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A Shenling Baizhu San-derived defined-component
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Abstract

Background: Cigarette smoke exposure is a major cause of chronic airway inflammation. Although smoking cessation remains the cornerstone intervention, established inflammatory and epithelial alterations may persist in some individuals. Shenling Baizhu San (SLBZS), a multi-component herbal formula, has demonstrated anti-inflammatory potential in experimental studies, but its complex composition limits standardization and mechanistic interpretation. This study evaluated the effects of a SLBZS-derived defined-component formula on cigarette smoke-induced airway inflammation in rats and preliminarily explored its association with p-p38/p-p53-related responses. **Methods:** Seventy 8-week-old male Sprague-Dawley rats weighing 220–250 g were randomly divided into seven groups (n = 10 per group): control, model, positive control (dexamethasone, 0.5 mg/kg/48 h, intraperitoneal injection), commercial SLBZS preparation (10 mL/kg/day, gavage), and low-, medium-, and high-dose defined-component formula groups (total mixture doses of approximately 3.29, 6.58, and 13.16 mg/kg/day, respectively, by gavage). The defined-component formula consisted of seven purified compounds: ginsenoside Rb1, atractylenolide III, pachymic acid, platycodin D, batatasin III, glycyrrhizic acid, and liquiritigenin. Except for the

control group, rats were exposed to cigarette smoke for 120 min daily for 8 consecutive weeks. Levels of inflammatory cytokines and chemokines, including IL-1 β , IL-6, TNF- α , CXCL1, and CCL2, as well as oxidative stress-related indicators, including 8-iso-PGF2 α , MDA, and SOD activity, were measured in lung tissue homogenates using commercial assay kits. p-p38 and p-p53 protein levels in lung tissue were detected by Western blot, and lung histopathology was examined by hematoxylin and eosin staining. **Results:** Compared with the control group, the model group showed increased levels of IL-1 β , IL-6, TNF- α , CXCL1, CCL2, and 8-iso-PGF2 α in lung tissue homogenates, decreased SOD activity, and increased p-p38 and p-p53 protein levels in lung tissue ($P < 0.05$). MDA levels did not differ significantly among groups. Compared with the model group, medium and high doses of the defined-component formula reduced IL-1 β , IL-6, TNF- α , CXCL1, CCL2, and 8-iso-PGF2 α levels, restored SOD activity, and decreased p-p38 and p-p53 protein levels ($P < 0.05$). Histopathological examination showed that the defined-component formula alleviated smoke-induced lung structural damage and inflammatory cell infiltration. The medium- and high-dose DCF groups showed improvement trends broadly similar to those observed in the commercial SLBZS preparation and dexamethasone groups. **Conclusion:** The SLBZS-derived defined-component formula attenuated cigarette smoke-induced airway inflammation in rats, as evidenced by reduced inflammatory cytokine and chemokine levels, improved lung histopathology, and restored SOD activity. The decrease in p-p38 and p-p53 levels suggests that a p-p38/p-p53-related mechanism may be involved. Because pathway-specific interventions were not performed, the mechanistic conclusion should be interpreted as exploratory.

Keywords: Shenling Baizhu San; defined-component formula; cigarette smoke; airway inflammation; p-p38/p-p53; rat

1 Background

Cigarette smoking is a major cause of chronic airway inflammation and remains an important contributor to respiratory disease burden [1, 2]. Long-term cigarette smoke exposure damages the airway epithelium and promotes oxidative and inflammatory responses, thereby contributing to persistent pathological changes in the airways [3–5]. These pathological changes are closely associated with the development and progression of chronic respiratory diseases.

Smoking cessation is the most important intervention for smoke-related airway injury. However, cessation alone may not fully reverse established inflammatory and epithelial abnormalities. Persistent smoke-related airway epithelial alterations and dysregulated gene expression have been reported in smokers and patients with COPD, suggesting that adjunctive interventions targeting residual inflammation may still be needed [6, 7].

Current pharmacological anti-inflammatory strategies have limitations in the context of chronic smoke-induced airway inflammation. Inhaled corticosteroids are widely used in selected chronic airway diseases, but their long-term application requires careful evaluation, especially in populations without clear steroid-responsive phenotypes

[8, 9]. Therefore, new interventions with clearer composition, acceptable safety, and anti-inflammatory potential remain worthy of investigation.

Previous experimental studies have provided increasing evidence supporting the anti-inflammatory, antioxidant, and tissue-protective effects of Shenling Baizhu San (SLBZS) in pulmonary inflammatory disorders. In a cigarette smoke extract (CSE)-induced COPD cellular model, Gao et al. identified serum-absorbed components of SLBZS using UPLC-Q-TOF-MS/MS and demonstrated that SLBZS-containing serum significantly reduced inflammatory mediator production and cellular injury in vitro. Mechanistically, SLBZS-containing serum regulated the TLR9/MyD88/TRAF6/NF- κ B/I κ B α signaling pathway, suggesting that modulation of smoke-related inflammatory signaling may contribute to its protective effects against COPD-associated airway injury [10].

