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# The Toxicity of Human Lung Epithelial Cells Exposure to $PM_{2.5}$ and Glucose Before or After Intervention of Guilu Erxian Jiao

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## ABSTRACT

$PM_{2.5}$ is known to be a potential risk factor for the progression of diabetes, particularly type 2 diabetes (T2D). Guilu Erxian Jiao (GEJ), a traditional Chinese medicine containing deer antlers and turtle shells, has been shown to have multiple health benefits. Given the synergistic association between $PM_{2.5}$ levels and T2D prevalence, as well as the therapeutic properties of GEJ, this study used treatment of $PM_{2.5}$ and glucose to assess the mitigating effects of GEJ intervention in A549 cells. This study aimed to mimic the effects of a GEJ intervention on cell growth, cell death, wound healing, and oxidative stress after T2D patients' exposure to $PM_{2.5}$. Our findings showed that A549 cells exposure to $PM_{2.5}$ or glucose led to a significant decrease in cell growth, an increase in cell death, and impaired wound healing, even at low levels of $PM_{2.5}$ (10 µg $mL^{-1}$) and glucose (20 mM). Cotreatment with $PM_{2.5}$ and glucose at 50 µg $mL^{-1}$ and 120 mM, respectively, exacerbated these effects. The administration of 200 µg $mL^{-1}$ GEJ resulted in the most significant improvement, regardless of the presence of $PM_{2.5}$ or glucose treatment. GEJ was revealed to upregulate antioxidant genes in A549 cells, such as MnSOD and CAT, indicating its potential radical-scavenging effects in cells treated with $PM_{2.5}$ and glucose. The findings also revealed that cotreatment with high levels of $PM_{2.5}$ and glucose in A549 cells leads to more severe health consequences, including reduced cell growth (decreased by 1.65–2.32%), increased cell death (increased by 4.8%–7.2%), impaired wound healing (reduced by –14–5.0%), and upregulation of reactive oxygen species. In contrast, GEJ intervention helped repair cellular damage (repaired by 3.2–7.9%), improving the wound healing rate from 51% to 63%. GEJ might have the potential to modulate oxidative stress and ameliorate the effects of high $PM_{2.5}$ and glucose.

**Keywords:** $PM_{2.5}$, A549 cells, Guilu Erxian Jiao or tortoiseshell and deer antler gelatin, High glucose, Human health

## 1 INTRODUCTION

With the increases in industrialization, urbanization, human activities, and climate change, air pollution has both directly and indirectly impacted the daily lives of people and animals. Notably, $PM_{2.5}$ is known to have a significant impact on human health and to be more detrimental than $PM_{10}$ (Zanobetti and Schwartz, 2009; Villar-Vidal *et al.*, 2014; Chao *et al.*, 2018; Tsai *et al.*, 2019; Thangavel *et al.*, 2022). The small particle size of $PM_{2.5}$ means it has a large relative surface area and strong ability to adsorb hazardous substances, such as polycyclic aromatic hydrocarbons (Xin *et al.*, 2021), halogenated persistent organic pollutants (Chao *et al.*, 2016; Su *et al.*, 2022; Amani Room *et al.*, 2024), and heavy metals (Potera, 2014), thus further contributing to its ability to increase health risks (Chao *et al.*, 2018; Thangavel *et al.*, 2022). Several studies have revealed that, upon deposition in human lungs, $PM_{2.5}$ triggers a cascade of biochemical events that lead to oxidative stress and inflammatory reactions (Lai *et al.*, 2017; Chao *et al.*, 2018; Tseng *et al.*, 2022), leading to damage to the liver (Oberdörster *et al.*, 2002) and the cardiovascular (Dabass *et al.*, 2018), central nervous (Kim *et al.*, 2020), and respiratory systems (Xing *et al.*, 2016). Moreover, outdoor workers in China, who are continuously exposed to high levels of $PM_{2.5}$, appear to have an increased risk of mortality due to cardiovascular and respiratory diseases (Wang *et al.*, 2017). Similarly, prolonged exposure to $PM_{2.5}$ exacerbates the mortality risk among the elderly, wherein there is a 7% increase for every 10 $\mu g\ m^{-3}$ rise in $PM_{2.5}$ concentration (Di *et al.*, 2017).

Various studies have explored toxicity of $PM_{2.5}$ particles via *in vitro* analysis. A neurological study revealed that the viability of olfactory ensheathing cells and SH-SY5Y cells is reduced when they are exposed to $PM_{2.5}$ due to decreased mitochondrial membrane potential, increased cytotoxicity, and cellular integrity impairment and subsequent apoptosis (Cristaldi *et al.*, 2024). In another study, lung epithelial cells (BEAS-2B) were utilized and exposed to $PM_{2.5}$, triggering mitochondrial damage and inhibiting cell mitophagy; this study further delineated the progression of pulmonary fibrosis (Liu *et al.*, 2024). In hepatoma cells, such as the HUH-7 and Hep3B cell lines, $PM_{2.5}$ exposure induces cellular reactive oxygen species (ROS) and is associated with poorer prognosis for the cellular phenotype of hepatocellular carcinoma (Li *et al.*, 2024). Several studies have conducted toxicity analysis on the effects of $PM_{2.5}$ on adenocarcinoma human alveolar basal epithelial cells, also known as A549 cells, and revealed that exposure induces cytotoxicity, DNA damage, endoplasmic reticulum and oxidative stress, pyroptosis, and apoptosis (Goudarzi *et al.*, 2019; Laiman *et al.*, 2022; Barzgar *et al.*, 2023; Wei *et al.*, 2023; Chen *et al.*, 2024). However, there is limited research on the combined effects of high $PM_{2.5}$ exposure and high glucose concentrations in the cellular models.

High levels of glucose in the bloodstream can be associated with various diseases including hyperglycemia and diabetes mellitus (DM). At high concentrations in the blood, glucose triggers biochemical reactions that lead to the formation of advanced glycation end-products and consequently cause both cell and organ damage. DM, especially type 2 DM (T2D) or non-insulin-dependent diabetes, is a chronic disease whereby glucose is elevated due to the failure of insulin to signal cellular uptake. As a result, plasma glucose concentrations are elevated in DM patients. Globally, T2D patients represent most diabetes cases. In 2017, an estimate revealed that around 425 million people were affected by this disease, with half of these cases being undiagnosed (Bai *et al.*, 2021). With 80% of the global diabetic population coming from low- and middle-income countries, Asian countries have experienced a rapid increase in the prevalence of DM (Beran and Higuchi, 2013; Ghisi *et al.*, 2022). In 2021, it was estimated that the disease affects 140 million people in China, which accounts for approximately a quarter of the total diabetic patients across the globe (Wang *et al.*, 2023). In Taiwan, it was estimated that DM affected 9.8% of the national population in 2013; this number is projected to increase to 13.1% by 2035 (Guariguata *et al.*, 2014).

Aside from in China and Taiwan, in 2021, approximately 4.3 million Filipinos were diagnosed with diabetes and an estimated 2.8 million were undiagnosed (Cando *et al.*, 2024). Indonesia is expected to be the only Southeast Asian country included in the top 10 countries with the highest prevalence of DM by 2035, with approximately 14.1 million cases (Guariguata *et al.*, 2014). Recent studies have indicated a certain correlation between $PM_{2.5}$ and DM, wherein the incidence of DM increases significantly among people who reside in areas with high $PM_{2.5}$ exposure (Valdez *et al.*, 2022; Zhou *et al.*, 2022; Liu *et al.*, 2023). $PM_{2.5}$ has also been linked to alterations in insulin secretion, affecting pancreatic cells' capacity to regulate blood sugar levels (Chen *et al.*, 2016). However, the biological mechanisms of the relationship between $PM_{2.5}$ and DM remain unclear, and only a few studies have shown that $PM_{2.5}$ can significantly exacerbate or increase the incidence of DM (Chung and Lin, 2024; Pan *et al.*, 2023; Potera, 2014; Zanobetti *et al.*, 2014; Hernandez *et al.*, 2018).

Herbal medicine, such as turmeric, constitutes the largest and most commonly used form of Traditional Chinese medicine (TCM). TCM has a history of approximately 3000 years in China, dating back to the early Zhou dynasty or even earlier. TCM focuses on achieving a balance of yin and yang to maintain health and prevent diseases. The sources of Chinese herbs include plants, animals, and minerals, which can be combined in various formulations to treat a wide range of illnesses. Herbal medicine, such as turmeric, constitutes the largest proportion of TCM and is the most commonly use (Jurenka, 2009; Chuang *et al.*, 2014; Liu *et al.*, 2022). Guilu Erxian Jiao (GEJ), or tortoiseshell and deer antler gelatin, is a TCM product primarily composed of semifluid extract derived from turtle shells, unossified deer antlers, lycii fructus, and ginseng. In China, GEJ has been widely used for thousands of years to treat orthopedic diseases including osteoporosis, degenerative joint disease, osteoarthritis, and joint pain. Plastrons, referring to the exoskeleton of the ventral side of the reptile, have been found to contain significant trace elements (e.g., Si, Ca, and Mg), as well as 16 amino acids, including high concentrations of glycine and glutamic acid, followed by proline, asparagine, and arginine (Li and Cheung, 2012). Deer antlers, specifically in an unossified state, are commonly used in TCM due to their substantial amounts of amino acids (e.g., glutamic acid, histidine, and glycine), mineral elements (e.g., Ca, P, Fe, and K), proteins, and lipids (Wu *et al.*, 2013). Previous studies revealed its immunomodulatory (Lei *et al.*, 2009), anti-inflammatory (Cheng *et al.*, 2022), and antioxidant (Zhou and Li, 2009) effects and ability to promote glucose homeostasis (Fang *et al.*, 2023).

