



## Short Communication

18- $\beta$ -glycyrrhetic acid-loaded polymeric nanoparticles attenuate cigarette smoke-induced markers of impaired antiviral response *in vitro*

Gabriele De Rubis<sup>a,b</sup>, Keshav Raj Paudel<sup>c</sup>, Stewart Yeung<sup>a,b</sup>, Siddiq Mohamad<sup>d</sup>, Swathi Sudhakar<sup>e</sup>, Sachin Kumar Singh<sup>b,f</sup>, Gaurav Gupta<sup>g,h,i</sup>, Philip Michael Hansbro<sup>c</sup>, Dinesh Kumar Chellappan<sup>d</sup>, Brian Gregory George Oliver<sup>j,k</sup>, Kamal Dua<sup>a,b,\*</sup>

<sup>a</sup> Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW 2007, Australia

<sup>b</sup> Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, Australia

<sup>c</sup> Centre for Inflammation, Centenary Institute and University of Technology Sydney, Faculty of Science, School of Life Sciences, Sydney 2007, Australia

<sup>d</sup> Department of Life Sciences, School of Pharmacy, International Medical University, Bukit Jalil, Kuala Lumpur 57000, Malaysia

<sup>e</sup> Department of Applied Mechanics and Biomedical Engineering, Indian Institute of Technology-Madras, Chennai, Tamil Nadu 600036, India

<sup>f</sup> School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab 144411, India

<sup>g</sup> Centre for Global Health Research, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India

<sup>h</sup> School of Pharmacy, Graphic Era Hill University, Dehradun 248007, India

<sup>i</sup> School of Pharmacy, Suresh Gyan Vihar University, Mahal Road, Jagatpura, Jaipur, India

<sup>j</sup> School of Life Science, University of Technology Sydney, Ultimo, NSW 2007, Australia

<sup>k</sup> Woolcock Institute of Medical Research, Macquarie University, Sydney, New South Wales, Australia

## ARTICLE INFO

## Keywords:

COPD  
Phytoceuticals  
18- $\beta$ -glycyrrhetic acid  
Advanced drug delivery  
Antiviral response  
Cigarette smoke

## ABSTRACT

Tobacco smoking is a leading cause of preventable mortality, and it is the major contributor to diseases such as COPD and lung cancer. Cigarette smoke compromises the pulmonary antiviral immune response, increasing susceptibility to viral infections. There is currently no therapy that specifically addresses the problem of impaired antiviral response in cigarette smokers and COPD patients, highlighting the necessity to develop novel treatment strategies. 18- $\beta$ -glycyrrhetic acid (18- $\beta$ -gly) is a phytoceutical derived from licorice with promising anti-inflammatory, antioxidant, and antiviral activities whose clinical application is hampered by poor solubility. This study explores the therapeutic potential of an advanced drug delivery system encapsulating 18- $\beta$ -gly in poly lactic-co-glycolic acid (PLGA) nanoparticles in addressing the impaired antiviral immunity observed in smokers and COPD patients. Exposure of BCI-NS1.1 human bronchial epithelial cells to cigarette smoke extract (CSE) resulted in reduced expression of critical antiviral chemokines (IP-10, I-TAC, MIP-1 $\alpha$ /1 $\beta$ ), mimicking what happens in smokers and COPD patients. Treatment with 18- $\beta$ -gly-PLGA nanoparticles partially restored the expression of these chemokines, demonstrating promising therapeutic impact. The nanoparticles increased IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$  levels, exhibiting potential in attenuating the negative effects of cigarette smoke on the antiviral response. This study provides a novel approach to address the impaired antiviral immune response in vulnerable populations, offering a foundation for further investigations and potential therapeutic interventions. Further studies, including a comprehensive *in vitro* characterization and *in vivo* testing, are warranted to validate the therapeutic efficacy of 18- $\beta$ -gly-PLGA nanoparticles in respiratory disorders associated with compromised antiviral immunity.

## 1. Introduction

According to the World Health Organization (WHO), tobacco use is the leading cause of preventable mortality worldwide, claiming more than eight million lives per year [1,2]. Cigarette smoke contains several

hundreds of toxic and carcinogenic chemicals [3], and exposure to tobacco smoke is known to cause a wide range of diseases including different types of cancer, strokes, heart conditions, diabetes, chronic-obstructive pulmonary disease (COPD), and other lung diseases [4]. Being smoking and inhalation the primary routes of tobacco

\* Corresponding author at: Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Sydney, NSW 2007, Australia.

E-mail address: [kamal.dua@uts.edu.au](mailto:kamal.dua@uts.edu.au) (K. Dua).