In addition to COPD-related cellular evidence, the pulmonary and airway protective effects of SLBZS have been investigated in several animal models of respiratory inflammation and injury. In a murine model of *Streptococcus pneumoniae*-induced pneumonia, SLBZS administration reduced pro-inflammatory cytokine production and alleviated histopathological lung injury, indicating its capacity to suppress excessive inflammatory responses and preserve pulmonary tissue integrity [11]. Furthermore, in a murine model of bronchial asthma, SLBZS treatment alleviated airway inflammatory responses by regulating Th17/Treg immune balance, accompanied by modulation of inflammatory cytokine profiles, suggesting its broader immunomodulatory effects in respiratory inflammatory conditions [12].

Oxidative stress represents another critical pathological process involved in cigarette smoke-induced airway injury. Recent animal evidence has suggested that SLBZS may protect pulmonary tissues against oxidative stress-associated damage by enhancing antioxidant defense pathways. In a rat model of obstructive sleep apnea-hypopnea syndrome induced by chronic intermittent hypoxia, SLBZS attenuated lung injury through activation of the Nrf2/HO-1 signaling pathway, restoration of antioxidant enzyme activity, and reduction of oxidative damage markers [13]. Although the pathological contexts differ among these models, these experimental findings collectively indicate that SLBZS possesses anti-inflammatory, antioxidant, and tissue-protective properties that overlap with the major pathological processes involved in cigarette smoke-induced airway inflammation, including excessive inflammatory activation and oxidative stress.

Experimental studies of several representative constituents of SLBZS provide further support for their potential relevance to pulmonary inflammatory and oxidative injury. Ginsenoside Rb1 attenuated cigarette smoke extract-induced inflammatory responses, oxidative stress, apoptosis, and lung injury in cellular and rat COPD models, accompanied by activation of Nrf2 and inhibition of NF- κ B signaling [14]. Platycodin D also reduced cigarette smoke-induced lung inflammation in mice [15]. In other experimental airway or pulmonary inflammatory models, atractylenolide III inhibited NLRP3 inflammasome activation and improved Th1/Th2 imbalance in bronchial epithelial cells and ovalbumin-induced asthmatic mice [16]; pachymic acid reduced inflammatory and apoptotic responses in an LPS-induced rat pneumonia model through NF- κ B- and MAPK-related regulation [17]; and glycyrrhizic acid

139 exerted anti-inflammatory and immunoregulatory effects in ovalbumin-sensitized mice
140 [18]. Collectively, these findings indicate that SLBZS and several of its representa-
141 tive constituents can modulate inflammatory, oxidative, and tissue-injury responses
142 relevant to cigarette smoke-induced pulmonary damage.

143 However, SLBZS is a chemically complex preparation containing multiple herbal
144 materials, which creates challenges for component standardization, experimental
145 reproducibility, and mechanistic interpretation. To improve component definition
146 while retaining selected multi-component characteristics of SLBZS, we constructed a
147 defined-component formula consisting of seven purified compounds: ginsenoside Rb1,
148 atractylenolide III, pachymic acid, platycodin D, batatasin III, glycyrrhizic acid, and
149 liquiritigenin. These compounds were selected as representative constituents of the
150 component herbs of SLBZS based on their chemical characterization and reported
151 pharmacological activities related to inflammation, oxidative stress, and tissue protec-
152 tion. The pharmacopoeial composition of SLBZS provided the quantitative framework
153 for constructing the formula, whereas the specific proportional design is described in
154 the Materials and Methods. However, whether a chemically defined SLBZS-derived
155 formula can reproduce the protective effects of SLBZS against chronic cigarette
156 smoke-induced airway inflammation *in vivo* remains unknown.

157 In addition to inflammatory and oxidative stress pathways, cigarette smoke expo-
158 sure can activate cellular stress-response signaling networks involved in airway injury.
159 Cigarette smoke or cigarette smoke extract has been associated with p38 MAPK acti-
160 vation in stress-related cellular responses and COPD-related inflammation, as well as
161 with p53-related stress responses [19–21]. Therefore, p-p38 and p-p53 were selected as
162 exploratory mechanistic indicators. This study aimed to evaluate the protective effects
163 of the SLBZS-derived defined-component formula against cigarette smoke-induced air-
164 way inflammation in rats and to preliminarily assess whether p-p38/p-p53-related
165 responses may be involved.

