

Salidroside attenuates PM_{2.5}-induced cardiac defects via modulation of RPL3 expression

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Author contributions

Ye LL and Chen JX drafted the original manuscript. Wang ZX supervised the study and performed manuscript review and editing. Zhang C, Liu YQ and Wang FM carried out software work. Xie WJ and Tang Y conducted formal analysis. Yang ZT, Li YH and Song YF developed the methodology. Wang YL and Li WF were responsible for data curation. Wu YC and Wang JW participated in study design and provided financial support for this research. All authors have read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

PaCO₂, Arterial partial pressure of carbon dioxide; PaO₂, Arterial partial pressure of oxygen; DEGs, Differentially expressed genes; ELISA, Enzyme-linked immunosorbent assay; FBS, Fetal bovine serum; PM_{2.5}, Fine particulate matter; GO, Gene Ontology; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IVS, Interventricular septal thickness; KEGG, Kyoto Encyclopedia of Genes and Genomes; LVEF, Left ventricular ejection fraction; LVEDD, Left ventricular end-diastolic diameter; LVFS, Left ventricular fractional shortening; LVPW, Left ventricular posterior wall thickness; MD, Molecular dynamics; NO, Nitric oxide; qPCR, Quantitative PCR; Rg, Radius of gyration; ROS, Reactive oxygen species; RPL3, Ribosomal protein L3; RMSD, Root-mean-square deviation; RMSF, Root-mean-square fluctuation; Sal, Salidroside; TNF-α, Tumor necrosis factor-α.

Citation

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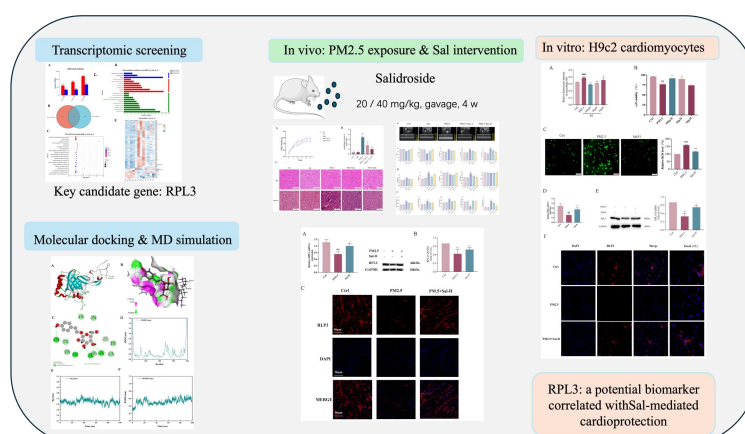
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Abstract

Background: Fine particulate matter (PM_{2.5}) exposure increases cardiovascular morbidity, yet effective clinical interventions for PM_{2.5}-related cardiac injury are lacking. Salidroside (Sal), the major bioactive component of *Rhodiola rosea*, has cardioprotective and anti-inflammatory properties. However, the relationship between Sal and key molecular regulators in PM_{2.5}-induced myocardial damage remains unclear, restricting its translational application. **Methods:** *In vivo* and *in vitro* models were constructed to investigate Sal's protective effects and identify its associated molecular targets. We assessed myocardial histology, inflammatory infiltration and pro-inflammatory cytokines *in vivo*, and detected nitric oxide (NO), reactive oxygen species (ROS) and cell viability *in vitro*. Transcriptome analysis was used to screen candidate genes, followed by immunofluorescence and qPCR validation. **Results:** Sal preserved myocardial structure, alleviated inflammatory infiltration and suppressed pro-inflammatory cytokines dose-dependently *in vivo*. *In vitro*, Sal reduced excess NO and ROS production to mitigate heart injury. Transcriptomics identified ribosomal protein L3 (RPL3); validation confirmed Sal upregulated RPL3 under PM_{2.5} stress. **Conclusion:** Sal may confer protection against PM_{2.5}-induced myocardial injury and modulate RPL3 expression. RPL3 may correlate with Sal-mediated cardioprotection, though its causal mediation remains unproven. This work highlights Sal's cardioprotective potential against PM_{2.5} toxicity and proposes RPL3 as a candidate molecular target, laying preliminary groundwork for further mechanistic research and preventive application of Sal in PM_{2.5}-associated cardiovascular diseases.

Keywords: salidroside; PM_{2.5}; myocardial injury; RPL3



Highlights

1. *In vivo* and *in vitro* models demonstrated that salidroside dose-dependently alleviates PM_{2.5}-induced myocardial inflammation.
2. Transcriptomic screening and validation revealed that salidroside alters the expression of RPL3 under PM_{2.5} exposure.
3. RPL3 may serve as a potential biomarker correlated with salidroside-mediated cardioprotection, though causal mediation remains unproven.

Medical history of objective

Rhodiola rosea, the raw herb of salidroside, was first recorded in the *Tibetan medical classic*. Traditionally, it is indicated for qi deficiency and blood stasis, chest bi and heart pain, which corresponds to modern cardiovascular disorders. Salidroside, its core bioactive constituent, has been demonstrated in modern pharmacological studies to possess anti-inflammatory, anti-oxidative, anti-hypoxic and cardioprotective properties.

Introduction

Fine particulate matter (PM_{2.5}) refers to airborne particles with an aerodynamic diameter of $\leq 2.5 \mu\text{m}$ and is characterized by a strong adsorptive capacity [1]. It is typically composed of a complex mixture of various toxic and hazardous pollutants and represents one of the major harmful components of ambient air pollution [2, 3]. A large body of epidemiological evidence has consistently demonstrated that both long-term and short-term exposure to PM_{2.5} is closely associated with increased cardiovascular morbidity and mortality [4–6]. Among adverse cardiovascular outcomes, myocardial injury and cardiac dysfunction are recognized as important pathological consequences of PM_{2.5} exposure [7]. These effects are primarily driven by excessive oxidative stress [8], enhanced inflammatory responses [9], and disruption of intracellular homeostasis [10]. Although accumulating studies have shown that PM_{2.5} can directly impair cardiac structure and function [11], the specific molecular mechanisms underlying PM_{2.5}-induced myocardial injury remain to be fully elucidated. In particular, SIRT6-associated endoplasmic reticulum stress has been implicated in cardiac developmental defects following maternal PM_{2.5} exposure [12], underscoring an urgent need to identify potential protective strategies and novel molecular targets to reduce the cardiovascular health risks associated with PM_{2.5} exposure.

Given the critical roles of excessive oxidative stress, enhanced inflammatory responses, and disruption of intracellular homeostasis in the development of PM_{2.5}-induced cardiovascular injury, increasing attention has been directed toward natural bioactive compounds with antioxidant and anti-inflammatory properties as potential protective or preventive agents [13–15]. Among these natural bioactive compounds, *Rhodiola* species (Hongjingtian), represented by *R. rosea* L. and *R. crenulata*, possess a long history of medicinal application in traditional Chinese and Tibetan medicine, with records dating back to the ancient medical classic *rGyud bzhi* (*The Four Medical Tantras*). Modern pharmacological studies have increasingly corroborated these traditional indications. For example, Chang et al. demonstrated that salidroside significantly attenuated myocardial ischemia–reperfusion injury in coronary artery occlusion-induced rats and Langendorff-perfused hearts by restoring energy-metabolism homeostasis through AMPK/PPAR- α /PGC-1 α activation and suppressing NF- κ B-mediated cardiac inflammation [16]. Consistently, Yan et al. reported that salidroside inhibited ischemia/reperfusion-induced cardiomyocyte apoptosis via the miR-378a-3p/IGF1R/PI3K/Akt axis [17], and a comprehensive review has further summarized the broad cardioprotective actions of *Rhodiola*, including anti-ischemia-reperfusion injury, antithrombotic,

and antiarrhythmic effects [18]. Among them, Salidroside (Sal), a major phenylpropanoid glycoside isolated from *Rhodiola rosea*, has been widely reported to exert cardioprotective effects under various pathological conditions, including ischemia–reperfusion injury [19], and oxidative stress-related cardiac dysfunction [18]. Previous studies suggest that salidroside can modulate cellular redox balance, suppress excessive inflammatory responses, and maintain intracellular homeostasis, thereby alleviating myocardial injury [17, 20]. However, whether salidroside can effectively mitigate myocardial injury induced by PM_{2.5} exposure, as well as the key molecular mechanisms underlying its potential protective effects, remains largely unexplored.