<https://doi.org/10.1016/j.prp.2024.155295>

Received 28 January 2024; Received in revised form 2 April 2024; Accepted 8 April 2024

Available online 9 April 2024

0344-0338/© 2024 The Author(s).

Published by Elsevier GmbH. This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0/>).

consumption, the lungs and the respiratory system are the sites that are majorly impacted, and COPD and lung cancer are the most common diseases caused by tobacco smoking [5]. In particular, cigarette smoke exposure has been shown to be the underlying cause of 70–90 % of lung cancer cases [6,7], and at least 50 % of smokers develop COPD in their lifetime [8].

Cigarette smoke causes inflammation and oxidative stress in the airways, and it damages the respiratory tract by impairing the function of epithelial cilia, increasing mucus secretion, and inducing an influx of immune cells such as macrophages and dendritic cells [9,10]. Considering the pivotal function of the airway ciliated epithelium and secreted mucus as a first line of defense against viral infections [11,12], cigarette smoke is known to severely impair the lung's ability to respond to these types of infections [9,10,13]. An impaired antiviral immunity is also observed in COPD patients [14], particularly among those with frequent disease exacerbations [15], as well as in “healthy” smokers who are not affected by COPD [10]. Furthermore, viral infections such as influenza A virus (IAV) or rhinovirus are among the principal triggers of COPD exacerbations [16], causing a sudden decline in lung function and contributing to disease progression in a positive feedback loop [17].

The respiratory tract's antiviral immune response is a complex and highly coordinated phenomenon involving both the innate and adaptive arms of the immune system [18]. The airway epithelium represents the first point of contact for viruses [18]. Together with dendritic cells, airway epithelial cells continuously sample the airway lumen with the aim of detecting the presence of viruses through pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs) TLR3, 7, and 9, which recognize viral double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and unmethylated CpG DNA sequences [19]. The activation of these PRRs triggers the early antiviral response, which is dominated by innate immune cells and mediated by the secretion of pleiotropic antiviral cytokines such as type I interferons (IFN- $\alpha$  and IFN- $\beta$ ), which in turn generate a cascade of immune cell recruitment, activation, and cytokine secretion [20]. This in turn leads to the activation of natural killer (NK) cells, which produce a type II interferon (IFN- $\gamma$ ) [21] and, ultimately, to the presentation of viral antigens to CD4<sup>+</sup> and CD8<sup>+</sup> T cells, marking the beginning of the adaptive immune response [18]. Numerous cytokines and chemokines, released by both epithelial and immune cells, orchestrate the airway's antiviral immune response. These include molecules such as IFN- $\gamma$ -induced protein 10 kDa (IP-10) [22], IFN-inducible T cell alpha chemoattractant (I-TAC) [23], and Macrophage inflammatory protein (MIP)-1 $\alpha$ /1 $\beta$  [24]. The production of both IP-10 and I-TAC is induced by IFN- $\gamma$  [22,23]. IP-10 contributes to the maturation of DCs and to the recruitment of virus-specific CD8<sup>+</sup> T cells [25], and I-TAC plays a general role as a chemoattractant for activated T cells [23]. Similarly, MIP-1 $\alpha$ /1 $\beta$  are involved in the recruiting of antigen-specific T and B cells, NK cells, monocytes, and neutrophils [24]. Numerous studies have shown that cigarette smoke exposure dampens the virus-induced production of these chemokines, providing an explanation of the mechanisms behind the impaired antiviral response observed in smokers and in COPD patients [10,15,26–29].

The current treatments available for COPD are mainly aimed at symptomatically targeting acute exacerbations and are limited by poor efficacy and severe side effects [5]. Importantly, there is currently no therapy available which specifically targets the impaired lung antiviral response observed among COPD patients and smokers. This underlines the necessity to develop innovative treatment strategies aimed at improving the patients' antiviral response. In this context, phytochemicals represent an endless source of potent therapeutic moieties to draw from [5]. 18- $\beta$ -glycyrrhetic acid (18- $\beta$ -gly), derived from licorice roots, is one of such molecules embedded with potent antioxidant, anti-inflammatory, and anticancer properties which has great potential in attenuating inflammation-driven pulmonary disorders [30]. However, its clinical translation is hampered by poor water solubility, which limits its bioavailability [30]. To overcome this problem, the use of advanced, nanoparticle-based drug delivery systems is set to

revolutionize the field by allowing improved delivery of molecules with suboptimal physicochemical properties and druggability [5]. Numerous classes of nanoparticle-based advanced drug delivery systems are currently available and being investigated. These include polymeric nanoparticles, liquid nanocrystals, nanoemulsions, liposomes, exosomes, dendrimers, polymeric micelles, metallic nanoparticles, and many others [5]. Among these, polymeric nanoparticles such as those composed of poly lactic-co-glycolic acid (PLGA) are particularly advantageous, as they are considered safe as nano-drug delivery systems for pulmonary targeting by the FDA [31]. Furthermore, PLGA-based nanoparticles are known to be non-toxic, as their main degradation by-products are water and carbon dioxide, which are quickly eliminated by the cell [32]. Finally, PLGA is a versatile polymer which is characterized by favorable biocompatibility, tunable mechanical characteristics, high biodegradability, and the possibility of numerous surface modifications and functionalizations [33,34].