166

167 2 Materials and methods

168

169 2.1 Animals and experimental grouping

170

171 Seventy 8-week-old specific-pathogen-free male Sprague-Dawley rats weighing 220–
172 250 g were obtained from Beijing Huafukang Bioscience Co., Ltd. (animal production
173 license No. SCXK (Beijing) 2024-0003). Animals were housed in an SPF facility under
174 a 12 h light/dark cycle at $22 \pm 2^\circ\text{C}$ and 40–60% relative humidity, with free access
175 to standard laboratory chow and water. Rats were housed in groups of five animals
176 per cage, with two cages assigned to each experimental group. Cages were equipped
177 with standard bedding and ventilation pipes. After one week of acclimation, the rats
178 were randomly divided into seven groups ($n = 10$ per group): control group (CON),
179 model group (MOD), dexamethasone group (DEX), commercial SLBZS preparation
180 group (SLBZS), and SLBZS-derived defined-component formula low-dose (DCF-L),
181 medium-dose (DCF-M), and high-dose (DCF-H) groups.

182 The experimental unit was an individual rat. No formal *a priori* sample size cal-
183 culation was performed. The sample size of 10 rats per group was determined based
184 on previous similar animal experiments, feasibility considerations, and the need to

ensure sufficient biological replication. After acclimation, rats were allocated to different groups using a random number table. Animals were included if they met the required strain, sex, health status, and body weight criteria before randomisation. No animals or data points were excluded from the final analysis.

The primary outcomes were inflammatory cytokine and chemokine levels in lung tissue homogenates, including IL-1 β , IL-6, TNF- α , CXCL1, and CCL2. Secondary outcomes included lung histopathological changes, oxidative stress-related markers, and p-p38/p-p53 protein expression.

Animals were monitored daily for general condition, activity, food and water intake, respiratory status, body condition, and signs of distress. Humane endpoints included severe dyspnea, persistent inability to access food or water, marked lethargy, or severe body weight loss. No unexpected adverse events occurred during the experiment.

All animal procedures were reviewed and approved by the Ethics Committee of Zunyi Hospital of Traditional Chinese Medicine (approval No. HS-2025-172), but the animal study protocol was not prospectively registered in a public registry. All procedures were conducted in accordance with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The experimental workflow is summarized in Fig. 1.

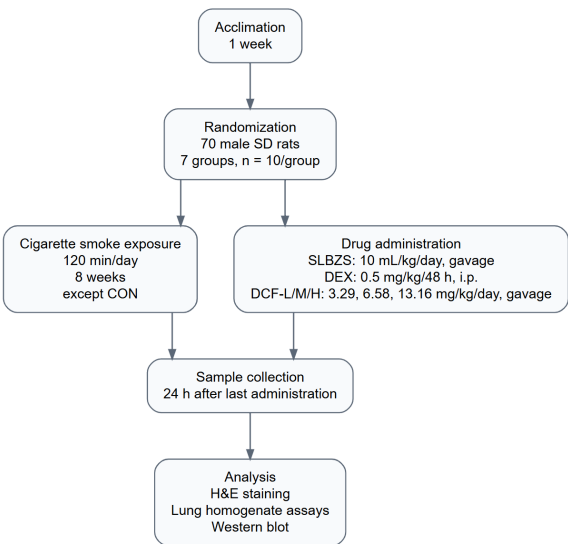


Fig. 1 Experimental workflow of cigarette smoke exposure, drug administration, sample collection, and outcome analysis.

2.2 Cigarette smoke exposure model

Except for the CON group, rats were exposed to cigarette smoke in a self-made plexiglass chamber with a volume of approximately 60 L. Huangguoshu brand cigarettes

(China Tobacco Guizhou Industrial Co., Ltd.; tar: 10 mg/cigarette; nicotine: 0.9 mg/cigarette; carbon monoxide: 12 mg/cigarette) were used. Ten cigarettes were lit simultaneously for each exposure session, and rats received passive smoke inhalation for 120 min once daily for 8 consecutive weeks.

2.3 Drugs and administration

2.3.1 Composition and design of the defined-component formula

The SLBZS-derived defined-component formula consisted of seven reference-standard compounds: ginsenoside Rb1 (CAS 41753-43-9), atractylenolide III (CAS 73030-71-4), pachymic acid (CAS 29070-92-6), platycodin D (CAS 58479-68-8), batatasin III (CAS 56684-87-8), glycyrrhizic acid (CAS 1405-86-3), and liquiritigenin (CAS 578-86-9). All compounds were purchased from Chengdu Mansite Bio-Technology Co., Ltd. (Chengdu, China) with a purity of $\geq 98\%$ as determined by HPLC.

The seven compounds were selected as representative constituents of the component herbal materials of SLBZS based on published chemical and pharmacological information. Their relative mass ratio was established with reference to the reported proportions of the corresponding herbal materials in SLBZS, available pharmacopoeial specifications, and published chemical-content information [22, 23]. The resulting ratio was intended to approximate the estimated relative abundance pattern of these constituents in the formula and did not represent direct quantitative analysis of the commercial SLBZS batch used in this study.

