.5}$ and organic matter on cells, while there are few reports on the cytotoxicity studies of atmospheric fine particulate matter WIS-$PM_{2.5}$ on cells. In this study, we mainly investigated the toxic effects of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ on cells to provide a basis for our understanding of the differences in cytotoxicity between WS-$PM_{2.5}$ and WIS-$PM_{2.5}$.

5 Conclusion

In this paper, we investigated the toxic effects of water-soluble and water-insoluble components of fine particulate matter on human lung epithelial cells A549 at the QianHu campus of Nanchang University, Nanchang, China. The effects of different components of fine particulate matter on A549 cells were investigated in terms of cell survival, cell membrane damage, oxidative stress, and inflammatory factors, respectively, in comparison. The results showed that both WS-$PM_{2.5}$ and WIS-$PM_{2.5}$ of atmospheric fine particulate matter had toxic effects on human lung epithelial cells A549, with significant differences in the magnitude of toxicity, mechanism of action, and degree of damage to the organism. The present study on the toxic effects of fine particulate matter can be improved, the cytotoxicity of particulate matter can be explored from other perspectives, and animal models can be introduced for staining and toxicity treatment.

Declarations

Acknowledgments

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Author information

Affiliations Key Lab of Environment and Resources Utilization of Poyang Lake, Education Department, School of Resources Environmental & Chemical Engineering, Nanchang University, Nanchang 330031, Jiangxi, People's Republic of China

Xiaozhen Liu, Lan Wang, Yufeng Ren.

Author Contributions Xiaozhen Liu and Yufeng Ren designed the study. Material preparation, data collection, and analysis were performed by Xiaozhen Liu with the assistance of Lan Wang and Yufeng Ren. The first draft of the manuscript was written by Xiaozhen Liu and Lan Wang. And all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability *The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.*

Corresponding author Correspondence to Xiaozhen Liu

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Ethics approval and consent to participate Data were all analyzed anonymously, so ethical approval was not needed.

Human and animal rights The article does not contain any studies with human and animals and this study was performed following institutional and national guidelines.

Competing Interests The authors have no relevant financial or non-financial interests to disclose