In the present study, we have encapsulated 18- $\beta$ -gly in PLGA nanoparticles and tested their impact on the secretion of antiviral chemokines by human bronchial epithelial cells exposed to cigarette smoke extract (CSE) *in vitro*. We show that CSE treatment reduces the secretion of IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$ , and that treatment with 18- $\beta$ -gly-PLGA nanoparticles attenuates the effect of CSE by partially restoring the expression of these chemokines. The results of this study provide proof of feasibility of novel, targeted therapies aimed at boosting the impaired pulmonary antiviral response of smokers and COPD patients, providing a blueprint for the future development of innovative plant-based therapies that protect these particularly susceptible patient cohorts from viral infections of the airways.

## 2. Methods

18- $\beta$ -gly-PLGA nanoparticles were synthesized using the emulsion- evaporation method and characterized for particle size, polydispersity index, and zeta potential as described previously [33,35,36]. 18- $\beta$ -gly (97 % purity) was purchased from Sigma-Aldrich, USA. Briefly, 5 mL of 10 mg/mL PLGA solution were prepared using 3:2 dichloromethane and acetone as solvent. Successively, 10 mg 18- $\beta$ -gly were added and a primary emulsion was obtained by sonicating at 200 W for 2 min. The primary emulsion was slowly mixed with 1 % w/v Poloxamer aqueous solution and sonicated at 200 W for 4 minutes to form a final O/W emulsion. This was then centrifuged at 14,000 RPM for 30 min, and the supernatant was removed. The precipitated nanoparticles were then washed three times with deionized water followed by centrifugation at 10,000 RPM for 20 minutes. Finally, the nanoparticles were resuspended in deionized water and freeze-dried. Minimally immortalized BCI-NS1.1 healthy human bronchial epithelial cells were grown in broncho-epithelial basal media (BEBM) (Lonza, USA) supplemented with insulin, bovine pituitary extract, retinoic acid, triiodothyronine, transferrin, human epidermal growth factor, gentamicin sulfate, and amphotericin (Lonza, USA), at 37° and in a humidified environment, maintaining the CO<sub>2</sub> level to 5 %. The cells were seeded in 6-well plates as indicated previously [37]. Following overnight adhesion, cells were treated with or without 18- $\beta$ -gly-PLGA nanoparticles at a final 18- $\beta$ -gly concentration of 5  $\mu$ M for one hour, and then exposed to 5 % CSE for 24 h. The CSE concentration and length of CSE exposure has been optimized in our laboratories. CSE was prepared and administered as described in previous reports by our research team [6,37–39]. Briefly, one 3R4F reference cigarette was burned and the smoke bubbled in 10 mL cell culture media to obtain 100 % CSE. This has been further diluted with fresh culture media to a final concentration of 5 %. The 5  $\mu$ M concentration of 18- $\beta$ -gly-PLGA nanoparticles was identified as the highest concentration to be used without significantly affecting the viability of human bronchial epithelial cells *in vitro* as assessed via MTT assay in our previously published study [33]. Successively, three washes with phosphate-buffered saline (PBS, Merck, Australia) were performed, and the cells were lysed using radioimmunoprecipitation assay (RIPA)

buffer (ThermoFisher Scientific, Australia) supplemented with cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Roche, Australia). The insoluble material was eliminated *via* centrifugation of the samples at 14,000 g for 15 min at 4 °C, and the protein content was quantified *via* bichinchonic acid assay (ThermoFisher Scientific, Australia). The relative expression of IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$  was measured using a Proteome Profiler Human XL Cytokine Array Kit (R&D Systems, USA), following the manufacturer's instructions and hybridizing 300  $\mu$ g protein per sample on the array membranes. The chemiluminescence acquisition was performed using a ChemiDoc™ MP system (Bio-Rad, Australia) and data analysis was conducted with ImageJ software (version 1.53c, Bethesda, MD, USA). Statistical analysis was conducted using PRISM v9.3 software (GraphPad, USA).