Ribosomal proteins are increasingly recognized as exerting functions beyond protein synthesis, particularly in cellular stress responses [21]. Consistent with this notion, ribosomal proteins other than RPL3, such as RPL22, have been implicated in cellular stress adaptation [22], and RPL3L, a cardiomyocyte-enriched paralog of RPL3, has been linked to the maintenance of cardiomyocyte homeostasis [23]. Altered expression of mitochondrial ribosomal proteins has also been observed in cardiomyocytes under inflammatory stress [24]. Given that PM_{2.5} is well documented to induce oxidative stress, inflammatory signaling and cellular damage across multiple organ systems, these observations raise the possibility that RPL3 may be involved in PM_{2.5}-induced myocardial injury. However, the functional involvement of RPL3 in this process, and its potential modulation by protective agents, remain largely unexplored. Elucidating whether ribosomal protein-mediated mechanisms contribute to PM_{2.5}-induced cardiac dysfunction may therefore provide novel insights into the molecular basis of air pollution-related cardiovascular diseases and aid in the identification of new therapeutic targets.

Methods**Materials and reagents**

FBS was obtained from Gibco (Australia, 10099-141). Rat TNF- α (QZ-10160), IL-6 (QZ-10049), IL-10 (QZ-10026) and IL-1 β (QZ-10037) ELISA kits were purchased from Bioengineering (Wuhan, China). RPL3 (66130-1-Ig) was purchased from Proteintech (Nanjing, China). GAPDH (ab8245) was purchased from Abcam (Cambridge, UK). HRP-labeled goat anti-mouse IgG secondary antibody (RGAM001) was purchased from Proteintech. Actin-F/R and RPL3-F/R primers (Q-02) were purchased from Accurate Biology (Hunan, China). Salidroside (purity $\geq 98\%$, Catalog No. TMSM-1792) was purchased from Topscience (Shanghai, China). Penicillin-streptomycin mixture (PB180120), DMEM culture medium (PM150210), ROS detection kit (S0033S), NO detection kit (S0020S), CCK-8 kit (C0038), LDH kit (C0019S), and ECL chemiluminescence kit (BL520A) were obtained from Beyotime Biotechnology (Shanghai, China). PAGE Gel Fast Preparation Kit (Cat. No. PG112) was purchased from EpiZyme (Shanghai, China).

PM_{2.5} and Sal preparation

PM_{2.5} particles (SRM 2786) were obtained from the National Institute of Standards and Technology (NIST, USA) and dispersed in saline to a final concentration of 5 mg/mL [25]. The suspension was sonicated for 30 minutes to ensure uniform dispersion, then stored at -80°C and used within 48 h. Prior to each use, the suspension was sonicated for 15 minutes to prevent aggregation. For *in vitro* experiments, salidroside (C₁₅H₂₀O₇) was dissolved in saline to prepare a 10 mM stock solution, which was stored at -20°C protected from light. For *in vivo* animal administration, salidroside powder was weighed and dissolved in saline to prepare the required working solution freshly before use.

Animal grouping and treatment protocol

Fifty male SD rats (120–160 g, SPF grade) were purchased from Chongqing Medical University. The animals were supplied with a quality certificate (No. 430727251100933087). Rats were housed in

SPF-controlled animal rooms at $23 \pm 2^\circ\text{C}$, $55 \pm 5\%$ humidity, with a 12-hour light/12-hour dark cycle and had free access to food and water. Experiments were conducted one week after acclimatization. All animal experimental procedures were reviewed and approved by the Animal Experiment Ethics Committee of Chongqing Medical University (Approval No. IACUC-CQMU-2024-0475) and were conducted in accordance with the guidelines for the care and use of laboratory animals.

Rats were randomly assigned to five groups ($n = 10$): control group, salidroside high-dose group, PM_{2.5} group, PM_{2.5} plus low-dose salidroside group (PM_{2.5} + Sal-L, 20 mg/kg), and PM_{2.5} plus high-dose salidroside group (PM_{2.5} + Sal-H, 40 mg/kg). Rats in the control group received normal saline, and those in the high-dose salidroside group were given salidroside (40 mg/kg) via oral gavage at an identical administration volume of 1.5 mL/kg. Treatments were administered once each morning for 4 consecutive weeks. They also received intratracheal instillation of normal saline, 1.5 mL/kg every other day. Rats in the PM_{2.5} group were exposed to PM_{2.5} via intratracheal instillation at a dose of 7.5 mg/kg (1.5 mL/kg) every other day in the afternoon for 4 consecutive weeks. In the PM_{2.5} + Sal treatment groups, rats were administered salidroside by oral gavage once daily in the morning at doses of 20 mg/kg (Sal-L) or 40 mg/kg (Sal-H). PM_{2.5} was intratracheally instilled in the afternoon at a dose of 7.5 mg/kg (1.5 mL/kg) every other day for 4 weeks.

Tracheal instillation procedure: Rats were anesthetized via intraperitoneal injection of 1.5% pentobarbital (2 mL/kg). Once fixed on the rat plate, the glottis was exposed using a laryngoscope, and a microsyringe was inserted approximately 0.5 cm into the trachea. The fluid was then slowly administered. Following instillation, rats were maintained upright, and their thorax was gently tapped to ensure even pulmonary distribution. After model establishment, rats were fasted overnight. Blood was collected post-euthanasia, and heart tissues were excised, rinsed with cold saline, and blotted dry. A portion of the left ventricle was fixed in 4% paraformaldehyde for histopathological analysis, and the remaining tissue was frozen in liquid nitrogen and stored at -80°C .

Molecular docking

The cryo-electron microscopy structure of the ribosome complex (PDB ID: 7QGG) was downloaded from the RCSB Protein Data Bank. 7QGG represents a stalled pre-translocation ribosome complex from *Rattus norvegicus*, in which the 60S ribosomal protein L3 (RPL3) is assigned to Chain B. The RPL3 monomer was extracted from the intact ribosome complex using PyMOL. The corresponding ligand structure was retrieved from PubChem in SDF format and converted to PDB format via OpenBabel. Prior to molecular docking, the extracted RPL3 structure was prepared in AutoDock Tools (version 1.5.6). Solvent molecules and redundant ribosomal components were removed, polar hydrogen atoms were added, and Gasteiger charges were assigned. The processed protein and ligand structures were then exported as PDBQT files.

Molecular docking was performed using AutoDock to explore the potential binding interactions between the ligand and RPL3. The docking grid box was centered at center_x = 143.597, center_y = 177.173, center_z = 222.704, with box dimensions of $86 \text{ \AA} \times 62 \text{ \AA} \times 126 \text{ \AA}$. The grid spacing was set to the default value of $0.375 \text{ \AA}$. The final protein–ligand binding models were analyzed and visualized in PyMOL (version 2.4.1).

Molecular dynamics simulation and binding free-energy calculation

Molecular dynamics simulations were performed using GROMACS 2020.3 to investigate the conformational stability and dynamic interactions of the protein–ligand complex. The amber99sb-ildn force field was employed for protein parameterization, while ligand topology parameters were generated based on the General Amber Force Field (GAFF).

The protein–ligand complex was placed in a periodic simulation box with a minimum distance of 1.0 nm between the solute and box

boundaries. The system was subsequently solvated using the explicit SPC216 water model, and charge neutrality was achieved by introducing appropriate amounts of Na⁺ and Cl[−] ions.

Prior to simulation, energy minimization was conducted using the steepest descent algorithm to eliminate steric clashes and unfavorable interactions. The minimized system was then subjected to equilibration under NVT and NPT ensembles for 100 ps each at 300 K and 1 bar.

A 100 ns production simulation was subsequently performed under periodic boundary conditions. During the simulation, temperature and pressure were regulated at 300 K and 1 bar using the V-rescale thermostat and Parrinello–Rahman barostat, respectively.

The equations of motion were integrated using the leapfrog algorithm with a 2 fs time step. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method with a Fourier grid spacing of 0.16 nm, and all covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm.