Given that the components of GEJ have the potential to alleviate oxidative stress and inflammatory responses, and considering the association between $PM_{2.5}$ exposure and diabetes, this study aimed to investigate the toxicity of both high $PM_{2.5}$ and high glucose concentrations through *in vitro* testing and examine the therapeutic potential of GEJ. Given the lungs' susceptibility to $PM_{2.5}$ exposure and the potential aggravation of oxidative stress and inflammatory responses by hyperglycemia, leading to compromised cellular functions and impaired wound healing process. Therefore, we chose the A549 cell line as our cell model in this study due to its stable representation of human alveolar type II pulmonary cells and responsiveness to various stimuli. An A549 cell is widely used as a model for alveolar type II pulmonary epithelium, which plays a key role in a variety of lung diseases. We hypothesize that $PM_{2.5}$ has adverse effects on A549 cells. High glucose levels exacerbate the detrimental effects of $PM_{2.5}$ on A549 cells, while GEJ mitigates the health impacts of both high $PM_{2.5}$ and glucose (Fig. S1).

## 2 MATERIALS AND METHOD

### 2.1 $PM_{2.5}$ Sampling

This study conducted air sampling and sample preparation following the methods described in our previous studies (Chung *et al.*, 2020; Lu *et al.*, 2023). Using quartz fiber filters, the $PM_{2.5}$ filters were preheated at 600°C for at least 2 hours prior to entering the electronic desiccator for 24 hours, both before and after gathering $PM_{2.5}$. The fiber filters were weighted using a six-digit balance with an accuracy of 0.1 µg, wherein the weight difference in the used and unused filters signifies the magnitude of $PM_{2.5}$. Two high-volume air samplers of SIBATA HV-1000R (Sibata Scientific Technology Ltd., Japan) were used to collect ambient $PM_{2.5}$ samples in the vicinity of the Taichung Thermal Power Plant from September 4 to September 6, 2022, following the U.S. EPA

Reference Method TO9A or Taiwanese EPA NIEA A205.11C. The flowrate was set at 800 L $min^{-1}$ for a total 36-hour sampling time. The samples were then transported to be stored in a refrigerator in our laboratory, the laboratory of National Pingtung University of Science and Technology, at –20°C prior to extraction to prevent volatile portion losses from evaporation.

## 2.2 Extraction of the $PM_{2.5}$ Samples

The procedures for extracting, cleaning up, and concentrating the $PM_{2.5}$ samples followed the methods outlined in our previous reports (Chung *et al.*, 2020; Lu *et al.*, 2023). The pooled $PM_{2.5}$ filters underwent sonicated extraction with dichloromethane (DCM) for 15 minutes. For purification, 15 mL of DCM was added to the extract and the mixture was passed through an acid-silica column for clean-up. The elute was concentrated to 1 mL and then further concentrated to near dryness via a gentle nitrogen gas stream. Subsequently, it was redissolved in 100 µL of dimethyl sulfoxide (DMSO) and stored in a –20°C freezer.

## 2.3 GEJ Extract Preparation

The GEJ was purchased from the Chuang Song Zong Pharmaceutical Co. Ltd., which is a GMP pharmaceutical factory in Pingtung, Taiwan. The GEJ samples were pre-frozen at –80°C for 15 minutes before being freeze-dried for 72 hours (Freeze Dryer, LABCONCO, Kansas City, MO, USA). The sample was then dissolved in DMSO and diluted with DMEM to achieve concentrations of 50, 100, 200, and 300 µg $mL^{-1}$, which were then stored in a 4°C refrigerator.

## 2.4 Cell Culture and Passage

Human A549 alveolar epithelial cells were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were grown in a monolayer culture on Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S). The cells were then incubated in a 5% $CO_2$ environment at 37°C for 48 hours. For cell passaging, Dulbecco's phosphate-buffered saline (DPBS) and accutase were used for cell washing and detachment, respectively.

## 2.5 Cell Growth and Death

The A549 cells were seeded in a 6-well transparent plate and exposed to DMEM culture medium for 5 days; the medium contained varying concentrations of $PM_{2.5}$ (10, 20, 50, and 100 µg $mL^{-1}$), glucose (20, 40, 80, and 120 mM), and GEJ (50, 100, 200, and 300 µg $mL^{-1}$). On day 3, 0.5 mL of DMEM was added. After the exposure periods, cell numbers were calculated, and cell death (%) is expressed as the ratio of dead cells to the total number of cells. This experimental procedure was conducted for all co- and single-exposure conditions.

## 2.6 Wound Healing Assay

A549 cells were seeded in a 3 cm culture dish with 3 replicates for each concentration of $PM_{2.5}$, glucose, and GEJ and for both single- and co-exposure conditions. After 24 hours, a vertical line was drawn in the center of the dish using a 1 mL sterile micropipette tip to mimic wound damage. The cells were then exposed for a total of 72 h, in DMEM mixed with the exposure agents, which was changed every 24 h. The wound repair status was monitored and documented every 24 h until the end of the exposure period.

## 2.7 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Assay

This study conducted a gene expression experiment to analyze the regulation of antioxidant-related genes in A549 cells under different exposure agents. The A549 cells were dispensed in 3 cm dishes and exposed to different concentrations of agents for 24 h. The cells were collected and resuspended in 500 µL of TRIzol (Gibco, Life Technologies, Carlsbad, CA, USA) and vortexed to ensure cell lysis. Subsequently, 1 mL of chloroform was added to the mixture, which was then incubated at room temperature for 10 mins and centrifuged at 13000 rpm at 4°C for 12 mins. The supernatant was removed, and 500 µL of isopropanol was added. This was then stored in a –20°C freezer for 45 mins prior to being centrifuged. The RNA pellet was then washed with 750 µL

**Table 1.** Primer Sequences.

| Gene Code | Forward Primer (5' to 3') | Reverse Primer (3' to 5') |
|---|---|---|
| CAT | CTGGGACTTCTGGAGCCTAC | CAACTGGGATGAGAGGGTAG |
| MnSOD | AGAAGTACCAGGAGGVGTTG | AGTGTCCCCGTTCCTTATTG |
| CuZnSOD | AGGGCATCATCAATTTCGAG | CCATCTTTGTCAGCAGTCAC |
| Gpx-1 | GAAGTGCGAGGTGAACGGTG | GGGATCAACAGGACCAGCAC |
| Gpx-2 | AGATGTGGCCTGGAACTTTG | CATTCTGTGAAGGCCCAGAG |
| GAPDH | TGGACCTGACCTGCCGTCTA | CCCTGTTGCTGTAGCCAAATTC |

of 75% ethanol and centrifuged under the same conditions. Samples were air-dried for 10–15 mins until the remaining ethanol evaporated and were then resuspended in 50 µL of DEPC water. The RNA concentrations were determined using a spectrophotometer, and the final concentration was adjusted to 1 µg $\mu L^{-1}$. RT Master Mix was utilized to reverse transcribe the RNA samples, and the cDNAs were diluted threefold. qRT-PCR was carried out using SYBR Advantage qPCR premix and a 7500 real-time PCR system for analysis. The sequences of the primers used are shown in Table 1.

### 2.8 Statistical Analysis

In this study, the data obtained were organized and analyzed using Microsoft Excel. Graphical representations (e.g., growth curves and death proportion histograms) were created using Prism-GraphPad7 (San Diego, CA, USA). Statistical analysis was conducted using Statistical Product and Service Solutions, version 12.0, employing Mann-Whitney *U* tests as the statistical approach. These analyses were used to explore the potential interactions between $PM_{2.5}$, high glucose, and GEJ in A549 cells. The statistical values are expressed as Mean ± standard deviation (SD), and ImageJ was used to analyze the wound area repair percentage.