2.3.2 Dosage and administration

The seven-component mixture was dissolved or suspended in 0.9% saline and administered by gavage once daily for 8 weeks. The low-dose group received the basal total dose of approximately 3.29 mg/kg/day. The medium- and high-dose groups received two- and four-fold the basal total dose, corresponding to approximately 6.58 and 13.16 mg/kg/day, respectively.

Table 1 Individual component doses in the basal defined-component formula.

Component	Dose in basal formula (mg/kg/day)
Ginsenoside Rb1	0.71875
Atractylenolide III	0.71875
Pachymic acid	0.575
Platycodin D	0.14375
Batatasin III	0.503125
Glycyrrhizic acid	0.0575
Liquiritigenin	0.575

The commercial SLBZS preparation used as the conventional-formula comparator was Shenling Baizhu granules manufactured by Beijing Tongrentang Pharmaceutical Co., Ltd. (Beijing, China; approval No. Z11020564; batch No. 24111035; 6 g per sachet). According to the manufacturer’s product information, the preparation contained ten herbal ingredients. Their scientific source names were identified according to the Chinese Pharmacopoeia, and the processing forms stated on the product label are provided in Table 2 [22]. The excipients were sucrose and dextrin. The granules were dispersed in warm water immediately before administration and administered by gavage at 10 mL/kg/day, corresponding to 1.89 g/kg/day.

Table 2 Scientific source names of the herbal ingredients contained in the commercial Shenling Baizhu granules.

Herbal ingredient	Scientific source name	Processing form
Ginseng (Renshen)	<i>Panax ginseng</i> C.A.Mey.	Not specified
Poria (Fuling)	<i>Wolfiporia cocos</i> (F.A.Wolf) Ryvarden & Gilb. [syn. <i>Poria cocos</i> (Schwein.) F.A.Wolf]	Not specified
Atractylodes (Baizhu)	<i>Atractylodes macrocephala</i> Koidz.	Bran-fried
Chinese yam (Shanyao)	<i>Dioscorea opposita</i> Thunb.	Not specified
White hyacinth bean (Baibiandou)	<i>Dolichos lablab</i> L. [syn. <i>Lablab purpureus</i> (L.) Sweet]	Stir-fried
Lotus seed (Lianzi)	<i>Nelumbo nucifera</i> Gaertn.	Not specified
Coix seed (Yiyiren)	<i>Coix lacryma-jobi</i> L. var. <i>ma-yuen</i> (Rom.Caill.) Stapf	Bran-fried
Amomi fruit (Sharen)	<i>Amomum villosum</i> Lour.; <i>Amomum villosum</i> Lour. var. <i>xanthioides</i> T.L.Wu & Senjen; or <i>Amomum longiligulare</i> T.L.Wu	Not specified
Platycodon root (Jiegeng)	<i>Platycodon grandiflorus</i> (Jacq.) A.DC.	Not specified
Licorice (Gancao)	<i>Glycyrrhiza uralensis</i> Fisch. ex DC.; <i>Glycyrrhiza inflata</i> Batalin; or <i>Glycyrrhiza glabra</i> L.	Not specified

Note: For Sharen and Gancao, the manufacturer’s product information did not specify the batch-specific source species. Therefore, all pharmacopoeially permitted source species are listed.

Dexamethasone sodium phosphate injection (Hainan Better Pharmaceuticals Co., Ltd., approval No. H32021561, 1 mL:5 mg) was administered intraperitoneally at 0.5 mg/kg once every 48 h for 8 weeks. The CON and MOD groups received an equal volume of saline by gavage.

2.4 Sample collection

Twenty-four hours after the last administration, rats were euthanized by intraperitoneal injection of an overdose of sodium pentobarbital (150 mg/kg). Right lung tissues were rapidly collected. A portion of the right lung was fixed in 4% paraformaldehyde for histopathological examination, and the remaining tissue was snap-frozen in liquid nitrogen and stored at -80°C for subsequent lung tissue homogenate assays and Western blot analysis.

2.5 Measurement of inflammatory and oxidative stress markers

Lung tissue homogenates were prepared according to the instructions of the corresponding assay kits. The levels of IL-1 β , IL-6, TNF- α , CXCL1, CCL2, and 8-iso-PGF2 α in lung tissue homogenates were measured using commercial rat ELISA kits purchased from Jiangsu Jingmei Biotechnology Co., Ltd. (China). Briefly, samples or standards were added to antibody-precoated microplate wells, followed by HRP-labeled detection antibody incubation, washing, TMB color development, and reaction termination. The optical density was measured at 450 nm, and the concentrations were calculated according to standard curves.