The generated MD trajectories were analyzed and visualized using VMD (version 1.9.3) and PyMOL (version 2.4.1). The ligand binding free energy was further estimated using the gmx_mmpbsa tool to evaluate the energetic contribution and stability of protein–ligand interactions.

Echocardiographic assessment

At the end of the experimental period, rats were weighed and anesthetized, followed by thoracic hair removal and application of a coupling gel. Transthoracic echocardiography was performed using an 18 MHz high-frequency ultrasound imaging system, which was prewarmed for 30 minutes prior to use. The echocardiography system used was the VINNO 6 LAB small animal high frequency ultrasound imaging system (VINNO, China), equipped with an X616L linear array probe. The imaging frequency was set to 18 MHz, and the imaging mode was the high resolution (HAR) mode. Key cardiac parameters, including left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular end-diastolic diameter (LVEDD), interventricular septal thickness (IVS), and left ventricular posterior wall thickness (LVPW), were measured to evaluate cardiac structure and function. All echocardiographic data were acquired and analyzed in a double-blind manner. When intergroup differences exceeded 5%, statistical comparisons were independently verified by a third-party analyst to ensure data reliability.

Measurement of inflammatory cytokines by ELISA

ELISA was conducted to quantify the levels of inflammatory cytokines, including TNF- α , IL-6, IL-1 β and IL-10 in serum samples. The assay was performed according to the manufacturer's recommended protocol.

Briefly, 10 μL of serum was loaded into each well of a 96-well microplate, followed by incubation with HRP-labeled secondary antibodies at 37°C for 1 h. After the reaction was terminated by adding 50 μL stop solution, the plates were incubated for an additional 15 min, and absorbance values were recorded at 450 nm using a microplate reader.

LDH activity in myocardial tissue

Myocardial tissue samples were homogenized in ice-cold lysis buffer and centrifuged to collect the supernatant. The lactate dehydrogenase (LDH) activity in myocardial tissue was determined with a commercial assay kit following the manufacturer's protocols. The absorbance was measured using a microplate reader, and tissue LDH activity was calculated to evaluate cardiomyocyte injury.

Blood gas and lactate analysis

Arterial blood samples were collected using a sterile heparinized syringe immediately after sampling to prevent blood coagulation and air bubble contamination. Whole blood parameters including pH, arterial partial pressure of oxygen (PaO₂), arterial partial pressure of carbon dioxide (PaCO₂), Glu, lactate, sodium, potassium, ionized calcium and chloride were measured within 3 min after blood

collection using the epoc® Blood Analysis System (Siemens Healthineers, USA). The epoc BGEM single-use test cards were applied following the manufacturer's standard operating procedure. Samples with obvious hemolysis or visible air bubbles were discarded and excluded from further statistical analysis.

Histopathological analysis

Heart tissues were collected and fixed in 4% paraformaldehyde, followed by routine dehydration, paraffin embedding, and sectioning. Paraffin-embedded tissues were cut into sections with a thickness of 4–5 µm and subsequently stained with hematoxylin and eosin (H&E) as well as Masson's trichrome staining according to standard protocols. Histopathological alterations were examined and imaged under a light microscope. H&E staining was used to assess myocardial damage, including fiber edema, disarrangement, rupture, inflammatory infiltration and tissue necrosis. A double-blind semi-quantitative scoring system was used to evaluate myocardial injury. For each section, five random and non-overlapping fields were examined, and the average score was calculated. The scoring scale ranged from 0 to 4: 0, normal myocardium with neatly arranged fibers and no inflammation or fibrosis; 1, mild injury with slight fiber edema and rare inflammatory cells; 2, mild-moderate injury with partial fiber disorder, moderate edema and sparse inflammatory infiltration; 3, moderate-severe injury with obvious fiber damage, prominent inflammatory infiltration and moderate interstitial fibrosis; 4, severe injury with widespread fiber rupture, massive inflammatory infiltration and severe fibrosis with focal necrosis. All histological evaluations and scoring were performed independently by two observers to ensure result reliability.

Immunofluorescence staining for RPL3

Immunofluorescence staining for RPL3 was performed on both paraffin-embedded myocardial sections and H9C2 cardiomyocytes. Paraffin myocardial tissues were cut into 4-µm slices. After deparaffinization and antigen retrieval, tissue sections and fixed H9C2 cell coverslips were permeabilized with 0.5% Triton X-100 and blocked with goat serum at room temperature for 1 h to reduce non-specific binding.

All samples were incubated with anti-RPL3 primary antibody at 4 °C overnight. After thorough washing, fluorophore-conjugated secondary antibodies were applied and incubated for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI for 5 min. Fluorescent signals were visualized and captured using a fluorescence microscope.

RNA extraction and quantitative PCR analysis

Total RNA was isolated from tissue samples and H9C2 cells. The input amount of total RNA for reverse transcription was adjusted to 500–1000 ng, and RNA purity was confirmed with an OD260/OD280 ratio ranging from 1.8 to 2.0. Reverse transcription was subsequently conducted following the manufacturer's protocol, and the generated cDNA samples were preserved at –80 °C until further experiments.

Specific primers targeting *RPL3* were designed, while *β-actin* was selected as the reference gene for normalization (Table 1). The relative abundance of *RPL3* mRNA was calculated using the $2^{-\Delta\Delta Ct}$ comparative threshold cycle method.

Heart tissue transcriptomic analysis

Total RNA was extracted from rat cardiac tissues, with 4 biological replicates per group. All samples were snap-frozen in liquid nitrogen and stored at –80 °C, then shipped on dry-ice to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for eukaryotic reference-based mRNA transcriptome sequencing. RNA purity was assessed by NanoDrop (OD260/OD280 = 1.8–2.2), and RNA integrity was

Table 1 Primers used for real-time quantitative PCR

Gene	Primer	Sequence (5'→3')
<i>RPL3</i>	Forward	ATGGCAAGATGACACAGGCA
<i>RPL3</i>	Reverse	CTTCTCAGCTACAGTGCCCC
<i>β-actin</i>	Forward	GGAGATTACTGCCCTGGCTCTTA
<i>β-actin</i>	Reverse	GACTCATCGTACTCTGCTTGCTG

evaluated using Agilent 2100 Bioanalyzer. Only samples with RIN ≥ 7.0 and 28S/18S ≥ 1.5 were used for library construction. mRNA was enriched by Oligo(dT) magnetic beads, fragmented and reverse-transcribed into double-stranded cDNA. After end-repair, A-tailing, adapter ligation and PCR amplification, cDNA libraries were sequenced on the Illumina NovaSeq X Plus platform in paired-end mode. Sequencing depth was ≥ 6 G clean data per sample (~40 M clean reads).

Raw reads were filtered by fastp to obtain clean reads. Clean reads were mapped to rat reference genome Rn6 (Ensembl) using HISAT2 software. Gene expression levels (TPM and read counts) were quantified by RSEM. Differential expression analysis was performed by DESeq2. Differentially expressed genes (DEGs) were screened with thresholds: FDR (padj) < 0.05 and $|\log_2(\text{fold-change})| \geq 1$, where FDR was corrected by the Benjamini-Hochberg method. GO and KEGG enrichment analyses were subsequently performed on the Majorbio Cloud Platform. Raw sequencing data will be deposited into the NCBI GEO database upon manuscript acceptance.

Western blot analysis

Protein expression levels were examined by western blotting. Briefly, cell and tissue samples were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors, and the extracted proteins were quantified using a BCA protein assay kit. Equivalent amounts of protein from each sample were resolved by SDS-PAGE and subsequently electrotransferred onto PVDF membranes.

Following transfer, membranes were incubated with 5% skim milk to block nonspecific binding sites. The membranes were then exposed to the corresponding primary antibodies at 4 °C overnight, followed by incubation with HRP-conjugated secondary antibodies. Target protein signals were developed using an ECL detection system.

The intensity of the detected bands was quantified using ImageJ software, and relative protein abundance was calculated after normalization to the appropriate loading control.