## 3 RESULTS AND DISCUSSION

### 3.1 Induced Toxicity of $PM_{2.5}$ and Glucose

During the 5-day exposure period, the growth rates and cell death were simultaneously assayed at different concentrations in order to assess the influence of $PM_{2.5}$ and glucose concentrations on A549 cells (Fig. 1). As shown in Fig. 1(A), on Day 1, none of the $PM_{2.5}$ concentrations (10, 20, 50, and 100 µg $mL^{-1}$) resulted in significant differences compared to the control. However, all the concentrations of $PM_{2.5}$ resulted in differences during the last 2 days of exposure. For example, 50 µg $mL^{-1}$ resulted in the most significant difference compared to the other $PM_{2.5}$ levels (growth rate (GR): 14.2 ± 1.44%), with a decline of 22.9% in the growth rate compared with the control. The trend of an insignificant difference on Day 1 and notable difference as the exposure period progressed remained consistent in the glucose exposure experiment (20, 40, 80, and 120 mM) and under co-treatment exposure (50 µg $mL^{-1}$ $PM_{2.5}$ and 120 mM glucose) (Figs. 1(C) and 1(E)). When the cells were exposed to 120 mM of glucose (Fig. 1(C)), the growth rate of the cells exhibited the earliest and most consistent decline, ending on a 35.5% difference on Day 5. Similarly, under co-treatment with 50 µg $mL^{-1}$ $PM_{2.5}$ and 120 mM glucose, and compared to the control (Fig. 1(E)), we observed a continuous significant decline in growth rate at Day 2, ending with a relative difference of 25.7% at Day 5 (GR: 12.6 ± 0.63% in co-treatment). In addition, co-treatment resulted in a significant difference compared to single treatments with $PM_{2.5}$ (GR difference of 15.6%) and glucose (GR difference of 11.7%), relative to the growth rate at the end of the exposure period.

The cell death ratio was also assessed in order to determine the cytotoxicity of the exposure agent. Over 5 days, A549 cells were also exposed under the same conditions and with the same varying concentrations. As shown in Fig. 1(B), all the $PM_{2.5}$ concentrations appeared to significantly increase cell death, starting from Day 3 and continuing through to Day 5. The cell death ratios (DRs) on Day 5 under $PM_{2.5}$ concentrations of 10, 20, 50, and 100 µg $mL^{-1}$ were 16.1 ± 1.77%, 16.9 ± 1.15%, 14.17 ± 1.44%, and 15.1 ± 1.01%, respectively. From Day 1, low and high glucose

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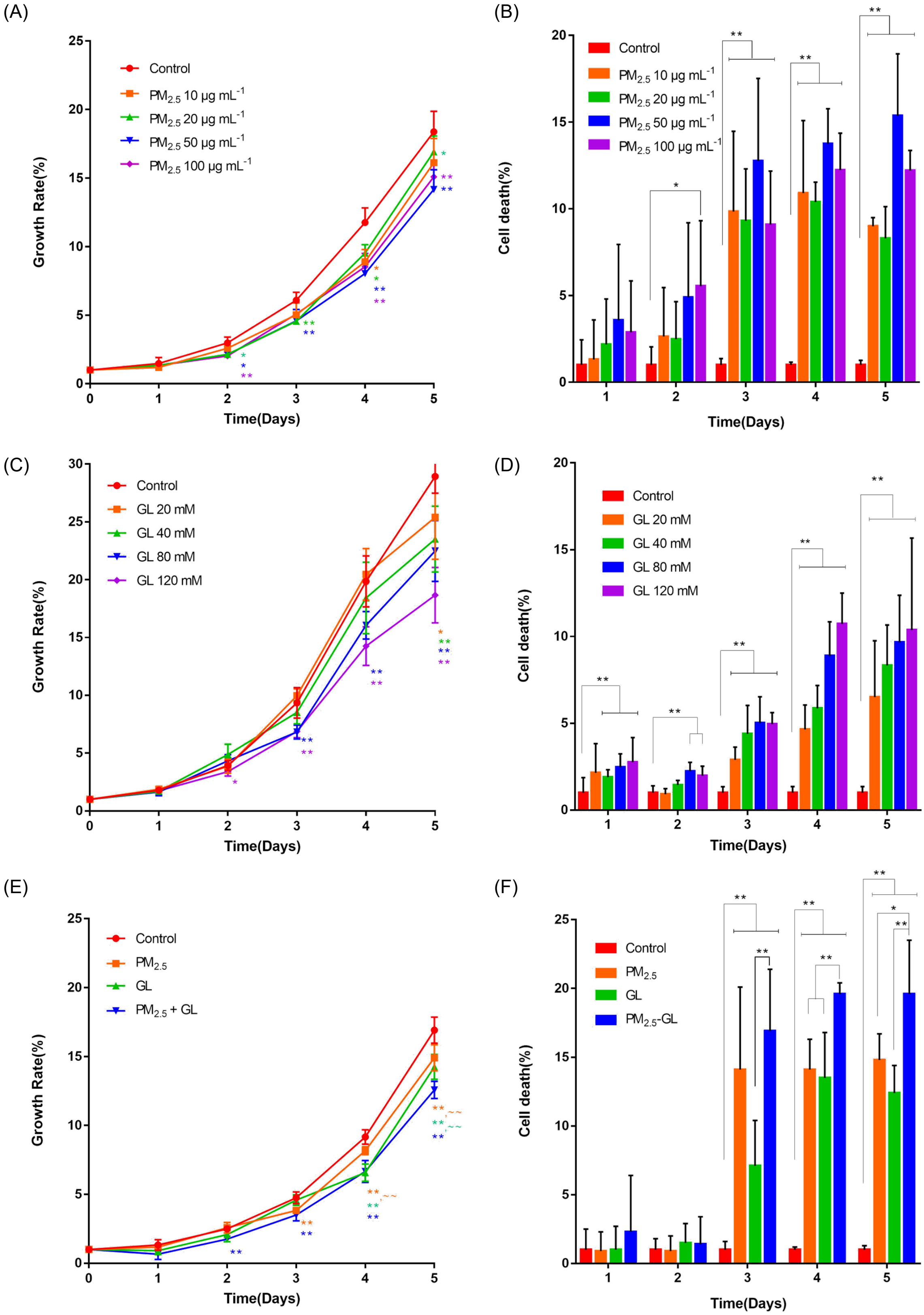



**Fig. 1.** Proliferation and death responses of A549 cells at different levels of $PM_{2.5}$ and glucose exposure; (A) cell growth under $PM_{2.5}$; (B) cell death ratio under $PM_{2.5}$; (C) cell growth under glucose; (D) cell death ratio under glucose; (E) cell growth under co-treatment with high $PM_{2.5}$ and glucose concentrations; (F) cell death ratio under co-treatment with high $PM_{2.5}$ and glucose concentrations. ($^{*}p < 0.05$, $^{**}p < 0.01$ compared to control; $^{\sim}p < 0.05$, $^{\sim\sim}p < 0.01$ compared to $PM_{2.5}$ + GL).

concentrations resulted in consistent significant differences against the control, ending with increased ratios of 14.4% and 11.2%, respectively, on Day 5 (Fig. 1(D)). Lastly, co-treatment with 50 µg $mL^{-1}$ $PM_{2.5}$ and 120 mM glucose notably increased cell death when compared to the control and single treatment with $PM_{2.5}$ or glucose. The highest increases in ratio were seen on Day 4 at 18.6%, 4.8%, and 7.2% against the control, $PM_{2.5}$, and glucose, respectively (Fig. 1(F)).

The present study showed that, after A549 cells were exposed to high levels of $PM_{2.5}$ or/and glucose, cell growth was reduced, cell death was enhanced, and wound healing was impaired. In a previous study involving a 72 h $PM_{2.5}$ exposure experiment, A549 cells appeared to have a lower percentage of cell viability when treated with 10, 50, and 200 µg $mL^{-1}$ $PM_{2.5}$ concentrations, and prolonged exposure further exacerbated this effect (Moonwiriyakit *et al.*, 2024). Additionally, in A549 cells, $PM_{2.5}$ concentrations (25, 50, 100, and 200 µg $mL^{-1}$) decreased cell viability; we identified this as being caused by DNA damage, oxidative stress, and apoptosis (Barzgar *et al.*, 2023). Moreover, another study observed a significant decrease in cell viability and a significant increase in the apoptosis rate in A549 cells exposed to $PM_{2.5}$ concentrations of 22.3, 44.7, and 89.3 µg $cm^2$ (Chen *et al.*, 2024). Only a few studies have investigated the toxic effects of a high-glucose diet on A549 cells. One such study employed different glucose concentrations and observed that 20 and 50 mM resulted in significant decreases in the cell viability of A549 cells after a 24 h exposure period (Ning *et al.*, 2022).