MDA levels were determined using a commercial microplate thiobarbituric acid assay kit (ADS-W-YH002, lot No. ADS20260224; Jiangsu Aidisheng Biotechnology Co., Ltd., China). SOD activity was measured using a commercial microplate WST-8-based SOD assay kit (ADS-W-KY011, lot No. ADS20260224; Jiangsu Aidisheng Biotechnology Co., Ltd., China). All procedures were performed according to the manufacturers' instructions.

For biochemical assays, including ELISA, MDA, and SOD measurements, n = 10 animals per group were included.

Table 3 Commercial assay kits used in this study.

Marker	Method	Manufacturer	Catalog number	Lot number
IL-1 β	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J2923-A	202602
IL-6	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J3066-A	202602
TNF- α	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J3056-A	202602
CXCL1	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J40040-A	202602
CCL2	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J2975-A	202602
8-iso-PGF2 α	Rat ELISA kit	Jiangsu Jingmei Biotechnology Co., Ltd., China	J9283-A	202602
MDA	Microplate TBA assay	Jiangsu Aidisheng Biotechnology Co., Ltd., China	ADS-W-YH002	ADS20260224
SOD	Microplate WST-8 assay	Jiangsu Aidisheng Biotechnology Co., Ltd., China	ADS-W-KY011	ADS20260224

2.6 Western blot analysis

Total protein was extracted from lung tissues using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Protein concentrations were determined using a BCA protein assay kit (P0012, Beyotime, China). Equal amounts of protein were separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% non-fat milk for 1 h at room temperature, the membranes were incubated overnight at 4°C with primary antibodies against p-p53 (Phospho-P53 Ser15, 28961-1-AP, Proteintech, China), p-p38 (Phospho-p38 MAPK-Y182, AP0057, ABclonal, China), and β -actin (AC026, ABclonal, China). After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Protein bands were visualized using an ECL chemiluminescence system and quantified using ImageJ software. The relative levels of p-p38 and p-p53 were normalized to β -actin. For Western blot analysis, $n = 3$ biological replicates per group were included.

2.7 Histopathological examination

Right lung tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated in graded ethanol, embedded in paraffin, and sectioned at 5 μ m thickness. Sections were stained with hematoxylin and eosin (HE) and examined under a light microscope (Olympus, Tokyo, Japan). Inflammatory cell infiltration and alveolar structural damage were assessed by a pathologist blinded to the group assignments.

2.8 Statistical analysis

Statistical analyses were performed using SPSS 26.0 (IBM, Armonk, NY, USA). Data are expressed as mean $\pm$ standard deviation (SD). Normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. For data that followed a normal distribution with homogeneous variances, one-way analysis of variance followed by Tukey's HSD post-hoc test was used. For data that did not meet the assumptions of normality or homogeneity of variances, the Kruskal-Wallis H test followed by Dunn's post-hoc test with Bonferroni correction was applied. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

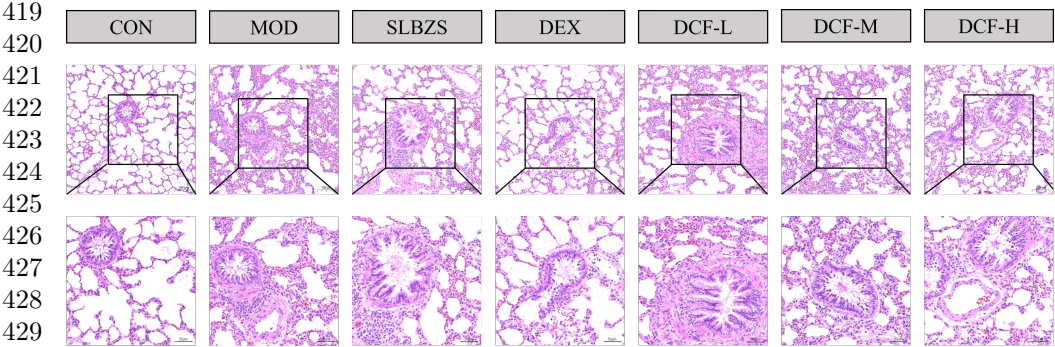
3 Results

3.1 The defined-component formula alleviated cigarette smoke-induced lung histopathological injury

H&E staining showed that the CON group had intact lung tissue structure, clear alveolar walls, and no obvious inflammatory cell infiltration (Fig. 2). In contrast, the MOD group showed marked histopathological injury, including alveolar wall thickening, reduced alveolar spaces, and extensive inflammatory cell infiltration in the peribronchial and interstitial regions. Compared with the MOD group, the DEX, SLBZS, and defined-component formula groups showed varying degrees of histopathological improvement. The DCF-M and DCF-H groups showed more evident amelioration,

415 characterized by relatively preserved alveolar structure and reduced inflammatory cell
 416 infiltration. Because no quantitative histological score was obtained, these observations
 417 should be interpreted as morphological evidence supporting the biochemical findings.