Cell culture and treatment

H9C2 cardiomyocytes were obtained from the Cell Bank of the Chinese Academy of Sciences and maintained in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin solution. Cells were cultured under standard conditions at 37 °C with 5% CO₂. The experimental groups were as follows: Control group: Cells cultured in normal medium. PM_{2.5} model group: Cells treated with PM_{2.5} at a concentration of 100 µg/mL for 24 hours. PM_{2.5} + low-dose Sal group (PM_{2.5} + Sal-L): Cells pre-treated with low-dose Sal (10 µM) for 24 hours, followed by PM_{2.5} exposure (100 µg/mL) for another 24 hours. PM_{2.5} + high-dose Sal group (PM_{2.5} + Sal-H): Cells pre-treated with high-dose Sal (50 µM) for 24 hours, followed by PM_{2.5} exposure (100 µg/mL) for another 24 hours.

Cell viability assay (CCK-8)

Cell viability was evaluated using a Cell Counting Kit-8 (CCK-8; Beyotime, C0038, China) assay. Briefly, H9C2 cells were seeded into 96-well plates and cultured overnight to allow adherence. Cells were treated with different concentrations of salidroside (100, 50, or 10 µM) for 24 h. For the PM_{2.5} exposure model, cells were divided into following groups: (i) the control group, treated with complete medium only; (ii) the PM_{2.5} group, exposed to 100 µg/mL PM_{2.5} for 24 h; (iii) the PM_{2.5} + Sal groups, in which cells were pre-incubated with salidroside (100, 50, or 10 µM) for 24 h and then co-exposed to 100 µg/mL PM_{2.5} for 24 h. 10 µL of CCK-8 solution was added to each well, and the plates were incubated at 37 °C for 1 h in the dark. The optical density (OD) value was measured at 450 nm using a microplate reader, and cell viability was expressed as a percentage of that in the untreated control group.

Measurement of NO and ROS in H9C2 cells

Intracellular NO production was evaluated using a DAF-FM DA-based Nitric Oxide Detection Kit (Beyotime, S0020S, China). H9C2 cells were treated with the DAF-FM DA probe solution following the

protocol provided by the manufacturer. After probe loading, cells were rinsed and maintained under appropriate conditions to allow complete intracellular de-esterification.

The fluorescence signal corresponding to NO production was subsequently recorded using a 96-well fluorescence microplate reader with excitation and emission wavelengths set at 495 nm and 515 nm, respectively. NO content was represented as relative fluorescence intensity and normalized against the untreated control group.

Intracellular ROS accumulation was detected using a ROS Detection Kit (Beyotime, S0033S, China). Briefly, H9C2 cells were incubated with the ROS-sensitive fluorescent probe in accordance with the manufacturer's protocol. After probe incubation, the cells were thoroughly washed to remove unbound probe. Cellular ROS fluorescence images were captured using a laser scanning confocal microscope. The intracellular ROS level was quantitatively analyzed based on the mean fluorescence intensity (MFI) and normalized to the control group.

Statistical analysis

Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons, or Student's t-test for pairwise comparisons. The normality of the data was assessed using the

Shapiro-Wilk test. A *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, USA). Weekly body weight was analyzed by two-way repeated-measures ANOVA with time and treatment as factors. Post-hoc multiple comparisons were conducted for pairwise group comparisons at each time point. Data are presented as mean $\pm$ SEM.

Results

Protective effect of solidoside on PM_{2.5}-induced myocardial injury in rats

Body weight was monitored weekly from model establishment to sample collection (Figure 1A). Two-way repeated-measures ANOVA revealed a significant main effect of time ($P < 0.01$) and a significant main effect of treatment ($P < 0.01$) on body weight. However, no significant time-treatment interaction was observed ($P = 0.9204$), indicating parallel body-weight changing trends among all experimental groups. Collectively, these results suggest that PM_{2.5} exposure adversely affected general health status, whereas solidoside mitigated the PM_{2.5}-induced decline in body weight.

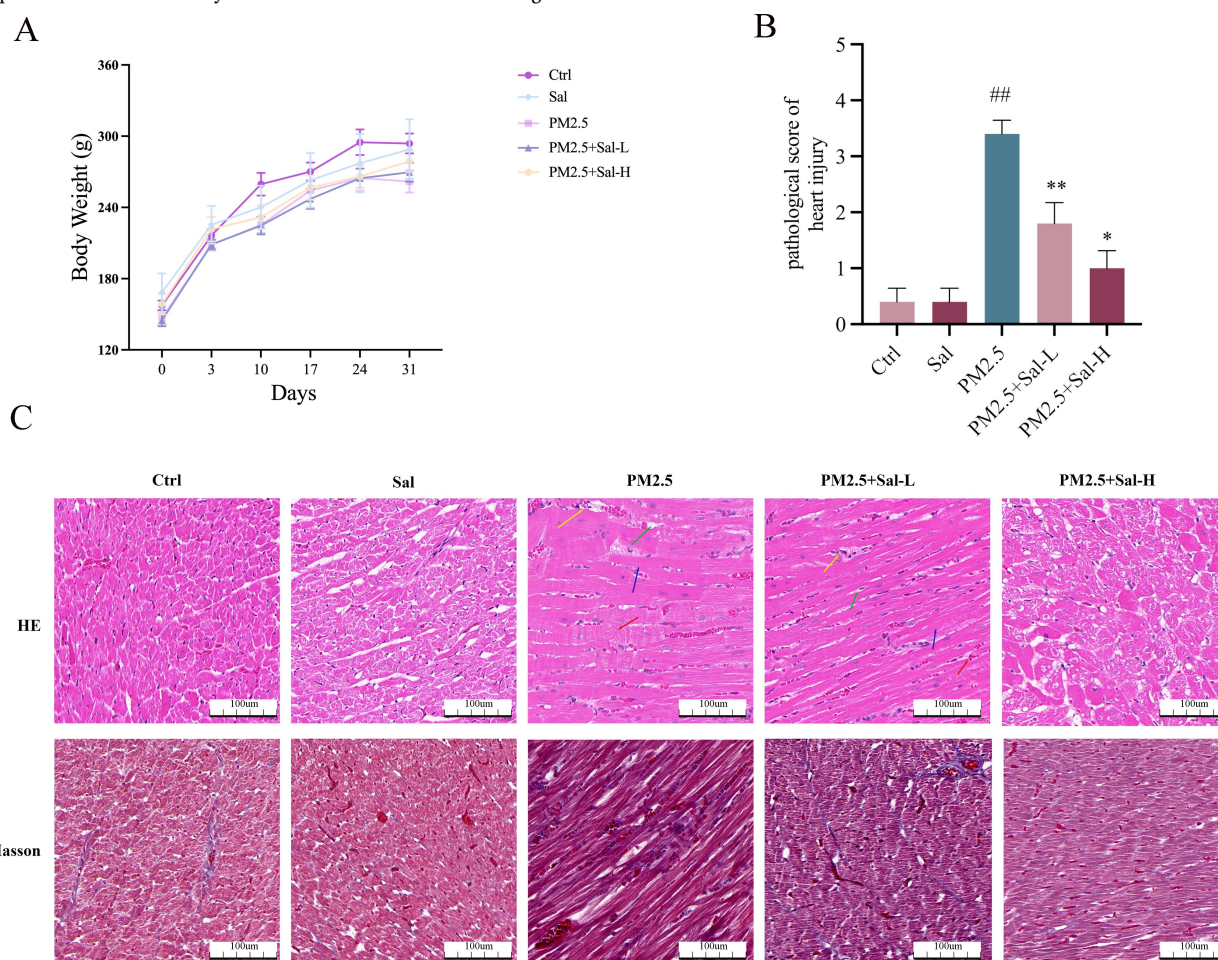


Figure 1 Solidoside alleviates PM_{2.5}-induced myocardial injury in rats.