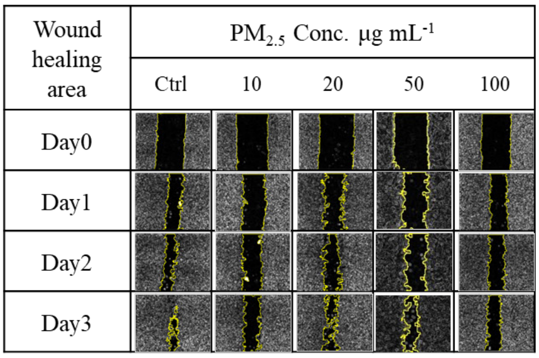
Exposure to high levels of $PM_{2.5}$ and glucose might alter the normal healing process of A549 cells. Comparing different concentrations of $PM_{2.5}$ and glucose, this study was able to analyze the recovery rate of wound healing impairment using ImageJ (Fig. 2). As shown in Figs. 2(A) and 2(B), on Day 3, the control had the highest cumulative wound gap closure (wound healing rate (WHR): 82 ± 3%), while 100 µg $mL^{-1}$ of $PM_{2.5}$ (WHR: 52 ± 5%) resulted in the lowest rate, with a difference of 57.7% in wound healing. In terms of glucose concentrations (Figs. 2(C) and 2(D)), the last day (Day 3) of exposure revealed that 80 mM (WHR: 80%) resulted in the highest wound closure rate, with a relative difference of 79.2% compared to the lowest, 120 mM (WHR: 63%). From Figs. 2(E) and 2(F), the wound closure ranking from the slowest to fastest was as follows—$PM_{2.5}$, $PM_{2.5}$ and glucose co-treatment, glucose, and the control group—with rates of 50 ± 10, 64 ± 4, 69 ± 5, and 81 ± 7% WHR, respectively, and a relative difference of 80.4% between $PM_{2.5}$ and the control.

In A549 cells, cell growth and death constitute a valuable toxicological index for assessing the short- and long-term effects of high $PM_{2.5}$ and glucose concentrations. Moreover, the wound healing rate is another significant indicator to consider and assess, given its relevance to DM patients, as well as the correlation between $PM_{2.5}$ exposure and the incidence and exacerbation of DM (Chen *et al.*, 2016; Valdez *et al.*, 2022; Zhou *et al.*, 2022; Liu *et al.*, 2023). The findings of the present study showed that exposure to a single treatment with high $PM_{2.5}$ or glucose resulted in a significant decline in A549 cell growth, an increase in cell death, and impaired wound healing. In Taiwan, several epidemiological studies have proven that long-term exposure to ambient $PM_{2.5}$ is positively associated with T2D prevalence (Lao *et al.*, 2019; Li *et al.*, 2019; Chung and Lin, 2024) and causes death (Lin *et al.*, 2022) in the general population. In male strepotozotocin-induced diabetic Sprague Dawley (SD) rats, exposure to high traffic-related air pollutant (TRAP) $PM_{2.5}$ (Lei *et al.*, 2005) caused endothelial dysfunction, induced oxidative stress, and generated ROS. In our previous study using an in vivo model of *Caenorhabditis elegans* (*C. elegans*), we found that the short-term co-exposure of nematodes to TRAP $PM_{2.5}$ and high levels of glucose caused reduced reproduction, disrupted locomotion, shortened longevity, and ROS species induction (Lu *et al.*, 2023). The present study provided further evidence, showing enhanced cell death, reduced cell growth, prolonged impaired wound healing, and increased oxidative stress in A549 cells co-treated with high $PM_{2.5}$ and glucose compared the control and single $PM_{2.5}$ or glucose treatments. These results might be reflected in T2D patients exposed to high $PM_{2.5}$, with adverse effects related to shortened longevity, impaired would healing, and the generation of ROS.

### 3.2 GEJ Intervention and Induced Toxicity

We pre-treated A549 cells with GEJ to investigate the therapeutic mitigation potential in terms of the toxicity induced by $PM_{2.5}$ and glucose (Fig. 3). Fig. 3(A) illustrates how GEJ 300 µg $mL^{-1}$ resulted in a significant difference compared to the control group during the early exposure periods, specifically on Days 1, 2, and 3, with respective increases in cell growth of 11.6, 20.6, and 13.1%.

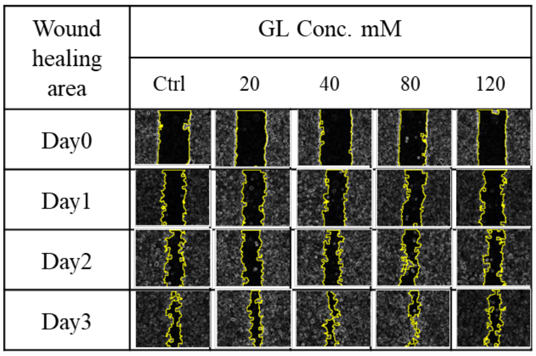
(A)




(B)

| Wound healing rate | PM$_{2.5}$ Conc. µg mL$^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Ctrl | 10 | 20 | 50 | 100 |
| Day0 | 0 % | 0 % | 0% | 0 % | 0 % |
| Day1 | 43 ± 7% | 31 ± 5% | 46 ± 5% | 28 ± 12% | 38 ± 8% |
| Day2 | 58 ± 8% | 43 ± 6% | 59 ± 4% | 44 ± 15% | 46 ± 7% |
| Day3 | 82 ± 3% | 55 ± 8%** | 74 ± 6% | 58 ± 17% | 52 ± 5%** |

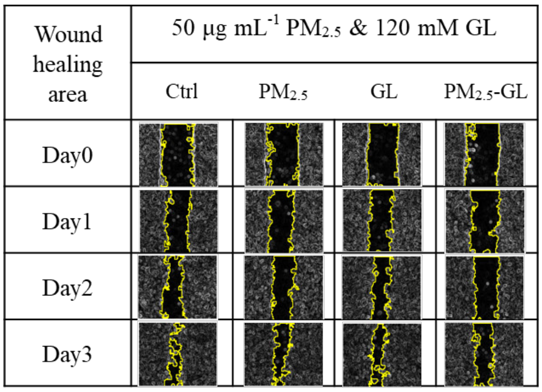
(C)




(D)

| Wound healing rate | GL Conc. mM | | | | |
|---|---|---|---|---|---|
| | Ctrl | 20 | 40 | 80 | 120 |
| Day0 | 0 % | 0 % | 0 % | 0 % | 0 % |
| Day1 | 43 ± 16% | 38 ± 4% | 27 ± 10% | 20 ± 6% | 28 ± 4% |
| Day2 | 64 ± 19% | 60 ± 13% | 62 ± 11% | 54 ± 20% | 53 ± 7% |
| Day3 | 70 % | 68 % | 78 % | 80 % | 63 % |

(E)




(F)

| Wound healing rate | 50 µg mL$^{-1}$ PM$_{2.5}$ & 120 mM GL | | | |
|---|---|---|---|---|
| | Ctrl | PM$_{2.5}$ | GL | PM-GL |
| Day0 | 0 % | 0 % | 0 % | 0 % |
| Day1 | 33 ± 1% | 21 ± 11% | 25 ± 4%* | 26 ± 5% |
| Day2 | 57 ± 3% | 36 ± 3%**~~ | 47 ± 3%* | 45 ± 0%** |
| Day3 | 81 ± 7% | 50 ± 10%* | 69 ± 5% | 64 ± 4%* |

**Fig. 2.** Wound repair of A549 cells at varying concentrations of $PM_{2.5}$ and glucose; (A) wound repair area map under $PM_{2.5}$; (B) wound repair rate under $PM_{2.5}$; (C) wound repair area map under glucose; (D) wound repair rate under glucose; (E) wound repair area map for co-treatment with high $PM_{2.5}$ and glucose concentrations; (F) wound repair area map for co-treatment with high $PM_{2.5}$ and glucose concentrations ($^{*}$ $p < 0.05$, $^{**}$ $p < 0.01$ compared to control; $^{\sim}p < 0.05$, $^{\sim\sim}$ $p < 0.01$ compared to $PM_{2.5}$ + GL).