418



430

431 **Fig. 2** Representative H&E staining images of lung tissues from each group. Cigarette smoke
 432 exposure induced lung histopathological injury, while treatment with the SLBZS-derived defined-
 433 component formula alleviated inflammatory cell infiltration and structural damage.

434

435

436 3.2 The defined-component formula reduced inflammatory 437 markers and restored SOD activity in lung tissue 438 homogenates 439

440 Cigarette smoke exposure significantly increased inflammatory cytokine, chemokine,
 441 and oxidative injury-related marker levels in lung tissue homogenates. Compared with
 442 the CON group, the MOD group showed higher levels of IL-1 β , IL-6, TNF- α , CXCL1,
 443 CCL2, and 8-iso-PGF2 α ($P < 0.05$; Fig. 3). Compared with the MOD group, the DEX,
 444 SLBZS, DCF-M, and DCF-H groups showed significantly lower levels of these markers
 445 ($P < 0.05$). The DCF-L group showed a downward tendency for several markers, but
 446 this trend did not consistently reach statistical significance.

447 For oxidative stress-related indicators, no significant differences in MDA levels were
 448 observed among groups ($P > 0.05$; Fig. 3). In contrast, SOD activity was significantly
 449 decreased in the MOD group compared with the CON group ($P < 0.05$). Compared
 450 with the MOD group, SOD activity was significantly increased in the DEX, SLBZS,
 451 DCF-M, and DCF-H groups ($P < 0.05$). These findings suggest that the defined-
 452 component formula, especially at medium and high doses, attenuated cigarette smoke-
 453 induced inflammatory responses, reduced 8-iso-PGF2 α levels, and partially improved
 454 endogenous antioxidant defense.

455

456 3.3 The defined-component formula decreased p-p38 and 457 p-p53 protein levels in lung tissue 458

459 Western blot analysis showed that p-p38 and p-p53 protein levels were significantly
 460 higher in the MOD group than in the CON group ($P < 0.05$; Fig. 4). Compared with

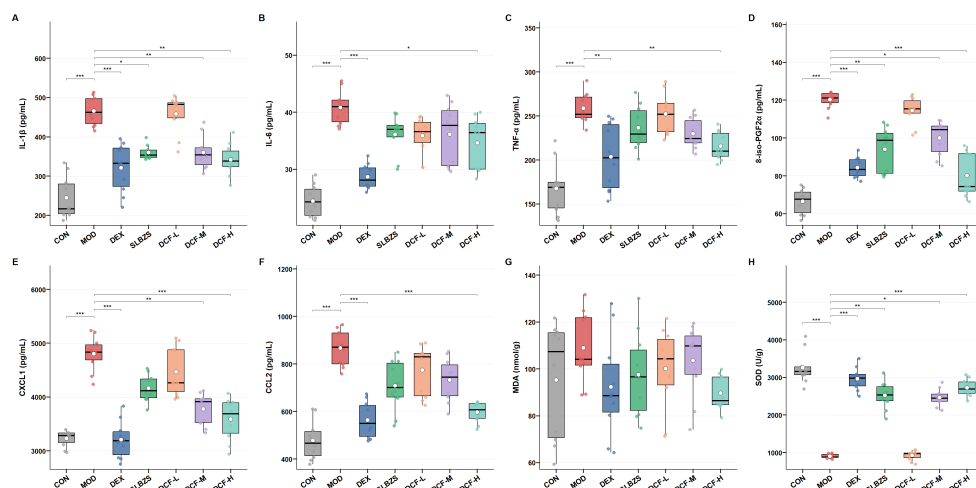


Fig. 3 Effects of the SLBZS-derived defined-component formula on inflammatory and oxidative stress-related markers in lung tissue homogenates. The measured indicators included IL-1 β , IL-6, TNF- α , 8-iso-PGF2 α , CXCL1, CCL2, MDA, and SOD. Data are presented as box-and-whisker plots with individual data points ($n = 10$ animals per group). Statistical comparisons were performed versus the MOD group. * $P < 0.05$.

the MOD group, the DEX, SLBZS, DCF-M, and DCF-H groups showed significantly reduced p-p38 and p-p53 levels ($P < 0.05$). The DCF-L group showed a decreasing trend for p-p38 but did not consistently show significant improvement across both proteins. These results suggest that the protective effect of the defined-component formula was accompanied by downregulation of p-p38 and p-p53-related responses.