(A) Weekly body-weight changes in rats. Statistical analysis was performed using two-way repeated-measures ANOVA. Main effect of time: $F(5, 225) = 164.3$, $P < 0.01$; main effect of treatment: $F(4, 45) = 9.028$, $P < 0.01$; time $\times$ treatment interaction: $F(20, 225) = 0.5836$, $P = 0.9204$. Post-hoc tests were used for pairwise comparisons at each time point. $n = 10$ per group. Data are shown as mean $\pm$ SEM. (B–C) Representative histopathological images and corresponding pathological scores showing the effects of Sal on PM_{2.5}-induced myocardial injury, as assessed by hematoxylin and eosin (H&E) staining and Masson's trichrome staining ($\times 400$ magnification; scale bar = 100 μ m). Red arrows: myofiber rupture, Yellow arrows: inflammatory infiltration, Blue arrows: interstitial edema and widened intercellular spaces, Green arrows: cardiomyocyte vacuolation. Data are presented as mean $\pm$ SEM. $^{##}P < 0.01$ versus Ctrl group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus PM_{2.5} group. Groups: Ctrl, control; Sal, solidoside; PM_{2.5}, PM_{2.5} exposure; PM_{2.5} + Sal-L, PM_{2.5} plus low-dose solidoside; PM_{2.5} + Sal-H, PM_{2.5} plus high-dose solidoside.

To assess the histopathological changes in the myocardium and the protective effects of Sal, H&E staining and Masson's trichrome staining were performed (Figure 1B, 1C). In the control group, myocardial architecture appeared intact, with regularly arranged cardiomyocytes and well-defined nuclei. In contrast, PM_{2.5} exposure induced pronounced myocardial injury, characterized by disrupted tissue architecture, cardiomyocyte swelling, disorganized myocardial fibers, and marked inflammatory cell infiltration. Sal treatment substantially attenuated these pathological alterations in a dose-dependent manner. Specifically, Sal improved myocardial fiber organization, reduced cellular swelling and interstitial edema, and decreased inflammatory cell infiltration. Notably, the high-dose Sal group exhibited myocardial morphology that was largely comparable to that of the control group, accompanied by a pronounced reduction in interstitial edema and inflammatory infiltration.

Salidroside ameliorates PM_{2.5}-induced myocardial dysfunction and inflammatory responses in rats

Echocardiographic analysis (Figure 2A) revealed significant impairment of cardiac function and structural alterations in PM_{2.5}-exposed rats. Compared with the control group, LVEF and LVFS were markedly reduced, whereas LVEDD was significantly increased ($P < 0.01$), indicating compromised systolic function and ventricular dilation. In addition, IVS and LVPW were altered in the PM_{2.5} group, suggesting myocardial structural remodeling. Salidroside administration significantly ameliorated these PM_{2.5}-induced

echocardiographic abnormalities. Both low- and high-dose Sal treatment increased LVEF and LVFS, reduced LVEDD, and partially normalized IVS and LVPW values compared with the PM_{2.5} group. These protective effects exhibited a dose-dependent pattern, with the high-dose Sal group showing more pronounced improvements ($P < 0.05$ or $P < 0.01$).

Blood gas analysis (Figure 2B) revealed that PM_{2.5} exposure induced systemic hypoxemia and acid-base imbalance closely associated with cardiac dysfunction. Compared with the control group, PM_{2.5}-exposed rats exhibited a significant reduction in arterial PaO₂ accompanied by an increase in arterial PaCO₂, resulting in decreased arterial pH. These alterations indicate impaired oxygen delivery and increased cardiac workload, which may exacerbate myocardial hypoxia and compromise cardiac performance under PM_{2.5} exposure. Salidroside treatment ameliorated these PM_{2.5}-induced disturbances in a dose-dependent manner. Sal administration increased PaO₂, reduced PaCO₂, and partially restored arterial pH toward physiological levels, suggesting improved systemic oxygenation and a reduced hypoxic burden on the myocardium. In parallel, blood lactate levels—elevated in PM_{2.5}-exposed rats as a consequence of myocardial hypoxia and enhanced anaerobic metabolism—were decreased following Sal treatment, indicating improved myocardial oxygen utilization and metabolic efficiency. Overall, PM_{2.5} induced hypoxia, hypercapnia, acidosis, and lactate accumulation, whereas Sal reversed these alterations, creating a favorable internal environment for myocardial protection.

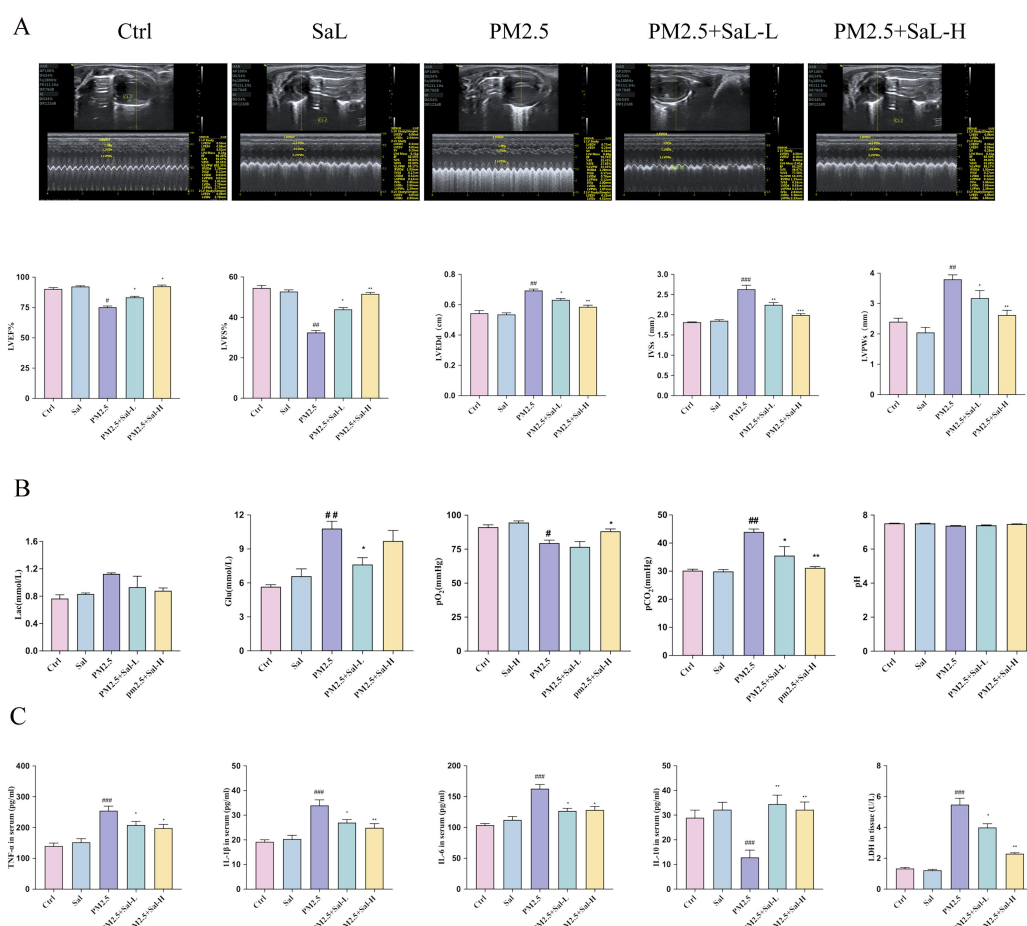
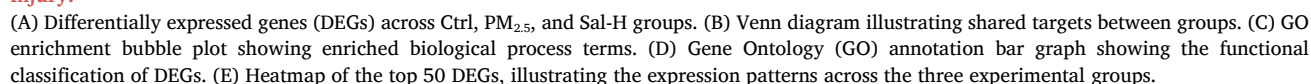


Figure 2 Salidroside improves myocardial function and reduces inflammatory responses in PM_{2.5}-induced myocardial injury.

(A) Representative M-mode echocardiography images and quantitative analysis of cardiac function indices (LVEF%, LVFS%, LVEDD, IVS, LVPW). (B) Quantification of blood-gas parameters including lactate, Glu, PaO₂, PaCO₂ and pH. (C) The levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and anti-inflammatory cytokine IL-10 in serum, as well as myocardial injury marker lactate dehydrogenase (LDH) in myocardial tissue homogenates, were measured. $n = 10$ per group. Data are expressed as mean $\pm$ SEM. ** $P < 0.01$, *** $P < 0.001$ versus Ctrl group; * $P < 0.05$, ** $P < 0.01$ versus PM_{2.5} group. Groups: Ctrl, control; Sal, salidroside; PM_{2.5}, PM_{2.5} exposure; PM_{2.5} + Sal-L, PM_{2.5} plus low-dose salidroside; PM_{2.5} + Sal-H, PM_{2.5} plus high-dose salidroside.