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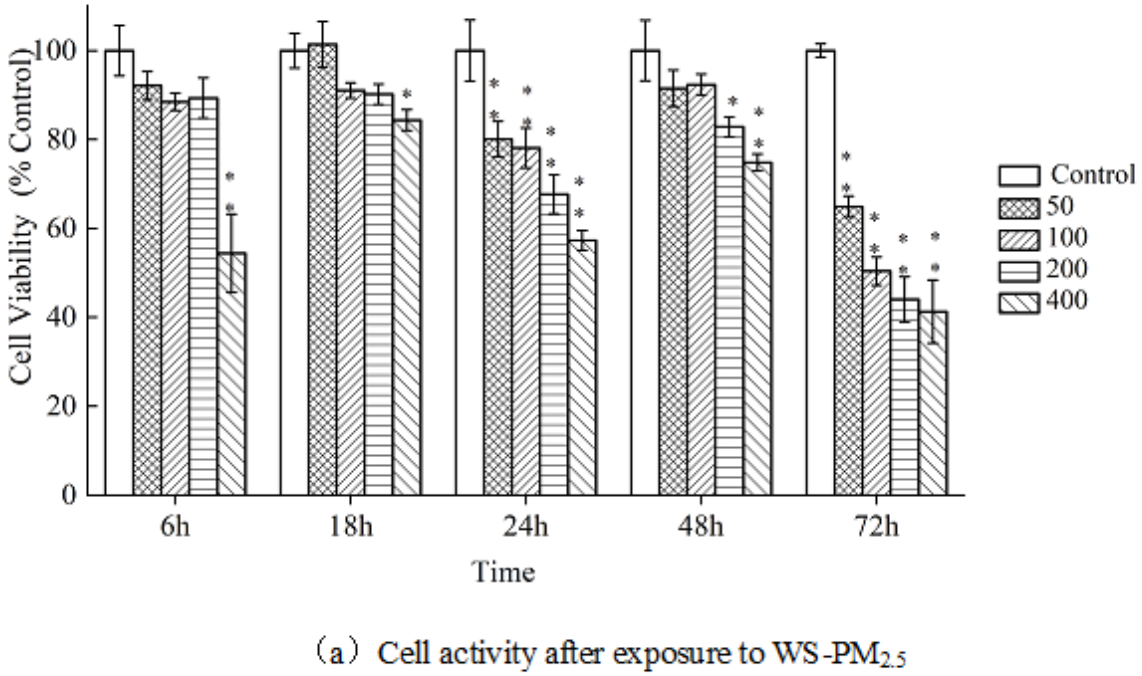
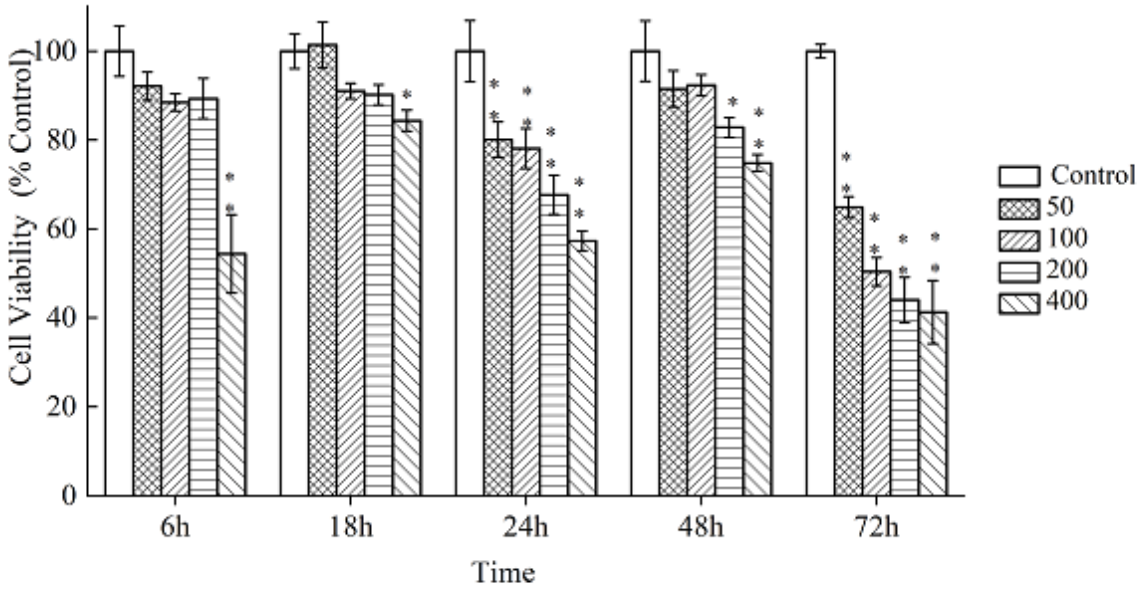
Table 2-8

Table 2-8 is not available with this version.

Figures



（a） Cell activity after exposure to WS-$PM_{2.5}$



（b） Cell activity after exposure to WIS-$PM_{2.5}$

Figure 1

Fig.3-1 The effects on the viability of A549 cells treated with various doses of different $PM_{2.5}$ components. Compared with the solvent control group（*：P＜0.05；**：P＜0.01）