4 Discussion

4.1 Principal findings

This study evaluated a SLBZS-derived defined-component formula in a rat model of cigarette smoke-induced airway inflammation. Cigarette smoke exposure induced obvious lung histopathological injury and increased the levels of IL-1 β , IL-6, TNF- α , CXCL1, CCL2, and 8-iso-PGF2 α in lung tissue homogenates. Medium and high doses of the defined-component formula reduced these inflammatory and oxidative injury-related markers and alleviated histopathological damage. The formula also restored SOD activity, whereas MDA levels were not significantly altered. In addition, treatment was accompanied by decreased p-p38 and p-p53 protein levels in lung tissue. These findings indicate that the defined-component formula has anti-inflammatory activity in this model, and that p-p38/p-p53-related responses may be involved.

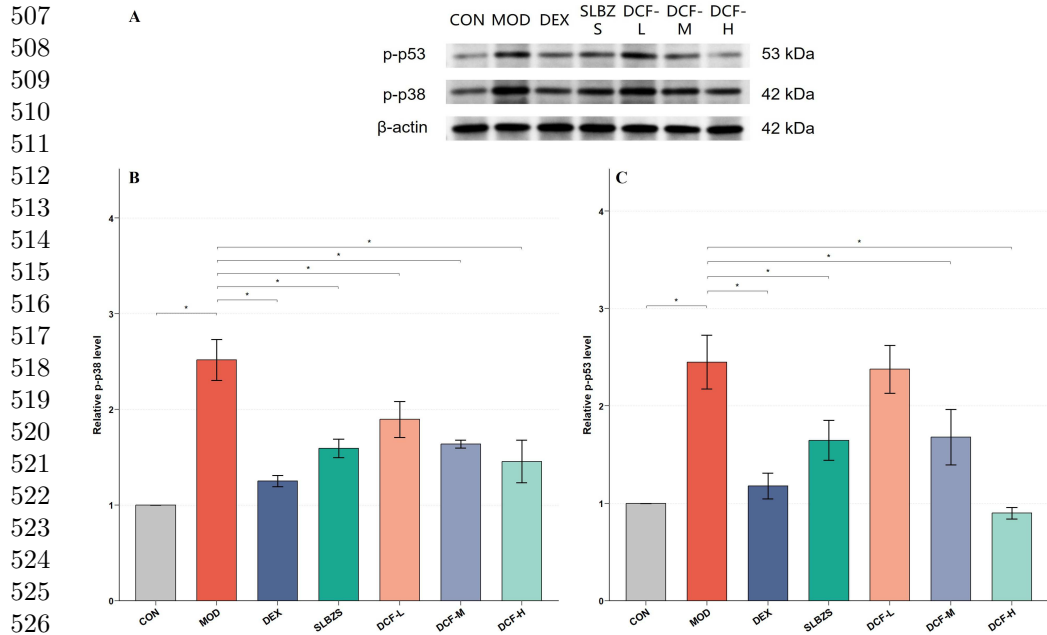


Fig. 4 Effects of the SLBZS-derived defined-component formula on p-p38 and p-p53 protein levels in lung tissue. (A) Representative Western blot bands of p-p53, p-p38, and β -actin. (B, C) Quantitative analysis of p-p38 and p-p53 protein levels. Protein levels were normalized to β -actin and expressed relative to the CON group. Data are presented as mean $\pm$ SD (n = 3 biological replicates per group). *P < 0.05 versus the MOD group.

4.2 Anti-inflammatory effects of the defined-component formula

The MOD group showed elevated levels of IL-1 β , IL-6, TNF- α , CXCL1, and CCL2 in lung tissue homogenates, together with inflammatory cell infiltration and alveolar structural injury, indicating successful establishment of a smoke-induced airway inflammation model. IL-1 β , IL-6, and TNF- α are representative pro-inflammatory cytokines involved in inflammatory amplification, epithelial injury, and immune cell activation. CXCL1 is an important neutrophil-associated chemokine acting through the CXCL1/CXCR2 axis in the lung, whereas CCL2/CCR2 signaling is closely related to macrophage regulation and inflammatory responses in COPD [24, 25]. The reduction of these cytokines and chemokines after treatment suggests that the formula attenuated both inflammatory mediator release and inflammatory cell recruitment.

Several components included in the formula have been reported to possess anti-inflammatory and antioxidant activities [14–18]. However, because this study did not include single-component groups or formal interaction analysis, synergistic effects among the components cannot be concluded. A more appropriate interpretation is that the combined pharmacological activities of these seven defined components may

contribute to the observed anti-inflammatory effect. This wording is also consistent with the exploratory nature of the current study.

4.3 Interpretation of 8-iso-PGF2 α , SOD restoration, and unchanged MDA levels

Oxidative stress is an important mechanism of cigarette smoke-induced airway injury [4, 5]. In the present study, cigarette smoke exposure increased 8-iso-PGF2 α levels and decreased SOD activity, whereas medium and high doses of the defined-component formula reduced 8-iso-PGF2 α levels and restored SOD activity. These findings suggest that the formula may help attenuate smoke-related oxidative injury and recover endogenous enzymatic antioxidant defense. However, MDA levels did not differ significantly among groups. This result indicates that lipid peroxidation-related changes may depend on the selected marker, exposure duration, and sampling time point. Therefore, the antioxidant effect of the formula should be described cautiously, mainly as reduced 8-iso-PGF2 α levels and restored SOD activity rather than broad suppression of all oxidative stress markers.