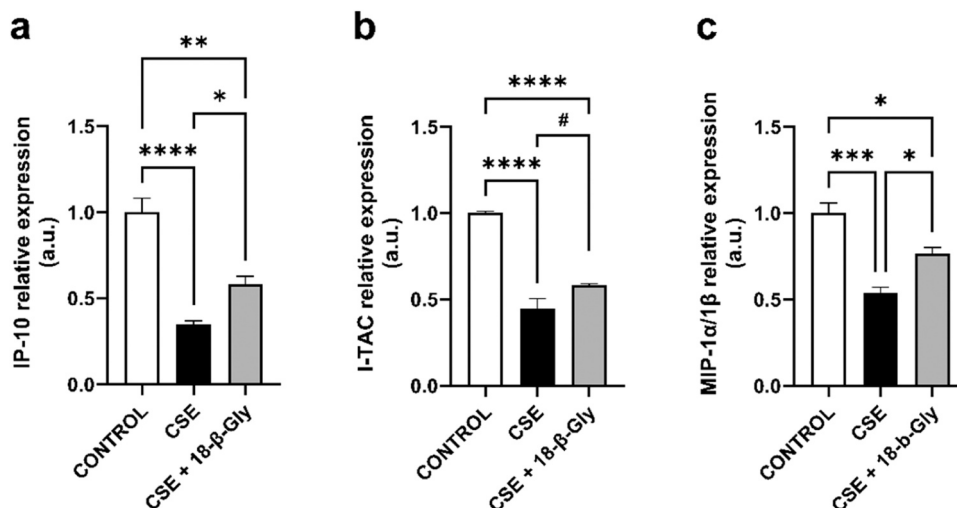
### 3. Results and discussion

The results of the present study are depicted in Fig. 1. Exposure to 5 % CSE resulted in a strong, significant reduction in the protein expression of IP-10 (65.4 %, Fig. 1a), I-TAC (55.5 %, Fig. 1b), and MIP-1 $\alpha$ /1 $\beta$  (46.4 %, Fig. 1c). Treatment with 5  $\mu$ M 18- $\beta$ -gly-PLGA nanoparticles significantly increased the expression of these three proteins compared to the CSE-exposed group, albeit not to level comparable to the untreated control (Fig. 1). In particular, 18- $\beta$ -gly-PLGA nanoparticles increased the levels of IP-10 by 67.7 % (Fig. 1a), the levels of I-TAC by 30.9 % (Fig. 1b), and the levels of MIP-1 $\alpha$ /1 $\beta$  by 43.0 % (Fig. 1c).

The findings of this study show that treatment with 5  $\mu$ M 18- $\beta$ -gly-PLGA nanoparticles can partially restore the expression of three fundamental mediators of pulmonary antiviral response: the chemokines IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$ , whose expression is downregulated by cigarette smoke exposure, potentially providing protection against the impaired antiviral response observed in cigarette smokers and COPD patients. It is worth mentioning that, despite 18- $\beta$ -gly-PLGA nanoparticles significantly increased the levels of the three indicated proteins compared to the CSE-treated group, in all the three cases the protein expression did not get back to the levels observed in the untreated control, therefore obtaining only a partial restoration of the expression of these proteins. Our results are in accordance with previous studies which showed, in different experimental systems, that cigarette smoke downregulates the expression of these three chemokines as part of its negative impact on lung antiviral response. Hudy et al. [28] showed that, in both primary and BEAS-2B human bronchial epithelial cells, CSE

exposure inhibited the Human Rhinovirus-16-induced production of IP-10. In another study, Duffney et al. [26] utilized an air-liquid interface culture of human primary small airway epithelia cells to demonstrate that whole cigarette smoke exposure dampened the production of IP-10 in response to IAV or poly I:C stimulation by impairing the correct activation of TLR3. More recently, Danov et al. [10] showed that cigarette smoke exposure completely suppressed the Influenza H1N1-induced secretion of several antiviral and proinflammatory cytokines, including IP-10 and I-TAC. Cigarette smoke has also been found to exert a similar effect on macrophages. Cigarette smoke condensate has been shown to decrease the expression of 8 cytokines, including IP-10 and MIP-1 $\alpha$ , in mouse Ana-1 macrophages [27], and CSE reduced the expression of IP-10 and MIP-1 $\alpha$ /1 $\beta$  in human THP-1 macrophages [29]. Interestingly, 18- $\beta$ -gly has been extensively studied for its promising antiviral activities against several viruses including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), herpes virus, and hepatitis virus [40], but to date no study has ever reported the potential of 18- $\beta$ -gly as a modulator of the body's own antiviral response. In this context, to the best of our knowledge, we are the first to report that 18- $\beta$ -gly has an impact on the production of IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$  in bronchial epithelial cells. The findings of this study are summarized in the Graphical Abstract of the manuscript.