A 200 µg mL$^{-1}$ GEJ concentration resulted in multiple highly significant differences compared to the control group on Days 2, 3, and 5, with increases in cell growth of 26.4, 31.3, and 25.0% (GR), respectively. However, no differences in growth rates were found between the cells exposed to 50 µg mL$^{-1}$ of GEJ and normal controls, while 100 µg mL$^{-1}$ of GEJ led to an increase in cell growth only at Day 4, with a difference of 19.3% compared to the control (GR: 18.2 ± 1.68%). When $PM_{2.5}$ (50 µg mL$^{-1}$) was used for co-treatment along with GEJ (200 µg mL$^{-1}$) (Fig. 3(C)), on Day 4, a notable difference in the cell growth rate of 22.4% was observed, which appeared to be a higher growth rate (GR: 10.4 ± 0.8%) compared to that observed with $PM_{2.5}$ (GR: 8.5 ± 1%) and the controls (GR: 12 ± 1%). Similarly, the trend for $PM_{2.5}$ and GEJ co-treatment on Day 4 persisted into Day 5, showing a notably higher growth rate compared to that observed with $PM_{2.5}$ alone (GR difference of 18.4%) and a lower rate compared to the control (difference of 17.1%). Fig. 3(E) reveals, across all the administered GEJ concentrations, significant differences in cell growth

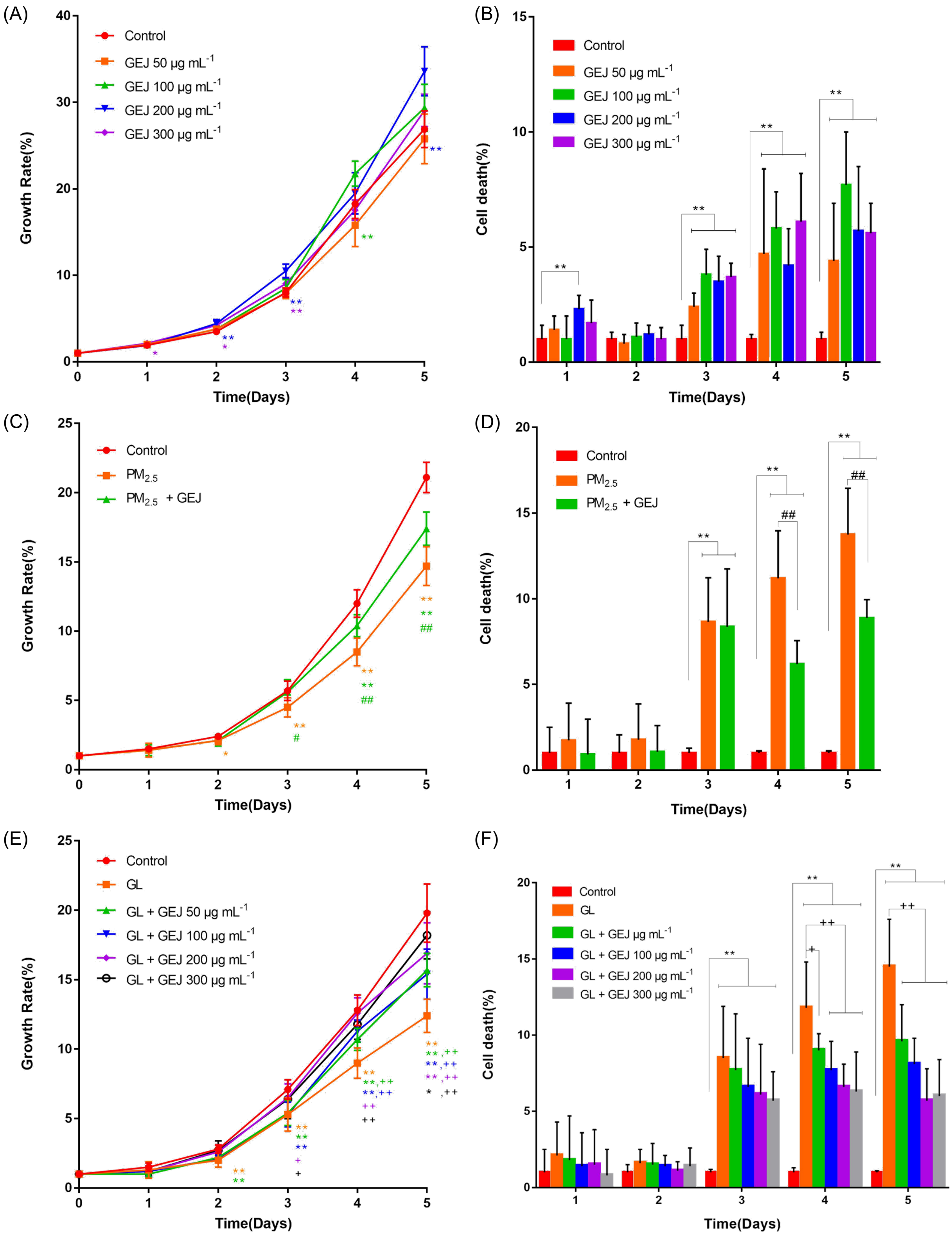


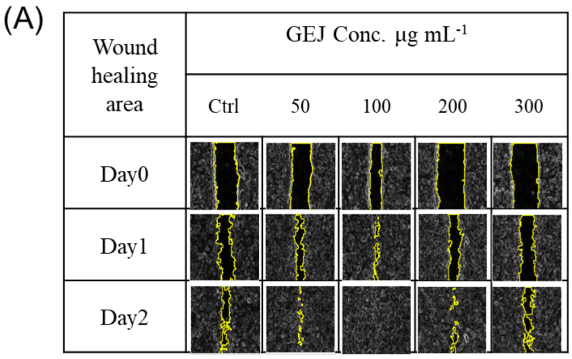

**Fig. 3.** Proliferation and death responses of A549 cells under GEJ intervention; (A) cell growth at varying GEJ concentrations; (B) cell death ratios at varying GEJ concentrations; (C) cell growth for co-treatment with high $PM_{2.5}$ and GEJ concentrations; (D) cell death ratios for co-treatment for high $PM_{2.5}$ and GEJ concentrations; (E) cell growth for co-treatment with high glucose and varying GEJ concentrations; (F) cell death ratios for co-treatment with high glucose and varying GEJ concentrations. ($^{*}p < 0.05$, $^{**}p < 0.01$ compared to control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to $PM_{2.5}$; $^{+}p < 0.05$, $^{++}p < 0.01$ compared to GL).

compared to a single exposure to 120 mM glucose, particularly on the last two days of exposure. On Day 4, compared to A549 cells exposed to glucose, those treated concurrently with GEJ concentrations of 50 (GR: 10.7 ± 0.8%), 100 (GR: 11.3 ± 0.8%), 200 (GR: 12.6 ± 1.1%), and 300 (GR: 11.8 ± 1.1%) µg $mL^{-1}$ had respective increases of 18.9, 25.6, 40, and 31.1% in cell growth. Similarly, there were also increases in the cell growth rate of 26.7 (GR: 15.7 ± 1.2% in 50 µg $mL^{-1}$), 24.2 (GR: 15.4 ± 1.8% in 100 µg $mL^{-1}$), 36.3 (GR: 16.9 ± 2.2% in 200 µg $mL^{-1}$), and 46.8% (GR: 18.2 ± 1.7% in 300 µg $mL^{-1}$), respectively, compared to the rate for glucose-exposed A549 cells.

Regarding cell death ratios, a significant difference can be observed between 200 µg $mL^{-1}$ of GEJ and the control on Day 1 in Fig. 3(B), while all the administered concentrations resulted in significant differences compared to the control from Day 3 to Day 5. Collectively, the cell death ratios ranged from 3.8% to 7.7% for all concentrations over the last 3 days of the exposure period. $PM_{2.5}$ and GEJ co-treatment resulted in no significant difference between Day 1 and Day 2 (Fig. 3(D)). In the last 2 days, this co-treatment led to a 5.19% (DR: 6.19 ± 1.37) decrease in cell growth on Day 4 and a 7% (DR: 8.88 ± 1.07) decrease on Day 5 when compared to $PM_{2.5}$ alone. Fig. 3(F) shows a similar trend to Fig. 3(D); various interventions of GEJ concentrations and a single treatment of glucose resulted in a significant decrease on the last 2 days of exposure. GEJ concentrations of 50, 100, 200, and 300 µg $mL^{-1}$ led to decreases of 23.7 (DR: 9 ± 1.1%), 35.8 (DR: 7.7 ± 1.9%), 44.1 (DR: 6.6 ± 1.5%), and 46.6% (DR: 6.3 ± 2.4%), respectively, relative to the death of A549 cells treated with glucose alone.

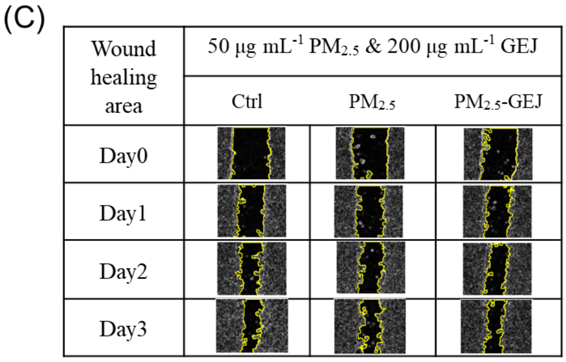
With GEJ treatment, the wound healing rate was increased (Figs. 4(A) and 4(B)); 100 µg $mL^{-1}$ of GEJ (WHR: 72 ± 16%) resulted in a relative difference of 28.6% compared to the control (WHR: 56 ± 20%) and a relative increase of 15.4% (WHR: 98 ± 2%) compared to the control (WHR: 85 ± 13%) on Day 2. As shown in Figs. 4(C) and 4(D), the co-treatment with 50 µg $mL^{-1}$ of $PM_{2.5}$ and 200 µg $mL^{-1}$ of GEJ induced a notable increase in wound healing progression on Days 1, 2, and 3 when compared to that of $PM_{2.5}$-exposed cells, with increases of 10.3% (WHR: 32 ± 4%), 18.6% (WHR: 51 ± 7%), and 7% (WHR: 61 ± 7%), respectively. As shown in Figs. 4(E) and 4(F), the wound repair rates of the control group and the glucose group with GEJ intervention were higher than those for A549 cells subjected a single 120 mM glucose treatment. On Day 3, the control group displayed the highest wound recovery rate (WHR: 77 ± 16%), followed by the groups administered 300 µg $mL^{-1}$ GEJ (WHR: 73 ± 15%), 200 µg $mL^{-1}$ GEJ (WHR: 62 ± 7%), and 50 µg $mL^{-1}$ GEJ (WHR: 61 ± 14%), with 100 µg $mL^{-1}$ GEJ resulting in the slowest rate (WHR: 58 ± 8%).