4.4 p-p38/p-p53-related responses may be involved in the protective effect

The p38 MAPK pathway is closely associated with stress and inflammatory responses related to cigarette smoke exposure and COPD pathogenesis [19, 20]. p53-related signaling is also linked to cigarette smoke-induced cellular stress and injury [21]. In this study, cigarette smoke exposure increased p-p38 and p-p53 protein levels in lung tissues, whereas treatment with the defined-component formula reduced their levels. These findings suggest an association between the protective effect of the formula and downregulation of p-p38/p-p53-related responses.

Nevertheless, the current data do not establish a direct causal relationship. Total p38 and total p53 protein levels were not detected, and no pathway inhibitor, gene knockdown, or rescue experiment was performed. Therefore, it cannot be concluded that the formula exerts its effects exclusively through inhibition of the p38/p53 pathway. The more cautious conclusion is that p-p38 and p-p53 downregulation accompanied the anti-inflammatory effect and may represent one of the mechanisms involved.

4.5 Comparison with the commercial SLBZS preparation and dexamethasone

Traditional SLBZS contains multiple herbal components and may exert multi-target pharmacological effects [23]. However, its complex composition creates challenges for quality control, dose standardization, and mechanistic interpretation. The defined-component formula used in this study was designed to improve compositional clarity while preserving selected pharmacological characteristics of SLBZS. In the present model, the medium- and high-dose defined-component formula groups showed improvement trends similar to those observed in the commercial SLBZS preparation

599 and dexamethasone groups. Because the study was not specifically designed or pow-
600 ered to prove therapeutic equivalence or non-inferiority, these findings should not be
601 interpreted as evidence that the defined-component formula is equivalent or superior
602 to dexamethasone or the commercial SLBZS preparation.

603

604 4.6 Strengths and limitations

605

606 A strength of this study is that it used a defined seven-component formula with clear
607 component composition and dose information, which may improve reproducibility
608 compared with complex herbal mixtures. The study also combined histopathological
609 observation, inflammatory cytokine and chemokine measurement, oxidative stress-
610 related indicators, and Western blot analysis to evaluate both pharmacological effects
611 and exploratory mechanisms.

612 Several limitations should be acknowledged. First, only p-p38 and p-p53 were
613 measured, and no pathway-specific intervention was used to verify causality. Second,
614 total P38 and total p53 were not detected, limiting interpretation of phosphory-
615 lation changes relative to total protein abundance. Third, no single-component or
616 component-removal groups were included, so the contribution of individual compounds
617 and possible synergistic interactions remain unknown. Fourth, histopathological
618 changes were assessed morphologically without a quantitative injury score. Fifth,
619 drug distribution, pharmacokinetics, and long-term toxicological profiles were not
620 examined. Finally, this was an animal study, and the findings cannot be directly
621 extrapolated to human smokers or patients with chronic respiratory diseases, nor
622 should the formula be considered equivalent to dexamethasone or the commercial
623 SLBZS preparation without further validation.

624

625 5 Conclusion

626

627 The SLBZS-derived defined-component formula attenuated cigarette smoke-induced
628 airway inflammation in rats. This protective effect was supported by reduced levels
629 of IL-1 β , IL-6, TNF- α , CXCL1, CCL2, and 8-iso-PGF2 α in lung tissue homogenates,
630 improvement of lung histopathological injury, and restoration of SOD activity. MDA
631 levels were not significantly altered, suggesting that the antioxidant conclusion
632 should be interpreted cautiously. The formula also reduced p-p38 and p-p53 pro-
633 tein levels in lung tissues, indicating that a p-p38/p-p53-related mechanism may be
634 involved. Further studies using pathway-specific interventions, total protein detection,
635 component-dissection designs, and long-term safety evaluation are needed to confirm
636 the mechanism and translational potential of this formula.

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641 Declarations

642

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Ethics approval and consent to participate. All animal procedures were reviewed and approved by the Ethics Committee of Zunyi Hospital of Traditional Chinese Medicine (approval No. HS-2025-172), but the animal study protocol was not prospectively registered in a public registry. All procedures were conducted in accordance with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Clinical trial number. Not applicable.

Consent for publication. Not applicable.

Data availability. The datasets generated and/or analyzed during the current study, including biochemical assay data and Western blot quantification data, are available from the corresponding author upon reasonable request.

Materials availability. Not applicable.

Code availability. Not applicable.

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