The results showcased in the present study underline the promising potential of 18- $\beta$ -gly-PLGA nanoparticles as a therapeutic agent aimed at boosting the pulmonary antiviral response, with particular applicability in patients whose antiviral response is impaired such as cigarette smokers and COPD patients. Despite the promising results shown, this study is not exempt from limitations. Firstly, the study shows the modulation of only three proteins involved in the antiviral response. The antiviral response is a complex and multi-step process and, to fully characterize the impact of 18- $\beta$ -gly-PLGA nanoparticles as a means to restore the CSE-impaired antiviral response, the expression of a larger panel of proteins should be investigated. Furthermore, a more thorough investigation would also include the analysis of the impact of 18- $\beta$ -gly-PLGA nanoparticles on the expression of the same antiviral factors at the mRNA level. Finally, functional assays should be performed to definitively assess to what extent the 18- $\beta$ -gly-PLGA nanoparticles restore the impaired antiviral immunity caused by cigarette smoke. These studies should include both *in vitro* and *in vivo* investigations on suitable virus infection models. The multifaceted therapeutic activity of 18- $\beta$ -gly, which encompasses antioxidant, anti-inflammatory, anticancer,



**Fig. 1.** 18- $\beta$ -gly-PLGA nanoparticles partially restore the expression of IP-10, I-TAC, and MIP-1 $\alpha$ /1 $\beta$  downregulated by CSE. BCI-NS1.1 human bronchial epithelial cells were treated with or without 5  $\mu$ M 18- $\beta$ -gly-PLGA nanoparticles for one hour, followed by 24 hours exposure to 5 % CSE. Cells were then lysed in RIPA buffer, and the extracted proteins analyzed *via* Proteome Profiler Human XL Cytokine Array Kit. The chemiluminescence, expressed as pixel density, was quantified with ImageJ software. One-Way ANOVA, n = 4, \*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001; \*\*\*\*: P < 0.0001. #: P = 0.0511 with One-Way ANOVA and P = 0.0286 with Mann-Whitney U-test.

antimicrobial, and antiviral activities [30,40,41], warrants a thorough investigation of the therapeutic potential of this 18- $\beta$ -gly-PLGA nanoparticle-based system, with the aim of developing it as a treatment for respiratory conditions characterized by the overlapping of several etiological and pathophysiological features such as COPD.

#### 4. Conclusion

The findings discussed in the present study highlight the enormous therapeutic potential of 18- $\beta$ -gly-PLGA nanoparticles as a treatment aimed at restoring the impaired antiviral immune response of smokers and COPD patients. Considering the limited amount of data presented, a more thorough characterization of the therapeutic activity of this formulation is required before proceeding to the pre-clinical and clinical testing of the efficacy of this product. This would include further *in vitro* studies aimed at fully characterizing the effects of 18- $\beta$ -gly-PLGA nanoparticles on the expression of genes and proteins related to inflammation and oxidative stress, particularly within epithelial cells and macrophages. Furthermore, testing this product on cigarette smoke-exposed air-lung interface cell culture systems would provide more physiologically relevant information. Ideally, another line of studies would involve *in vivo* testing of the 18- $\beta$ -gly-PLGA formulation on animal models of COPD such as the cigarette smoke-exposed mice [42]. In conclusion, the result of this study provide a promising blueprint for the development of plant-based engineered nanosystems for the treatment of respiratory disorders.

#### CRedit authorship contribution statement

**Sachin Kumar Singh:** Writing – review & editing, Validation, Methodology. **Swathi Sudhakar:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Philip Michael Hansbro:** Writing – review & editing, Visualization, Methodology. **Gaurav Gupta:** Writing – review & editing, Visualization, Validation, Resources, Methodology. **Stewart Yeung:** Writing – review & editing, Visualization, Methodology. **Keshav Raj Paudel:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Siddiq Mohamad:** Writing – review & editing, Visualization, Methodology, Investigation. **Kamal Dua:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Dinesh Kumar Chellappan:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Conceptualization. **Gabriele De Rubis:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Brian Gregory George Oliver:** Conceptualization, Methodology, Visualization, Writing – review & editing.

#### Declaration of Competing Interest

The authors of the manuscript “18- $\beta$ -glycyrretinic acid-loaded polymeric nanoparticles attenuate cigarette smoke-induced markers of impaired antiviral response *in vitro*”, submitted to the journal “Pathology - Research and Practice”, have no conflict of interest to declare.