In the present study, co-treatment with $PM_{2.5}$, glucose, and GEJ significantly mitigated the deterioration of cell death and growth and wound healing compared to co-exposure to $PM_{2.5}$ and glucose. The TCM of GEJ is primarily composed of a semifluid extract from turtle shells and unossified deer antlers; it is known to contain high amounts of amino acids, trace elements, minerals, proteins, and lipids (Li and Cheung, 2012; Wu *et al.*, 2013). In our study, GEJ exposure alone led to a significant increase in cell growth and decrease in cell death in A549 cells (see Figs. 3(A) and 3(B)); it also notably increased wound healing performance, even at low concentrations (see Figs. 4(A) and 4(B)). Although, at present, there is no documented study indicating that GEJ affects A549 cells, few researchers have delved into the therapeutic effects of GEJ or explored the underlying mechanisms of different cellular and animal models. However, some previous reports in the scientific literature support our current findings (Fang *et al.*, 2023; Ding *et al.*, 2023; Yang *et al.*, 2023). In the mouse myoblast cell line, myogenic differentiation and growth are promoted upon exposure to 0.01 to 1 µg $mL^{-1}$ of GEJ for 24 h (Fang *et al.*, 2023). Similarly, GEJ was observed to induce chondrogenesis among mesenchymal stem cells, which delayed cell aging (Yang *et al.*, 2023). In a study that utilized mice spermatogonia, GEJ effectively ameliorated the oxidative damage caused by ROS induced by $H_2O_2$, leading to autophagy inhibition in stem cells (Ding *et al.*, 2023). As shown in the present study, GEJ might be able to accelerate wound healing after A549 cells have been damaged by high $PM_{2.5}$ and blood glucose levels. Our results also potentially imply that GEJ might elicit a cellular defense mechanism to alleviate such damage. This investigation was undertaken due to GEJ's reported antioxidant and anti-inflammatory effects and capacity to modulate glucose levels (Fang *et al.*, 2023; Zhou and Li, 2009; Cheng *et al.*, 2022). Presently, there are no published data exploring the potential interactions between GEJ, $PM_{2.5}$, and a high-glucose diet, specifically in cellular models. Referring to previous studies highlighting the therapeutic impacts of GEJ, our study's results might indicate GEJ's potential effectiveness in



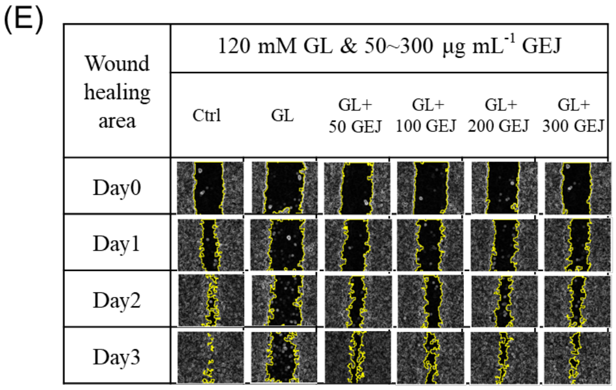

(B)

| Wound healing rate | GEJ Conc. $\mu g\ mL^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Ctrl | 50 | 100 | 200 | 300 |
| Day0 | 0% | 0% | 0% | 0% | 0% |
| Day1 | 56 ± 20% | 68 ± 6% | 72 ± 16% | 68 ± 7% | 54 ± 12% |
| Day2 | 85 ± 13% | 97 ± 3% | 98 ± 2% | 95 ± 2% | 81 ± 9% |




(D)

| Wound healing rate | $PM_{2.5}$ & GEJ $\mu g\ mL^{-1}$ | | |
|---|---|---|---|
| | Ctrl | $PM_{2.5}$ | $PM_{2.5}$-GEJ |
| Day0 | 0 % | 0 % | 0 % |
| Day1 | 34 ± 2% | 29 ± 4% | 32 ± 4% |
| Day2 | 52 ± 0% | 43 ± 5%* | 51 ± 7% |
| Day3 | 67 ± 6% | 57 ± 6% | 61 ± 7% |




(F)

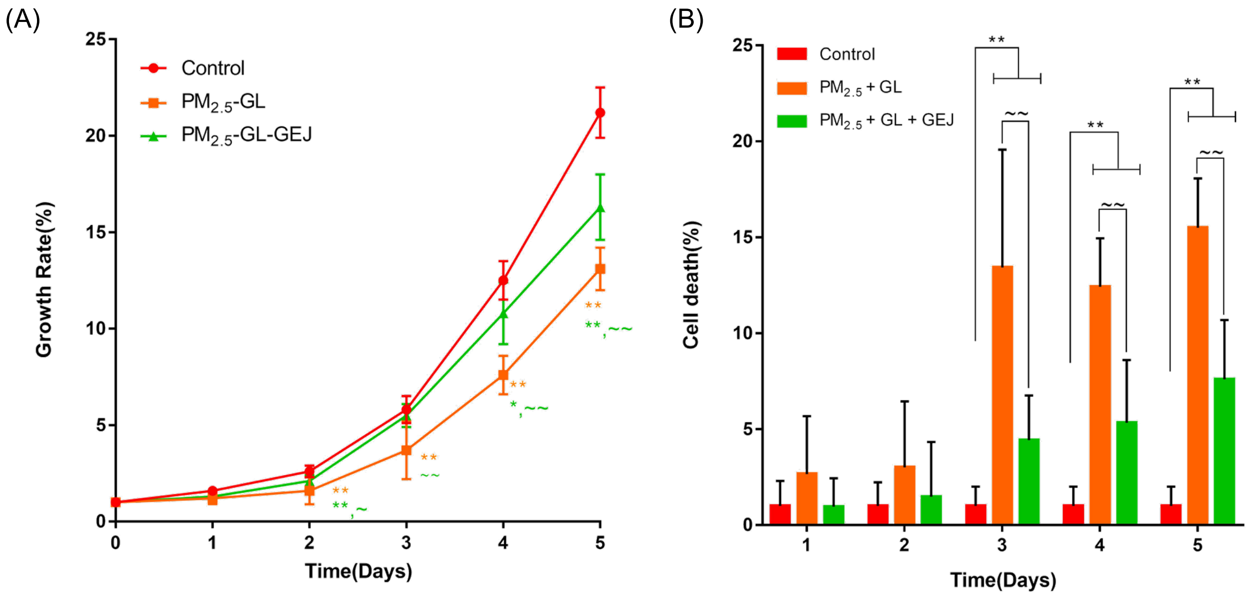
| Wound healing rate | GL mM & GEJ $\mu g\ mL^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Ctrl | GL | GL+ 50 GEJ | GL+ 100 GEJ | GL+ 200 GEJ | GL+ 300 GEJ |
| Day0 | 0 % | 0 % | 0 % | 0 % | 0 % | 0 % |
| Day1 | 37 ± 11% | 18 ± 7% | 21 ± 3% | 24 ± 5% | 23 ± 4% | 29 ± 6% |
| Day2 | 61 ± 5% | 35 ± 1%** | 45 ± 2%**++ | 43 ± 5%* | 46 ± 5%*+ | 32 ± 3%** |
| Day3 | 77 ± 16% | 48 ± 4%* | 61 ± 14% | 58 ± 8% | 62 ± 7%+ | 73 ± 15%+ |

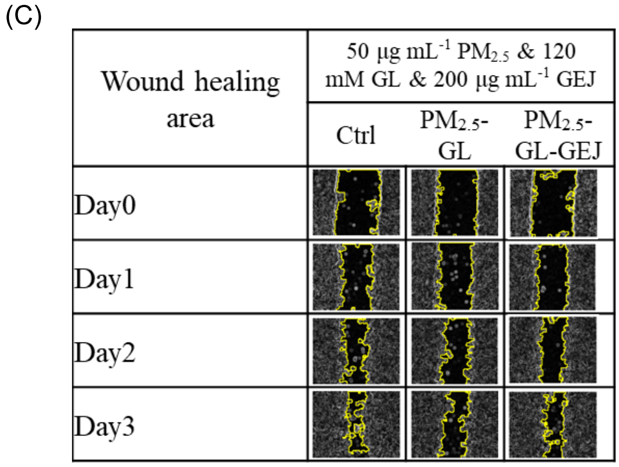
**Fig. 4.** Wound repair of A549 cells under GEJ intervention; (A) wound repair area map for varying GEJ concentrations; (B) wound repair rates at varying GEJ concentrations; (C) wound repair area map for co-treatment with high $PM_{2.5}$ and GEJ concentrations; (D) wound repair rate for co-treatment with high $PM_{2.5}$ and GEJ concentrations; (E) wound repair area map for co-treatment with high glucose and varying GEJ concentrations; (F) wound repair area map for co-treatment with high glucose and varying GEJ concentrations ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{+}p < 0.05$, $^{++}p < 0.01$ compared to GL).

ameliorating the symptoms of diseases brought about by $PM_{2.5}$ and blood glucose and minimizing the damage associated with T2D, such as impaired wound healing. From previous findings and our results, GEJ or its components and constituents have the potential to be used in developing novel treatments and as candidates for future clinical trials.