Transcriptomic profiling of myocardial tissues was performed to explore the molecular mechanisms underlying the cardioprotective effects of salidroside (Sal) against PM_{2.5}-induced injury. Genes with $|\log_2\text{FC}| \geq 1$ and $\text{FDR} < 0.05$ were defined as differentially

Intersection analysis of these DEG sets identified 12 overlapping genes that were reversed or modulated by Sal treatment under PM_{2.5} exposure. Raw overlapping DEGs were first obtained by intersection. Subsequently, we screened reversal genes by filtering genes with opposite expression tendencies: genes downregulated by PM_{2.5} but upregulated upon salidroside treatment, or genes upregulated by PM_{2.5} but downregulated upon salidroside intervention. Genes with consistent expression trends in two comparisons were excluded. GO enrichment analyses of these shared DEGs (Figure 3C, 3D) revealed significant enrichment in pathways related to ribosomal function and cellular homeostasis. Notably, RPL3 emerged as a potential key target associated with the cardioprotective effects of Sal in PM_{2.5}-induced myocardial injury.



Computational analysis of the Salidroside–RPL3 interaction

Molecular docking analysis revealed that salidroside binds to RPL3 with a binding energy of -6.2 kcal/mol, indicating a favorable interaction (Figure 4A). Detailed interaction analysis showed that salidroside formed multiple conventional hydrogen bonds with key amino acid residues of RPL3, including PRO18, LYS20, SER22, HIS275, HIS276, and THR278 (Figure 4B, 4C). These hydrogen-bond interactions contribute to the stable binding conformation between salidroside and RPL3.

Molecular dynamics simulations were performed to evaluate the stability and interaction characteristics of the RPL3–salidroside complex (Figure 4D). The RMSD of the protein remained stable throughout the simulation, with an average value of 0.5182 ± 0.0782 nm, indicating a well-equilibrated and stable complex (Figure 4F). Consistently, radius of gyration (Rg) showed a slight decreasing tendency, suggesting increased structural compactness of the protein upon ligand binding (Figure 4E). RMSF analysis showed limited residue-level fluctuations, with an average RMSF value of 0.2437 nm, further supporting stable protein–ligand interactions. Hydrogen bond analysis revealed persistent interactions between the protein and ligand, with an average of 1.32 hydrogen bonds and a maximum of six observed during the simulation. Moreover, MM/PBSA binding free energy analysis demonstrated a negative binding free energy of

-64.805 kJ/mol, indicating a thermodynamically favorable interaction dominated primarily by van der Waals forces. Collectively, these results support the structural stability and favorable binding of salidroside to RPL3.

Sal ameliorated PM_{2.5}-induced myocardial injury accompanied by increased RPL3 expression

To examine whether changes in RPL3 expression accompany the protective effects of Sal against PM_{2.5}-induced myocardial injury, western blotting, qPCR, and immunofluorescence analyses were performed (Figure 5A–5C). Immunofluorescence results revealed strong and uniformly distributed RPL3 signals in the myocardial tissues of control rats. In contrast, PM_{2.5} exposure markedly attenuated RPL3 fluorescence intensity, indicating suppressed RPL3 expression in myocardium. Following high-dose Sal treatment, the RPL3 fluorescence intensity was increased relative to the PM_{2.5} group. Consistently, WB and qPCR analyses demonstrated that RPL3 protein and RPL3 mRNA levels were remarkably reduced in PM_{2.5}-challenged rats, whereas Sal administration significantly restored its expression. These consistent transcriptional and protein results indicate that Sal may ameliorate PM_{2.5}-induced myocardial injury, at least in part, through modulating RPL3 upregulation.

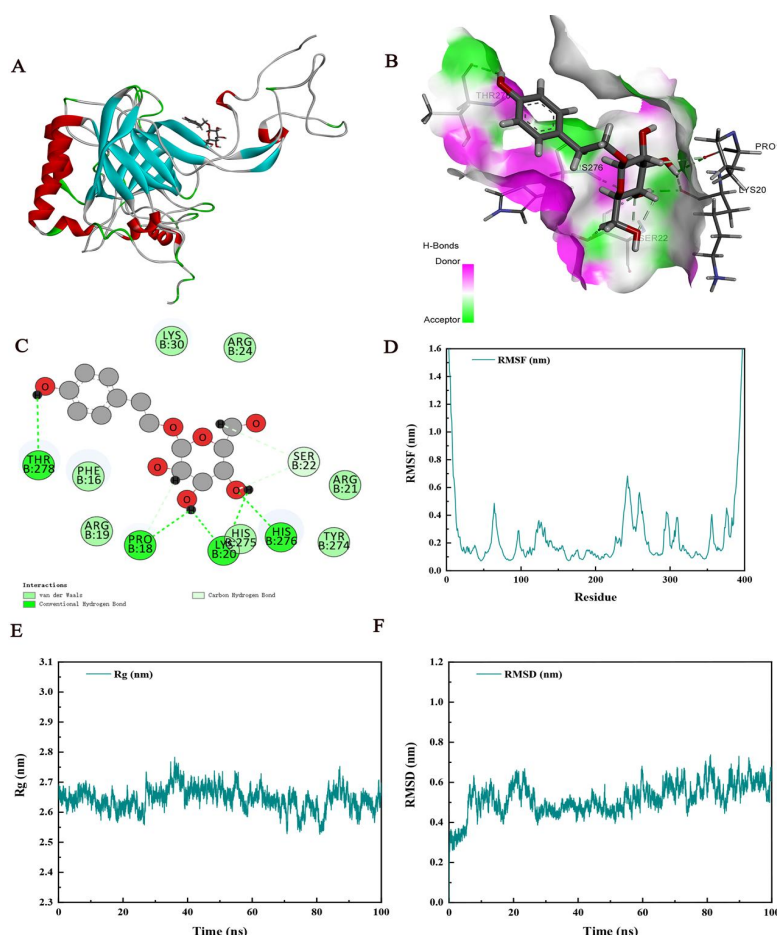


Figure 4 Molecular docking and dynamics analyses of the salidroside–RPL3 complex.

(A) Overall structure of the protein-ligand complex. (B) Binding mode of the ligand in the protein pocket, with key interacting residues and hydrogen bond properties shown. (C) 2D diagram of ligand-receptor non-covalent interactions. (D) RMSF profile of the protein residues over 100 ns simulation. (E) Time evolution of the radius of gyration (Rg) of the protein. (F) RMSD plot of the protein backbone during 100 ns molecular dynamics simulation.