#### Acknowledgments

The authors are thankful to the Graduate School of Health, University of Technology Sydney, Australia. KD, GDR, and KRP are supported by the Maridulu Budyari Gumal Sydney Partnership for Health, Education, Research and Enterprise (SPHERE) RSEOH CAG and Triple I Seed and Extension Grants, Triple I Skills Advancement Grants 2023 and 2024 and Extension Grants, the UTS Faculty of Health Industry Partnership Grant 2023, and the UTS Faculty of Health Category 1 Seeding grants for Early Career Researchers 2024.. GDR, KD, and SY are also supported by the UTS 2023 Key Technology Partner (KTP) grants.

#### References

- [1] E. Banks, G. Joshy, M.F. Weber, B. Liu, R. Grenfell, S. Egger, E. Paige, A.D. Lopez, F. Sitas, V. Beral, Tobacco smoking and all-cause mortality in a large Australian cohort study: findings from a mature epidemic with current low smoking prevalence, *BMC Med.* 13 (2015) 38.
- [2] A. Peruga, M.J. López, C. Martínez, E. Fernández, Tobacco control policies in the 21st century: achievements and open challenges, *Mol. Oncol.* 15 (3) (2021) 744–752.
- [3] S.S. Hecht, Tobacco carcinogens, their biomarkers and tobacco-induced cancer, *Nat. Rev. Cancer* 3 (10) (2003) 733–744.
- [4] P. National Center for Chronic Disease, S. Health Promotion Office on, Health, Reports of the Surgeon General, The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General, Centers for Disease Control and Prevention (US), Atlanta (GA), 2014.
- [5] G. De Rubis, K.R. Paudel, L. Corrie, S. Mehndiratta, V.K. Patel, P.S. Kumbhar, A. S. Manjappa, J. Disouza, V. Patravale, G. Gupta, B. Manandhar, R. Rajput, A. K. Robinson, R.J. Reyes, A. Chakraborty, D.K. Chellappan, S.K. Singh, B.G. G. Oliver, P.M. Hansbro, K. Dua, Applications and advancements of nanoparticle-based drug delivery in alleviating lung cancer and chronic obstructive pulmonary disease, *Naunyn Schmiede Arch. Pharm.* (2023).
- [6] V. Malyla, K.R. Paudel, G. De Rubis, N.G. Hansbro, P.M. Hansbro, K. Dua, Cigarette smoking induces lung cancer tumorigenesis via upregulation of the WNT/ $\beta$ -catenin signaling pathway, *Life Sci.* 326 (2023) 121787.
- [7] T. Walser, X. Cui, J. Yanagawa, J.M. Lee, E. Heinrich, G. Lee, S. Sharma, S. M. Dubinett, Smoking and lung cancer, *Proc. Am. Thorac. Soc.* 5 (8) (2008) 811–815.
- [8] B. Lundbäck, A. Lindberg, M. Lindström, E. Rönmark, A.C. Jonsson, E. Jönsson, L. G. Larsson, S. Andersson, T. Sandström, K. Larsson, Not 15 but 50 % of smokers develop COPD?—report from the obstructive lung disease in Northern Sweden studies, *Respir. Med.* 97 (2) (2003) 115–122.
- [9] T.W. Marcy, W.W. Merrill, Cigarette smoking and respiratory tract infection, *Clin. Chest Med.* 8 (3) (1987) 381–391.
- [10] O. Danov, M. Wolff, S. Bartel, S. Böhlen, H. Obernolte, S. Wronski, D. Jonigk, B. Hammer, D. Kovacevic, S. Reuter, S. Krauss-Etschmann, K. Sewald, Cigarette smoke affects dendritic cell populations, epithelial barrier function, and the immune response to viral infection with H1N1, *Front. Med.* 7 (2020) 571003.
- [11] P.G. Quie, Lung defense against infection, *J. Pediatr.* 108 (5 Pt 2) (1986) 813–816.
- [12] X.M. Bustamante-Marin, L.E. Ostrowski, Cilia and mucociliary clearance, *Cold Spring Harb. Perspect. Biol.* 9 (4) (2017).