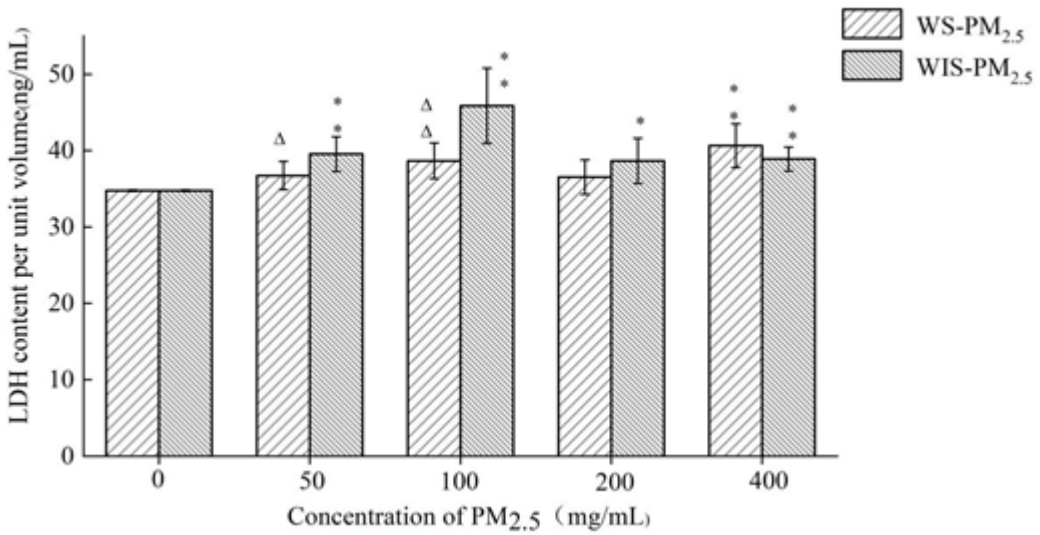


Figure 2

Fig.3-2 The effects on the release of LDH in A549 cells exposed to different $PM_{2.5}$ components. Compared with the solvent control group（*：P＜0.05；**：P＜0.01）. Comparison of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$（△：P＜0.05；△△：P＜0.01）

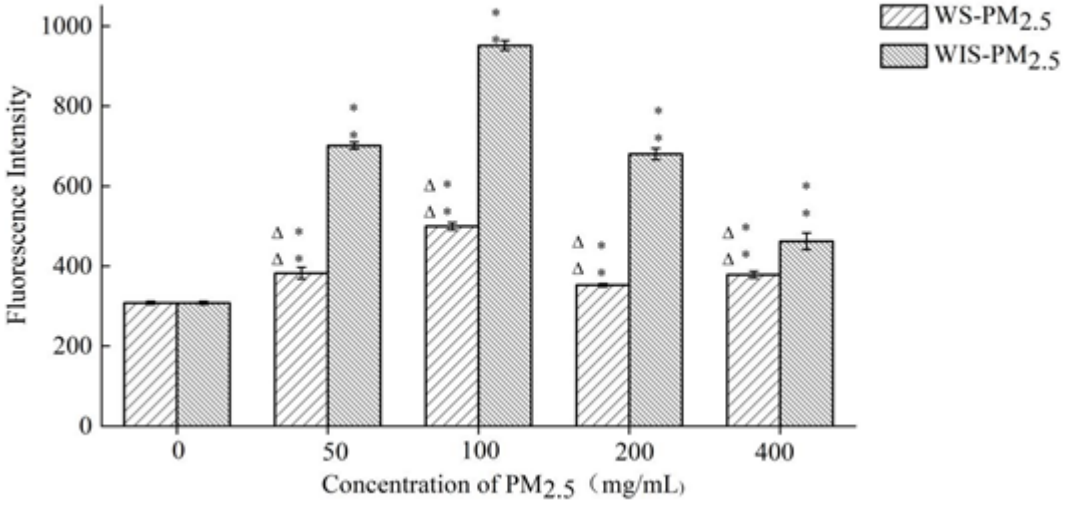


Figure 3

Fig.3-3 Relative ROS production after A549 cells exposed to different components of $PM_{2.5}$.

Compared with the solvent control group（*：P＜0.05；**：P＜0.01）

Comparison of water-soluble and non-water-soluble components of PM2.5（△ *P* 0.05，△△ *P* 0.01）

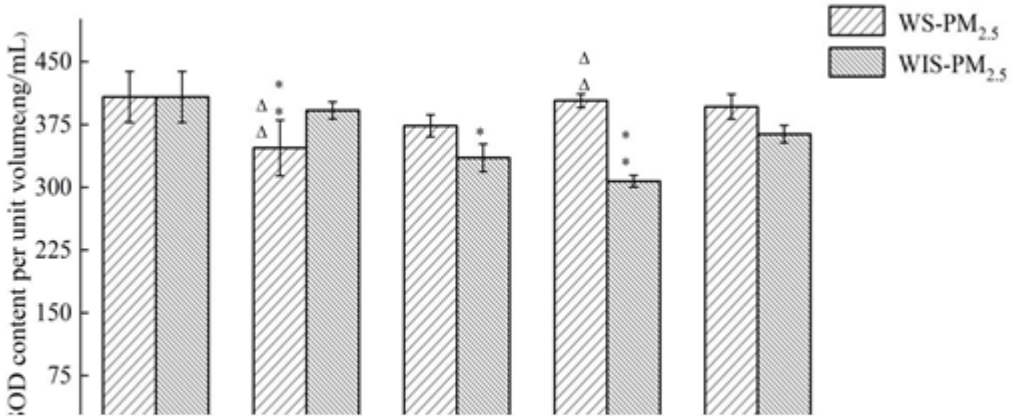


Figure 4

Fig.3-4 The effects on SOD contents after A549 cells were exposed to different $PM_{2.5}$ components.