### 3.3 Efficacy of GEJ Pre-treatment Regarding Co-treatment with High $PM_{2.5}$ and Glucose Concentrations

As shown in Fig. 5(A), from Day 2, co-treatment with $PM_{2.5}$, glucose, and GEJ resulted in significant increases in growth compared to co-treatment with only $PM_{2.5}$ and glucose. On Days 2, 3, 4, and 5, the respective increases in the cell growth rate were 31.3 (GR: 2.1 ± 0.3%), 48.7 (GR: 5.5 ± 0.6%), 42.1 (GR: 10.6 ± 1.6%), and 24.4% (GR: 16.3 ± 1.7%). As shown in Fig. 5(B), co-exposure to $PM_{2.5}$, glucose, and GEJ resulted in a significant decrease in the cell death ratio compared to




(D)

| Wound healing rate | PM2.5 & GL & GEJ | | |
|---|---|---|---|
| | Ctrl | PM2.5-GL | PM2.5-GL-GEJ |
| Day0 | 0% | 0% | 0% |
| Day1 | 32 ± 3% | 25 ± 5% | 26 ± 4% |
| Day2 | 51 ± 1% | 41 ± 2%** | 45 ± 1%**~ |
| Day3 | 71 ± 5% | 51 ± 4%** | 63 ± 4%~ |

**Fig. 5.** Effects of GEJ intervention on co-treatment with high $PM_{2.5}$ and glucose concentrations; (A) cell growth; (B) cell death ratio; (C) wound repair area map; (D) wound repair rate ($^{*}p < 0.05$, $^{**}p < 0.01$ compared to control; $^{\sim}p < 0.05$, $^{\sim\sim}p < 0.01$ compared to $PM_{2.5}$ + GL).

co-treatment with $PM_{2.5}$ and glucose between Day 3 and Day 5. On Days 3, 4, and 5, relative differences were observed at 67.01 (DR: 1.48 ± 2.85%), 57 (DR: 5.34 ± 3.26%), and 50.97% (DR: 7.6 ± 3.09%), respectively, for co-treatment with $PM_{2.5}$ and glucose and the GEJ intervention. In terms of wound healing, Figs. 5(C) and 5(D) show the increases in wound healing for co-treatments with high $PM_{2.5}$ and glucose concentrations when GEJ was administered; on the final day of exposure, an increase of 23.5% (WHR: 63 ± 4%) was observed.

In this study, A549 cells pre-treated with GEJ were assessed to determine whether the latter alleviated the toxic effects of cell growth, cell death, and wound healing induced by high $PM_{2.5}$ and glucose. Though high $PM_{2.5}$ or/and high blood glucose levels could induce inflammation and oxidative stress, leading to cellular proptosis and apoptosis (Lei *et al.*, 2005; Schneider *et al.*, 2010; Nääv *et al.*, 2020; Lu *et al.*, 2023), GEJ was still able to mitigate these adverse effects (Fang *et al.*, 2023; Zhou and Li, 2009; Cheng *et al.*, 2022). These potential effects imply that GEJ may elicit cellular defense mechanisms to alleviate such damage. To further understand the mitigating impact of GEJ on $PM_{2.5}$ and hyperglycemia, especially for the DM patients, this study investigated the expression of antioxidant-related genes in A549 cells under exposure to $PM_{2.5}$, different glucose concentrations, and GEJ through a real-time PCR assay. While there are no existing studies examining the expression of these genes after GEJ administration, there are also few studies exploring various traditional medicines and foods in terms of promoting gene expression as a cellular defense mechanism after treatment with high $PM_{2.5}$ or glucose concentrations. TCMs, such as chicory (*Cichorium intybus*) (Pushparaj *et al.*, 2007), *Cornus officinalis* (Huang *et al.*, 2018), and Rhodolia (*Rhodiola rosea*) (Zheng *et al.*, 2019), have been reported to exhibit anti-diabetic properties. In our previous study, tempeh, a traditional Indonesian fermented soybean food, upregulated the antioxidant pathway (e.g., SKN-1/Nrf2) in nematodes (*C. elegans*) and demonstrated potential to protect against the damage caused by TRAP $PM_{2.5}$ and a high-glucose diet (Lu *et al.*, 2023).

### 3.4 Gene Expression Analysis

Fig. 6 displays the expression of antioxidant-related genes (CuSOD, MnSOD, CAT, GPX-1, and GPX-2) before and after treatment with high levels of $PM_{2.5}$ (50 µg $mL^{-1}$), glucose (120 nM), or GEJ (200 µg $mL^{-1}$). To investigate the influence of GEJ on the upregulated expression of antioxidant-related genes in A549 cells, a qRT-PCR assay was conducted wherein GAPDH was used as the reference gene. Among these antioxidant-related genes, only the MnSOD and CAT genes displayed significant differences when high $PM_{2.5}$ and glucose concentrations were administered with GEJ. MnSOD is a mitochondrial enzyme that scavenges superoxide and catalyzes the dismutation of two superoxide anions ($O_2^-$) into hydrogen peroxide ($H_2O_2$) and oxygen. MnSOD helps protect cells from oxidative damage caused by ROS. CAT is active in tissues or cells throughout the body, where it decomposes hydrogen peroxide into oxygen and water. MnSOD was upregulated by 77.8% (1.78 ± 0.17-fold), while no significant difference in CAT was observed when comparing the control groups with $PM_{2.5}$ and glucose co-treatment, as can be seen in Figs. 6(B) and 6(C). When GEJ was administered along with $PM_{2.5}$, MnSOD and CAT exhibited upregulations of 58.93 (1.78 ± 0.27-fold) and 368.7% (7.86 ± 1.29-fold), respectively, when compared to the levels observed with $PM_{2.5}$ exposure alone. Compared to glucose exposure, co-treatment with glucose and GEJ resulted in increases in MnSOD and CAT expression of 46.67 (1.54 ± 0.21-fold) and 366.75% (9.69 ± 3.35-fold), respectively. Similarly, when compared to that for co-treatment with $PM_{2.5}$ and glucose, significant increases in gene expression were also found for co-treatment with high $PM_{2.5}$, high glucose, and GEJ concentrations; these increases were 4.78% (1.7 ± 0.18-fold) in MnSOD and 312.23% (6.74 ± 0.18-fold) in CAT.

GEJ is composed of several TCM herbs, including turtle shells, unossified deer antlers, lycii fructus, and ginseng (Fig. S2). Ginseng is rich in ginsenosides, phenolic compounds, and flavonoids, which are known for their antioxidant properties (Yao *et al.*, 2019). Lycii fructus also contains important bioactive compounds such as carotenoids, flavonoids, and polysaccharides. In terms of antioxidant activity, flavonoid components are the most effective in scavenging DPPH· and ABTS+ free radicals, chelating metal ions, and exhibiting reduced power. Carotenoids and polysaccharides are particularly notable for their ability to scavenge hydroxyl radicals and superoxide anions, respectively (Wang *et al.*, 2010). Additionally, lycii fructus extracts have been shown to inhibit the free radical-induced DNA damage caused by peroxyl (ROO•) radicals (Skenderidis *et al.*, 2018). The upper sections of unossified deer antlers showed higher levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) compared to the middle and base sections (Cheng *et al.*, 2017). The main components of turtle shell are minerals and vitamin B3, and its free radical-scavenging ability is lower than that of muscle and liver (Islam *et al.*, 2021). However, incorporating it into the "turtle-deer combined gel" formula may enhance its synergistic effects. As the results of this study were shown, only the MnSOD and CAT genes were significantly upregulated in A549 cells pre-treated with GEJ after they were exposed to high $PM_{2.5}$ and/or glucose concentrations. Previous studies identified the potential mechanism of the two upregulated genes in expressing protein enzymes that catalyze the breakdown of ROS such as superoxides and hydrogen peroxide ($H_2O_2$) (Bonetta Valentino, 2022), while CAT converts hydrogen peroxide into water (Sultan *et al.*, 2023).