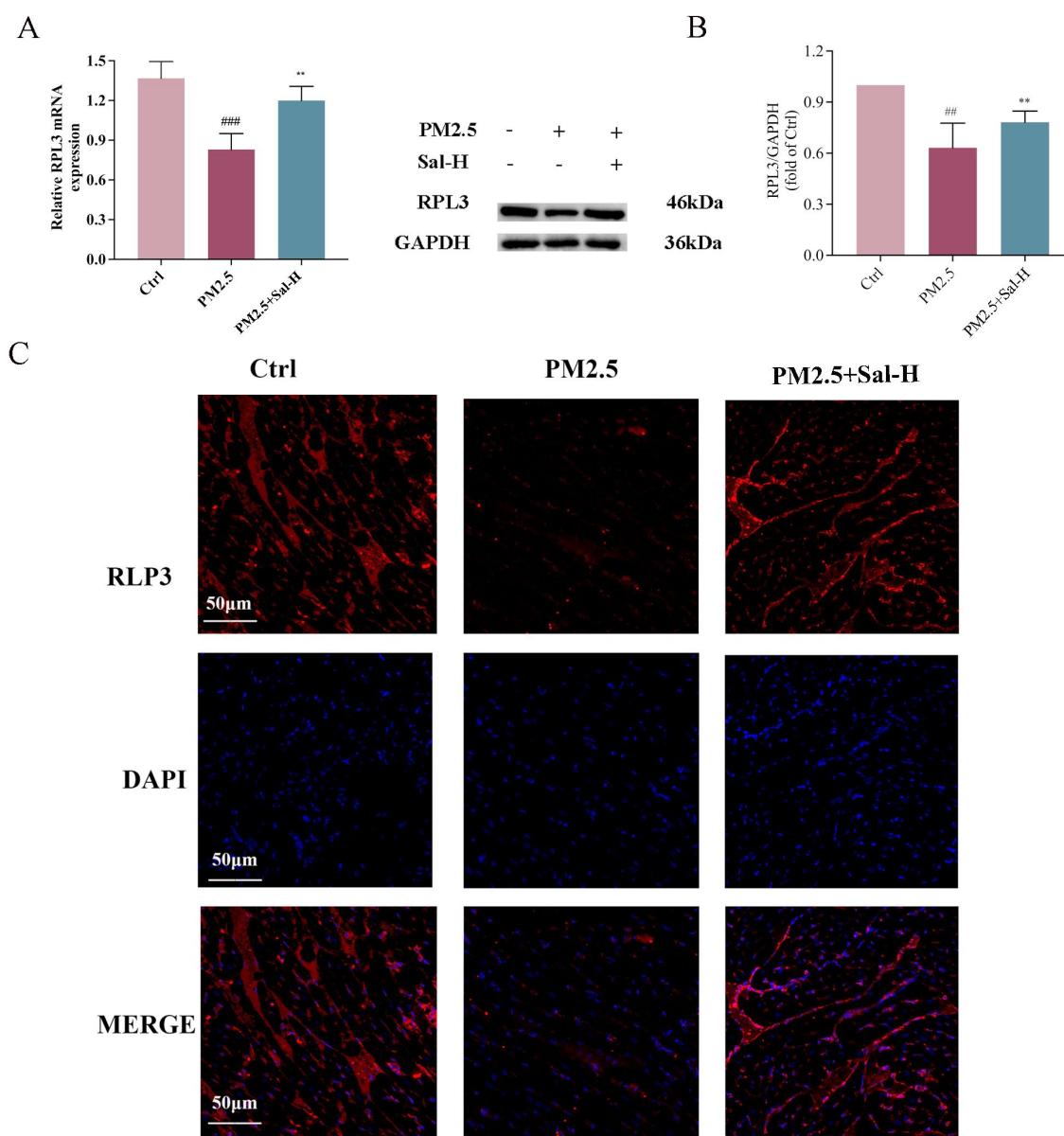


Figure 5 Salidroside may attenuate PM_{2.5}-induced myocardial injury, along with elevated RPL3 expression.

(A) Relative mRNA expression of *RPL3* detected by qPCR. (B) Representative western-blot bands and quantitative analysis of RPL3 protein level, with GAPDH as internal reference. (C) Representative immunofluorescence images for RPL3 (red) and DAPI-stained nuclei (blue). Scale bar: 50 μ m. $n = 10$ per group. Data are presented as mean $\pm$ SEM. ^{###} $P < 0.001$, ^{##} $P < 0.01$ versus Ctrl group; ^{*} $P < 0.05$ versus PM_{2.5} group. Groups: Ctrl, control; PM_{2.5}, PM_{2.5} exposure; PM_{2.5}+Sal-H, PM_{2.5} plus high-dose salidroside.

Sal alleviated PM_{2.5}-induced myocardial injury *in vitro*, accompanied by restored *RPL3* expression

The *in vivo* findings described above suggest that salidroside (Sal) alleviates PM_{2.5}-induced myocardial injury, and that the upregulation of *RPL3* expression is associated with this protective effect. To further investigate this observation and explore the associated cellular mechanisms, additional *in vitro* experiments were performed. The protective effects of Sal were first evaluated by assessing oxidative stress-related indicators, including NO and ROS, in PM_{2.5}-exposed H9C2 cells (Figure 6A–6C). Compared with the control group, PM_{2.5} exposure significantly increased intracellular NO and ROS levels. In contrast, Sal treatment markedly reduced NO and ROS production to levels comparable to those in the control cells, suggesting that PM_{2.5}-induced oxidative stress was attenuated. These results suggest

that Sal may protect cardiomyocytes against PM_{2.5}-induced oxidative injury, although whether *RPL3* is directly required for this effect remains to be determined by loss-of-function experiments.

Furthermore, transcriptomic findings were integrated with subsequent quantitative PCR (Figure 6D), western blot (Figure 6E) and immunofluorescence analyses (Figure 6F) to verify changes in *RPL3* expression in H9C2 cells. Consistent with the *in vivo* results, PM_{2.5} exposure significantly suppressed *RPL3* expression at both the mRNA and protein levels, whereas Sal treatment effectively restored *RPL3* expression. Together, these results provide further evidence that the protective effect of Sal against PM_{2.5}-induced myocardial injury is associated with upregulated *RPL3* expression and alleviation of oxidative stress at the cellular level.

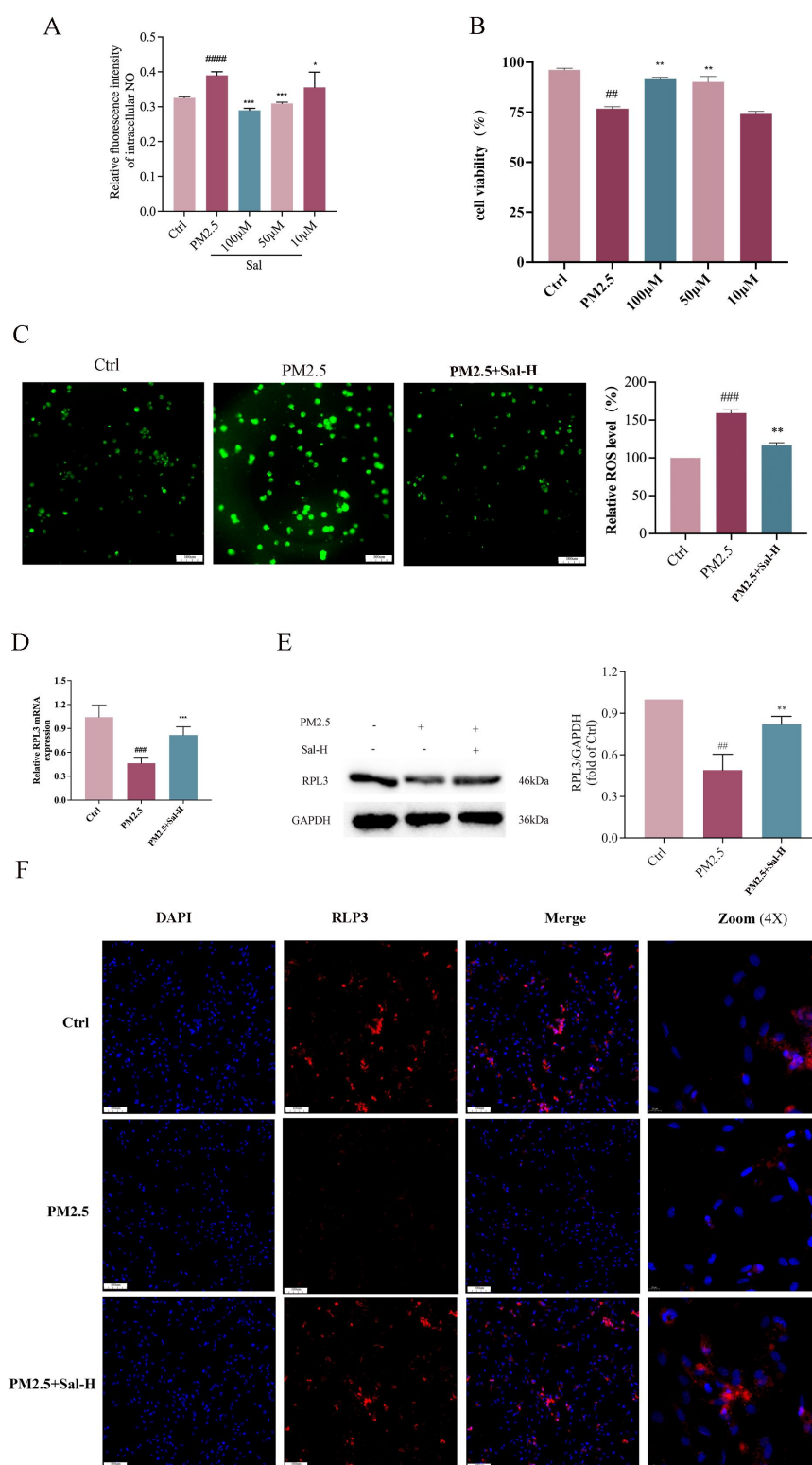


Figure 6 Salidroside regulated NO-associated fluorescence, cell viability, ROS production and RPL3 expression in H9C2 cardiomyocytes exposed to PM_{2.5} *in vitro*.