Compared with the solvent control group（* *P* 0.05，** *P* 0.01）

Comparison of water-soluble and non-water-soluble components of PM2.5（△ *P* 0.05，△△ *P* 0.01）

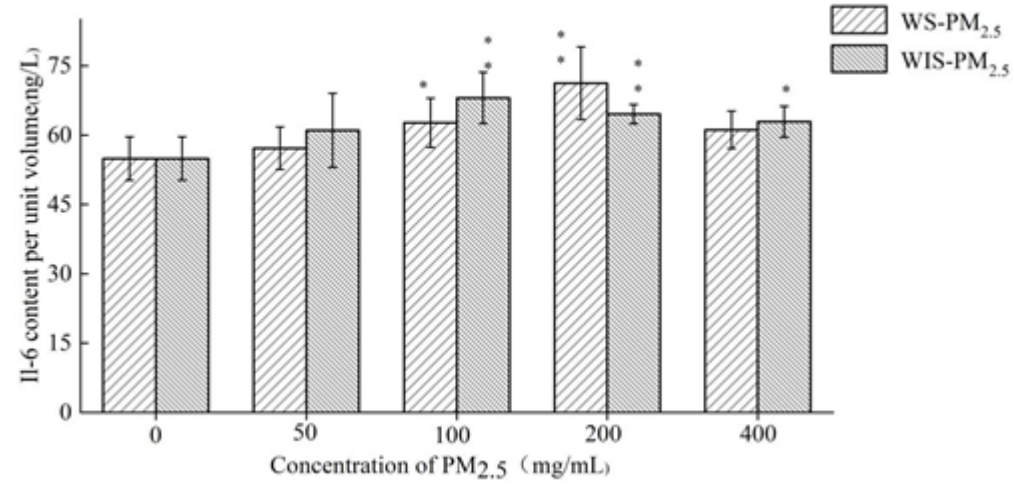


Figure 5

Fig.3-5 The effects on IL-6 contents after A549 cells were exposed to different $PM_{2.5}$ components.

Compared with the solvent control group（* *P* 0.05，** *P* 0.01）

Comparison of water-soluble and non-water-soluble components of $PM_{2.5}$（△ *P* 0.05，△△ *P* 0.01）

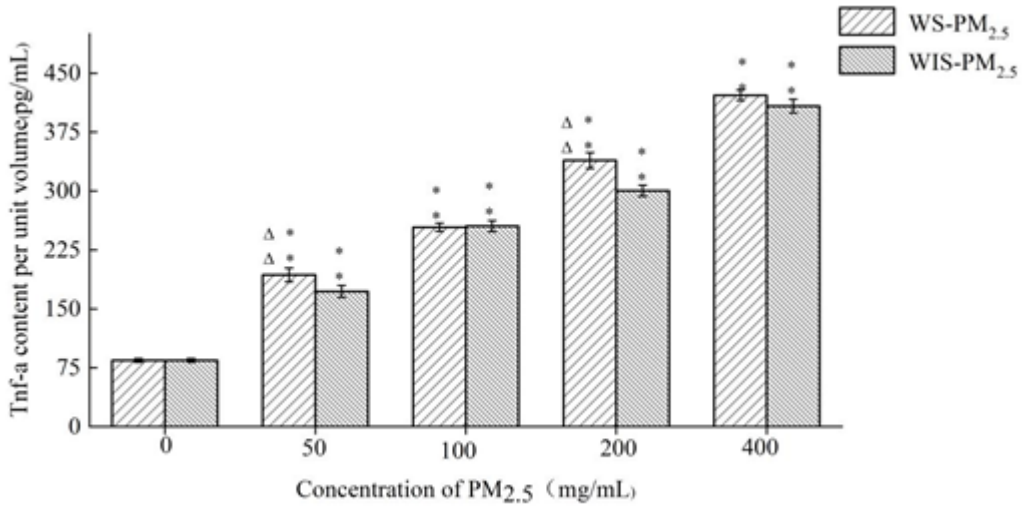


Figure 6

Fig.3-6 The effects on TNF-α contents after A549 cells were exposed to different $PM_{2.5}$ components. Compared with the solvent control group：*：$P<0.05$；**：$P<0.01$.