- [13] H. Lawrence, A. Hunter, R. Murray, W.S. Lim, T. McKeever, Cigarette smoking and the occurrence of influenza – systematic review, *J. Infect.* 79 (5) (2019) 401–406.
- [14] A.C. Hsu, K. Parsons, F. Moheimani, D.A. Knight, P.M. Hansbro, T. Fujita, P. A. Wark, Impaired antiviral stress granule and IFN- $\beta$  enhanceosome formation enhances susceptibility to influenza infection in chronic obstructive pulmonary disease epithelium, *Am. J. Respir. Cell Mol. Biol.* 55 (1) (2016) 117–127.
- [15] A. Singanayagam, S.L. Loo, M. Calderazzo, L.J. Finney, M.B. Trujillo Torralbo, E. Bakhsoliani, J. Girkin, P. Veerati, P.S. Pathinayake, K.S. Nichol, A. Reid, J. Footitt, P.A.B. Wark, C.L. Grainge, S.L. Johnston, N.W. Bartlett, P. Mallia, Antiviral immunity is impaired in COPD patients with frequent exacerbations, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 317 (6) (2019) L893–L903.
- [16] G. Rohde, A. Wiethage, I. Borg, M. Kauth, T.T. Bauer, A. Gillissen, A. Bufe, G. Schultze-Werninghaus, Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study, *Thorax* 58 (1) (2003) 37–42.
- [17] A.I. Ritchie, J.A. Wedzicha, Definition, causes, pathogenesis, and consequences of chronic obstructive pulmonary disease exacerbations, *Clin. Chest Med.* 41 (3) (2020) 421–438.
- [18] J.E. Kohlmeier, D.L. Woodland, Immunity to respiratory viruses, *Annu. Rev. Immunol.* 27 (2009) 61–82.
- [19] A. Iwasaki, R. Medzhitov, Toll-like receptor control of the adaptive immune responses, *Nat. Immunol.* 5 (10) (2004) 987–995.
- [20] C. Asselin-Paturel, A. Boonstra, M. Dalod, I. Durand, N. Yessaad, C. Dezutter-Dambuyant, A. Vicari, A. O’Garra, C. Biron, F. Brière, G. Trinchieri, Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology, *Nat. Immunol.* 2 (12) (2001) 1144–1150.
- [21] X.S. He, M. Draghi, K. Mahmood, T.H. Holmes, G.W. Kemble, C.L. Dekker, A. M. Arvin, P. Parham, H.B. Greenberg, T cell-dependent production of IFN-gamma by NK cells in response to influenza A virus, *J. Clin. Invest.* 114 (12) (2004) 1812–1819.
- [22] M.P. Alves, A. Schögler, R. Muster, M.-N. Kronig, E. Kieninger, B.S. Kopf, A. Stokes, N. Regamey, IP-10 is selectively produced in the airways upon respiratory virus infection, *Eur. Respir. J.* 42 (Suppl. 57) (2013) SP3877.
- [23] K.E. Cole, C.A. Strick, T.J. Paradis, K.T. Ogborne, M. Loetscher, R.P. Gladue, W. Lin, J.G. Boyd, B. Moser, D.E. Wood, B.G. Sahagan, K. Neote, Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3, *J. Exp. Med.* 187 (12) (1998) 2009–2021.
- [24] E. Jones, D.A. Price, M. Dahm-Vicker, V. Cerundolo, P. Klenerman, A. Gallimore, The influence of macrophage inflammatory protein-1alpha on protective immunity mediated by antiviral cytotoxic T cells, *Immunology* 109 (1) (2003) 68–75.
- [25] D.M. Lindell, T.E. Lane, N.W. Lukacs, CXCL10/CXCR3-mediated responses promote immunity to respiratory syncytial virus infection by augmenting dendritic cell and CD8(+) T cell efficacy, *Eur. J. Immunol.* 38 (8) (2008) 2168–2179.
- [26] P.F. Duffney, C.E. McCarthy, A. Nogales, T.H. Thatcher, L. Martinez-Sobrido, R. P. Phipps, P.J. Sime, Cigarette smoke dampens antiviral signaling in small airway