(A) NO concentration detected in H9C2 cells. (B) Cell viability was measured using a CCK-8 assay after treatment with different concentrations of salidroside. (C) Representative fluorescence images and quantitative analysis of cellular ROS level. (D) Relative RPL3 mRNA expression measured by qPCR. (E) Western-blot bands and quantitative analysis of RPL3 protein. (F) Immunofluorescence staining of RPL3 (red), DAPI-stained nuclei (blue), merged images and 4-fold zoomed view. Scale bar: 100 μm. n = 3 per group. Data are presented as mean ± SEM. #### $P < 0.0001$, ### $P < 0.001$, ## $P < 0.01$, versus Ctrl group; *** $P < 0.0001$, ** $P < 0.01$ versus PM_{2.5} group. Groups: Ctrl, control; PM_{2.5}, PM_{2.5} exposure; Sal-H, PM_{2.5} plus high-dose salidroside.

Discussion

Fine particulate matter exposure has increasingly been recognized as a major environmental risk factor contributing to elevated cardiovascular morbidity and mortality worldwide [26, 27]. In this study, we employed integrated *in vivo* and *in vitro* approaches to systematically evaluate the cardiotoxic effects of PM_{2.5} and the potential protective role of salidroside. Our results demonstrate that PM_{2.5} exposure induces pronounced myocardial dysfunction and histopathological damage, accompanied by enhanced oxidative stress, metabolic disturbance, and inflammatory responses. Notably, Sal intervention effectively ameliorated these pathological alterations, indicating its potential protective effects against PM_{2.5}-associated cardiac injury. Further mechanistic investigations revealed that RPL3 may serve as a key molecular target involved in the cardioprotective effects of Sal.

PM_{2.5}-induced cardiac injury represents a complex pathological process involving multiple interacting mechanisms, including oxidative stress, inflammatory responses, hypoxic conditions, and disturbances in energy metabolism [28, 29]. Consistent with previous epidemiological and experimental studies, PM_{2.5} exposure in the present study resulted in marked impairment of cardiac function, disruption of myocardial structure, and elevated levels of systemic pro-inflammatory cytokines [30, 31]. Further blood gas analysis revealed decreased PaO₂, increased PaCO₂, reduced arterial pH, and elevated lactate levels following PM_{2.5} exposure, indicating compromised oxygen delivery and a metabolic shift toward anaerobic glycolysis. These alterations have been reported to increase myocardial workload and thereby exacerbate cardiac injury. In addition, our results demonstrated that IL-10 level was markedly decreased in the model group, which reflected impaired anti-inflammatory compensation and insufficient capacity to counteract tissue injury mediated by pro-inflammatory cytokines including TNF- α and IL-1 β . After salidroside intervention, the upregulated expression of IL-10 indicated that salidroside could restore anti-inflammatory compensation by elevating IL-10, thereby alleviating PM_{2.5} triggered inflammatory injury. Notably, Sal intervention significantly ameliorated these abnormalities in blood gas and metabolic parameters, suggesting that Sal may alleviate PM_{2.5}-induced myocardial stress and injury by improving systemic oxygenation, reducing oxidative burden, and suppressing excessive inflammatory responses.

This study suggests that RPL3 is potentially associated with PM_{2.5}-induced myocardial injury. Ribosomal proteins have traditionally been regarded as structural components involved in protein synthesis; however, increasing evidence indicates that they also exert important noncanonical functions, including regulation of cellular stress responses and maintenance of intracellular homeostasis under adverse environmental conditions [21]. Transcriptomic analysis in the present study revealed that RPL3 expression was markedly downregulated in myocardial tissues following PM_{2.5} exposure, whereas Sal treatment significantly restored its expression. These findings were consistently validated by quantitative PCR and immunofluorescence analyses in both cardiac tissues and H9C2 cardiomyocytes, demonstrating that PM_{2.5}-induced suppression of RPL3 expression represents a stable and reproducible molecular event that can be effectively reversed by Sal intervention.

Under PM_{2.5}-induced stress conditions, restoration of RPL3 expression by Sal may contribute to enhanced adaptive capacity of cardiomyocytes. RPL3 has been implicated in cellular stress responses, including the regulation of autophagy under nucleolar stress [32]. Whether the reduction in RPL3 expression observed in the present study contributes to oxidative stress and inflammatory injury in cardiomyocytes remains to be determined. In this context, Sal-mediated upregulation of RPL3 may help stabilize intracellular homeostasis and preserve essential cellular functions, ultimately exerting cardioprotective effects during PM_{2.5} exposure and

contributing to the maintenance of myocardial structural and functional integrity. Collectively, these findings further expand the current understanding of ribosomal protein-mediated mechanisms involved in environmental pollutant-induced cardiovascular injury.

To further support the potential mechanistic link between Sal and RPL3, molecular docking and molecular dynamics (MD) simulations were performed. Computational analyses indicated a favorable binding affinity between Sal and RPL3, with the binding conformation stabilized by multiple hydrogen bonds and predominantly driven by van der Waals interactions. Subsequent MD simulations demonstrated that the protein-ligand complex maintained stable structural characteristics throughout the simulation, as reflected by convergent root-mean-square deviation (RMSD) values, reduced radius of gyration, limited residue fluctuations, and persistent hydrogen-bond interactions. MM/PBSA analysis further confirmed a negative binding free energy, indicating a thermodynamically favorable interaction between Sal and RPL3. Although computational approaches cannot replace direct experimental validation, these findings provide important complementary structural and energetic evidence supporting a potential direct interaction between Sal and RPL3.

Accumulating evidence reveals that Sal confers cardioprotection by suppressing oxidative stress, inflammation and ferroptosis [33, 34]. Nevertheless, how Sal coordinates these multiple protective programs remains incompletely understood. In this work, we tentatively identify RPL3 as a candidate target of Sal in cardiomyocytes. RPL3 may not represent an independent parallel pathway; instead, it is potentially involved in upstream signaling upon Sal stimulation. Upregulation of RPL3 may, in turn, contribute to the activation of downstream antioxidant, anti-inflammatory and anti-ferroptosis responses, thereby alleviating PM_{2.5}-induced cardiac injury. Future investigations are warranted to uncover the direct molecular interactions between RPL3 and key components of these canonical pathways.

Several limitations of the present study should be acknowledged. First, although our findings strongly suggest the involvement of RPL3 in the cardioprotective effects mediated by Sal, direct genetic manipulation of RPL3 (such as knockdown or overexpression) was not performed to establish a causal relationship. Second, the downstream signaling pathways linking RPL3 regulation to oxidative stress, energy metabolism, and inflammatory responses remain to be fully elucidated and warrant further investigation. Future studies incorporating targeted genetic approaches and pathway-specific analyses are required to more comprehensively define the role of RPL3 in PM_{2.5}-associated cardiovascular injury and its underlying molecular mechanisms. Despite these limitations, the integration of *in vivo* and *in vitro* experimental data with computational simulations in this study provides a solid theoretical and experimental foundation for further exploration of RPL3 as a potential molecular target in PM_{2.5}-related cardiovascular damage.

Conclusion

In conclusion, the present study shows that Sal alleviates PM_{2.5}-induced myocardial injury, an effect accompanied by the restoration of RPL3 expression and by partial reductions in oxidative stress, inflammatory responses, and metabolic disturbances. Although the present data do not establish a causal role for RPL3, they identify RPL3 as a potential mediator in PM_{2.5}-associated cardiac injury and provide clues toward understanding the cardioprotective effects of Sal. From an environmental health perspective, our results suggest that intracellular homeostasis regulators such as RPL3 warrant further investigation as candidate targets for mitigating the adverse cardiovascular effects of environmental PM_{2.5} exposure, and that their functional contribution should be tested directly in future studies using loss-of-function and gain-of-function approaches.

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