Comparison of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$：△：$P<0.05$；△△：$P<0.01$

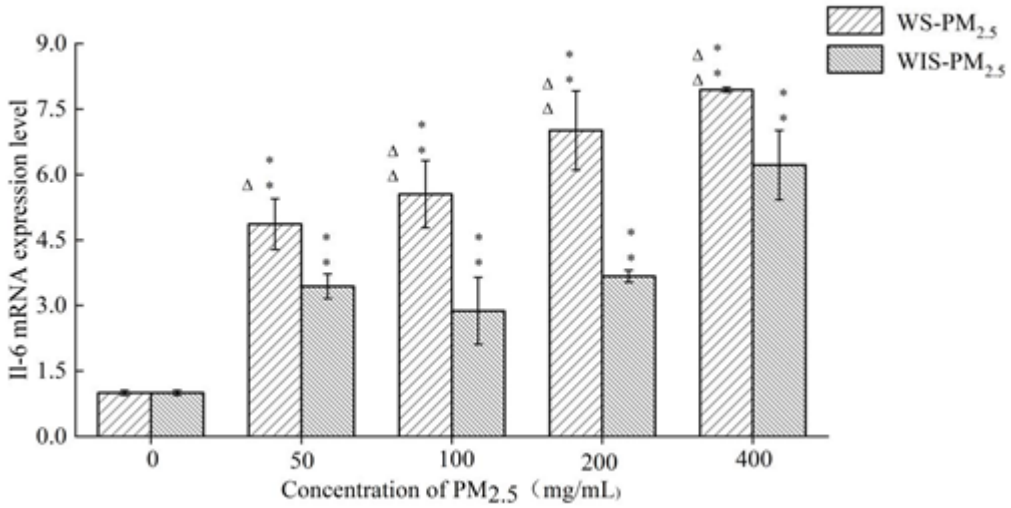


Figure 7

Fig.3-7 The relative expression of intracellular IL-6 m RNA after A549 cells exposed to different $PM_{2.5}$ components.

Compared with the solvent control group：*：$P<0.05$；**：$P<0.01$.

Comparison of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$：△：$P<0.05$；△△：$P<0.01$

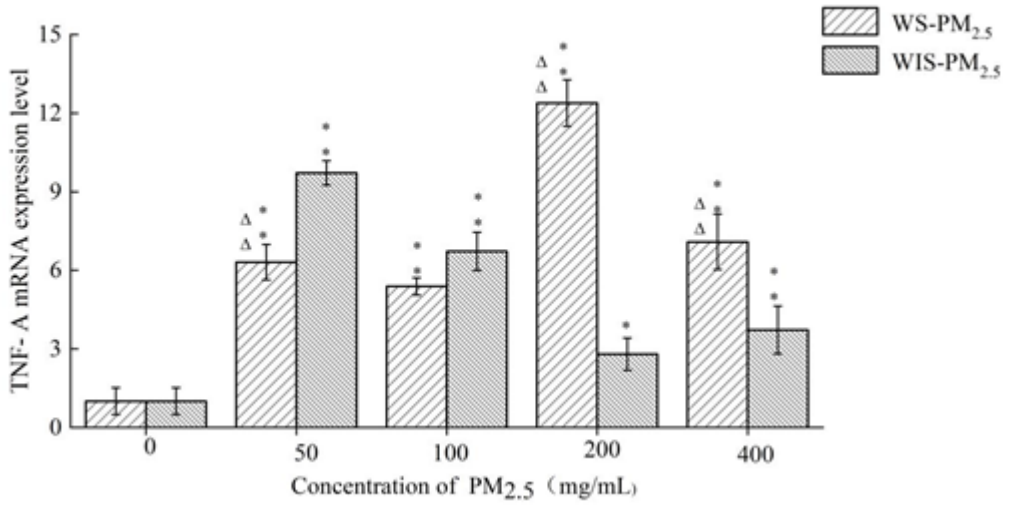


Figure 8

Fig.3-8 The relative expression of intracellular TNF-α mRNA after A549 cells were exposed to different $PM_{2.5}$ components.

Compared with the solvent control group，*，P＜0.05；**，P＜0.01．

Comparison of WS-$PM_{2.5}$ and WIS-$PM_{2.5}$，△，P＜0.05；△△，P＜0.01．