The traditional herbal product GEJ is focused on treating bone diseases, primarily due to the high calcium content in tortoiseshell and deer antler, which provides approximately 0.19% calcium. This abundant calcium is thought to supply essential calcium ions to patients (Ho *et al.*, 2023, 2024). Recent studies in Taiwan (Fang *et al.*, 2023; Ho *et al.*, 2023, 2024) suggest that GEJ can enhance the bioavailability of calcium, peptides, proteins, and bioactive compounds in both the human bone and skeletal muscle systems. Several studies have also shown a strong correlation

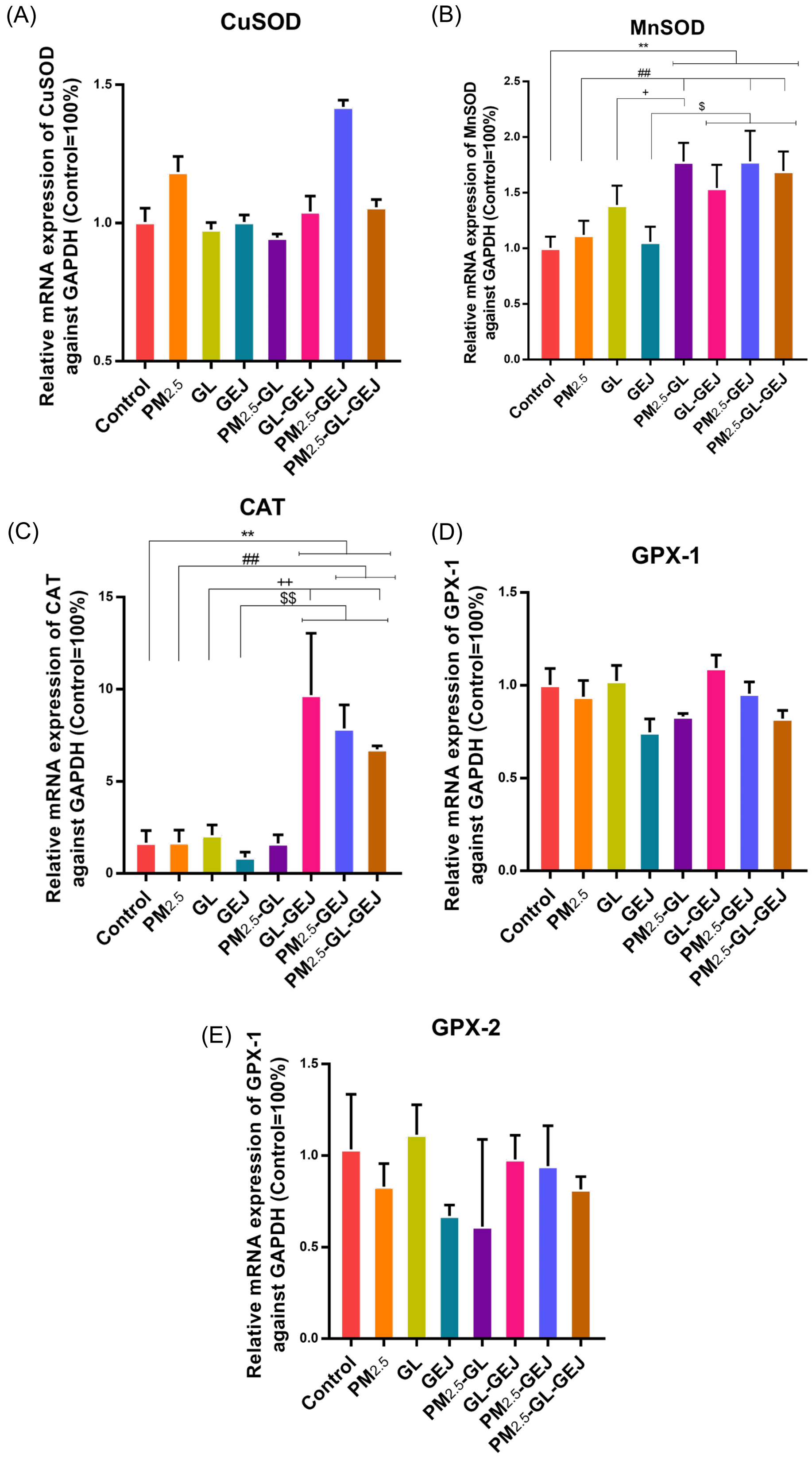



**Fig. 6.** GEJ modulation of A549 cells' antioxidant-related genes in the presence or absence of high $PM_{2.5}$ and glucose concentrations ($^{*}p < 0.05$, $^{**}p < 0.01$ compared to control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to $PM_{2.5}$; $^{+}p < 0.05$, $^{++}p < 0.01$ compared to GL; $^{\$}p < 0.05$; $^{\$\$}p < 0.01$ compared to GEJ).

between the development of T2D and exposure to high levels of $PM_{2.5}$ (Hernandez *et al.*, 2018; Valdez *et al.*, 2022; Zhou *et al.*, 2022; Liu *et al.*, 2023). In this study, it was hypothesized that A549 cells exposed to high levels of $PM_{2.5}$ and glucose would show positive responses to GEJ treatment, particularly in terms of cell growth, cell death, wound healing, and antioxidant effects (e.g., MnSOD and CAT). The therapeutic ability of GEJ is available to mitigate the A549 cellular damage induced by $PM_{2.5}$ and glucose; it could also be a potential candidate in developing treatments for respiratory diseases and metabolic disorders such as DM. Our findings indicated that GEJ might have therapeutic potential in accelerating wound healing, increasing cell growth, decreasing cell death, and enhancing antioxidant responses at the cellular level in T2D patients. Despite the valuable findings, the further studies shall be conducted to deepen the understanding towards the underlying molecular mechanism of cellular response, induced by high $PM_{2.5}$ and glucose concentrations, with or without administration of GEJ or other traditional Chinese medicine. However, *in vivo* studies are necessary to offer more relevant and comprehensive insights, enhancing the scientific depth and significance of our findings. To address this limitation, we plan to conduct subsequent animal studies to validate our *in vitro* results and further explore the potential therapeutic effects of GEJ. This approach will enable us to make a more robust and meaningful contribution to understanding impaired wound healing in lung epithelial cells due to high $PM_{2.5}$ and glucose concentrations.

This investigation was motivated by the need to address the increasing concerns over the detrimental health effects brought about by the continuous increases in air pollution and human metabolic disorders, such as DM. In terms of in vitro models, A549 cells represent human alveolar type II pulmonary cells, providing insights into the human lung cellular responses triggered by environmental stressors and aiding in investigating potential therapeutic strategies involving TCM like GEJ. With these findings, our study hypothesized the following: (1) $PM_{2.5}$ and hyperglycemia (high glucose) increase ROS, thus exacerbating impaired wound healing in DM patients; (2) GEJ possibly aids in reducing and modulating the highly reactive molecules generated and oxidative stress triggered by $PM_{2.5}$ and hyperglycemia; and (3) by upregulating antioxidant genes, GEJ potentially contributes to significantly reducing the adverse effects induced by $PM_{2.5}$ and hyperglycemia. Additionally, this study highlighted the importance of evaluating environmental stressors and dietary factors in triggering molecular mechanism responses, including cell growth, cell death, oxidative stress, and the downregulation of key metabolic pathways or enhancement of ROS generation.

## 4 CONCLUSION

This study mimicked the exposure of T2D patients to high $PM_{2.5}$ and glucose concentrations, exploring their effects on wound healing and radical scavenging in A549 cells. Furthermore, we examined the potential mitigating effects of GEJ administration. This investigation addressed the increasing concerns regarding the detrimental health effects brought about by the continuous growth of $PM_{2.5}$ and human metabolic disorders, such as DM. This study revealed that co-exposure to high $PM_{2.5}$ and glucose concentrations resulted in more severe health consequences, exacerbating the adverse effects in terms of reduced cell growth (decreased by 1.65–2.32%), enhanced cell death (increased by 4.8%–7.2%), impaired wound healing (reduced by –14–5.0%), and induced oxidative stress. In $PM_{2.5}$- and glucose-treated A549 cells, we proved the potential therapeutic ability of GEJ in terms of increased cell growth (decreased by 3.2%), decreased cell death (increased by 7.9%), accelerated wound healing (increased by 12% or from 51% to 63%), and upregulated MnSOD and CAT gene expression, clearing free radicals. Our findings indicated that GEJ might have therapeutic potential to accelerate wound healing, increase cell growth, decrease cell death, and enhance antioxidant responses at the cellular level in T2D patients. Furthermore, the therapeutic effects of GEJ persisted even after T2D patients were exposed to $PM_{2.5}$.

## DISCLAIMER

The authors of this paper declare no conflicts of interest.

## SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version at https://doi.org/10.4209/aaqr.240165

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