- epithelial cells by disrupting TLR3 cleavage, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 314 (3) (2018) L505–L513.
- [27] J. Zhao, X. Li, F. Xie, Z. Yang, X. Pan, M. Zhu, P. Shang, C. Nie, H. Liu, J. Xie, Immunomodulatory effects of cigarette smoke condensate in mouse macrophage cell line, *Int. J. Immunopathol. Pharm.* 30 (3) (2017) 315–321.
- [28] M.H. Hudy, S.L. Traves, S. Wiehler, D. Proud, Cigarette smoke modulates rhinovirus-induced airway epithelial cell chemokine production, *Eur. Respir. J.* 35 (6) (2010) 1256–1263.
- [29] R. Hamon, H.B. Tran, E. Roscioli, M. Ween, H. Jersmann, S. Hodge, Bushfire smoke is pro-inflammatory and suppresses macrophage phagocytic function, *Sci. Rep.* 8 (1) (2018) 13424.
- [30] M.S.B. Mohamad, R.J. Reyes, G. De Rubis, K.R. Paudel, P.M. Hansbro, K. Dua, D. K. Chellappan, The versatility of 18 $\beta$ -glycyrrhetic acid in attenuating pulmonary inflammatory disorders, *Excli J.* 22 (2023) 188–190.
- [31] J. Saxena, M. Bisen, A. Misra, V.K. Srivastava, S. Kaushik, A.J. Siddiqui, N. Mishra, A. Singh, A. Jyoti, Targeting COPD with PLGA-based nanoparticles: current status and prospects, *BioMed Res. Int.* 2022 (2022) 5058121.
- [32] B. Boltnarova, J. Kubackova, J. Skoda, A. Stefela, M. Smekalova, P. Svacinova, I. Pavkova, M. Dittrich, D. Scherman, J. Zbytovska, P. Pavek, O. Holas, PLGA based nanospheres as a potent macrophage-specific drug delivery system, *Nanomaterials* 11 (3) (2021).
- [33] K.R. Paudel, M.S.B. Mohamad, G. De Rubis, R.-J. Reyes, N. Panth, H. Dureja, G. Gupta, S.K. Singh, T. Madheswaran, T. Collet, P.M. Hansbro, K. Dua, D. K. Chellappan, 18- $\beta$ -glycyrrhetic acid encapsulated PLGA nanoparticles attenuate lung cancer proliferation and migration, *J. Drug Deliv. Sci. Technol.* 95 (2024) 105523.
- [34] C. Lin, X. Wang, N. Liu, Q. Peng, Y. Li, L. Zhang, Y. Gao, Characterization and evaluation of HGF-loaded PLGA nanoparticles in a CCl<sub>4</sub>-induced acute liver injury mouse model, *J. Nanomater.* 2019 (2019) 7936143.
- [35] N. Vij, Synthesis and evaluation of airway targeted PLGA nanoparticles for drug delivery in obstructive lung diseases, *Methods Mol. Biol.* 906 (2012) 303–310.
- [36] Synthesis and characterization of dose-dependent antioxidants and antimicrobial activity of phloretin loaded PLGA nanoparticles, *Biointerface Res. Appl. Chem.*, 2021.
- [37] K.R. Paudel, N. Panth, B. Manandhar, S.K. Singh, G. Gupta, P.R. Wich, S. Nammi, R. MacLoughlin, J. Adams, M.E. Warkiani, D.K. Chellappan, B.G. Oliver, P. M. Hansbro, K. Dua, Attenuation of cigarette-smoke-induced oxidative stress, senescence, and inflammation by berberine-loaded liquid crystalline nanoparticles: in vitro study in 16HBE and RAW264.7 cells, *Antioxidants* 11 (5) (2022).
- [38] G. De Rubis, K.R. Paudel, B. Manandhar, S.K. Singh, G. Gupta, R. Malik, J. Shen, A. Chami, R. MacLoughlin, D.K. Chellappan, B.G.G. Oliver, P.M. Hansbro, K. Dua, Agarwood oil nanoemulsion attenuates cigarette smoke-induced inflammation and oxidative stress markers in BCI-NS1.1 airway epithelial cells, *Nutrients* 15 (4) (2023).
- [39] K.R. Paudel, D.D. Clarence, N. Panth, B. Manandhar, G. De Rubis, H.P. Devkota, G. Gupta, F.C. Zacconi, K.A. Williams, L.G. Pont, S.K. Singh, M.E. Warkiani, J. Adams, R. MacLoughlin, B.G. Oliver, D.K. Chellappan, P.M. Hansbro, K. Dua, Zerumbone liquid crystalline nanoparticles protect against oxidative stress, inflammation and senescence induced by cigarette smoke extract in vitro, *Naunyn Schmiede Arch. Pharm.* 397 (4) (2024) 2465–2483.
- [40] J. Zuo, T. Meng, Y. Wang, W. Tang, A review of the antiviral activities of glycyrrhizic acid, glycyrrhetic acid and glycyrrhetic acid monoglucuronide, *Pharmaceuticals* 16 (5) (2023).
- [41] A. Kowalska, U. Kalinowska-Lis, 18 $\beta$ -Glycyrrhetic acid: its core biological properties and dermatological applications, *Int. J. Cosmet. Sci.* 41 (4) (2019) 325–331.
- [42] J. Shrestha, K.R. Paudel, H. Nazari, V. Dharwal, S.R. Bazaz, M.D. Johansen, K. Dua, P.M. Hansbro, M.E. Warkiani, Advanced models for respiratory disease and drug studies, *Med. Res. Rev.* 43 (5) (2023) 1470–1503.