Lily bulb Nectar Produces Expectorant and Anti-Tussive Activities, and Suppresses Cigarette Smoke-Induced Inflammatory Response in the Respiratory Tract in Mice

Hoishan Wong¹, Shiyu Zou², Jiangping Li², Chungwah Ma², Jihang Chen¹, Poukuan Leong¹, Hoiyan Leung¹, Wingman Chan¹, Kamming Ko¹

¹Division of Life Science, Hong Kong University of Science and Technology, Hong Kong, China
²Infinitus (China) Company Ltd., Guangzhou, China

Email: *bcrko@ust.hk

Received 23 March 2015; accepted 30 May 2015; published 2 June 2015

Abstract

Air pollutants pose a major environmental threat to the respiratory system. Pathogen invasion and the exposure to particulate matters in atmospheric air, particularly, cigarette smoke (CS), have been found to be associated with acute and chronic respiratory diseases, including asthma. Therefore, the search for agents that can protect the respiratory system against potentially harmful substances is of interest in preventive health. Lily bulb Nectar (LBN), which contains Lily bulb, Pyrus pyrifolia N., Siraitia grosvenorii and Apricot kernel as its ingredients, is a health supplement intended to improve the wellness of the respiratory system in humans. Lily bulb, Pyrus pyrifolia N., Siraitia grosvenorii and Apricot kernel are commonly prescribed for the treatment of respiratory tract disorders such as bronchitis, pneumonia and cough in the practice of traditional Chinese medicine. Pharmacological studies have shown that these herbs can produce beneficial effects on the respiratory tract or even the lungs. In the present study, we investigated the effects of LBN on mouse respiratory tract function under normal and challenged conditions. LBN was first examined for its expectorant and anti-tussive activities in mice. The effect of LBN on long-term exposure to CS was also investigated. Our findings showed that long-term LBN treatment enhanced the expectorant activity and suppressed the SO₂-induced coughing in mice. LBN treatment also suppressed the CS-induced inflammation in the respiratory tract, as assessed by differential cell count and cytokine production. In conclusion, long-term LBN consumption may produce beneficial effects on the respiratory tract function in humans, particularly in the face of challenge by irritants in the inhaling air.

*Corresponding author.

How to cite this paper: Wong, H.S., et al. (2015) Lily bulb Nectar Produces Expectorant and Anti-Tussive Activities, and Suppresses Cigarette Smoke-Induced Inflammatory Response in the Respiratory Tract in Mice. Chinese Medicine, 6, 136-146. http://dx.doi.org/10.4236/cm.2015.62015
Keywords

*Lily bulb*, *Expectoration*, *Anti-Tussion*, *Anti-Inflammation*

1. Introduction

Atmospheric air, which is the main source of oxygen, is essential for the survival of aerobic organisms. In addition to nitrogen, oxygen, carbon dioxide and trace amounts of other gases, the inhaled air also contains many air pollutants, of which the over-exposure poses a threat to the well-being of respiratory system [1][2]. The lungs, which provide the gas exchange surface of our body, are exceptionally vulnerable to such insult because of the close proximity to the potentially harmful substances in the inhaled air [3][4]. Air pollutants, including particular matters (PMs), especially PMs smaller than 2.5 micrometer (also known as fine PMs), and airborne microbial pathogens, can impair the functioning of the respiratory system by physical irritation and/or the induction of inflammatory response in the respiratory tract [5][6]. The air microbes and pollutants were also found to be associated with a broad spectrum of acute and chronic illnesses, such as lung cancer, chronic obstructive pulmonary disease, asthma and even cardiovascular diseases [7]. To cope with these challenges, the lungs are equipped with sophisticated defense mechanisms, which operate in a coordinated fashion to expel foreign particles from the respiratory tract.

Mucociliary clearance and coughing are two of the defensive mechanisms that mechanically remove foreign particles from the respiratory tract [8][9]. Conducting airways, including nasal passage and tracheobronchial tree, are covered with a layer of mucus secreted by goblet cells, submucosal glands and Clara cells. The mucous blanket provides a surface for the deposition of inhaled particles. The particle-laden mucus, also known as sputum, is transferred by the coordinated movement of cilia to the naso-/oropharynx where it is swallowed [8][9]. Cough reflex is triggered by mechanical or chemical stimuli on the epithelium of larynx or tracheobronchial tree. It prevents the accumulation of mucus secretion and thus helps eliminate the inhaled particles from the lower respiratory tract [8][9]. However, while mucociliary clearance and coughing are effective in removing most of the inhaled particles in healthy individuals, their clearance efficiency is far from sufficient when the respiratory system is affected by the exposure to cigarette smoke (CS), which exists as the most frequently occurring air pollutant [10].

CS, with over 4000 chemical components, is the major etiological factor of chronic lung inflammation [11]-[14]. The CS-induced lung inflammation is mediated by a complex mechanism that involves various types of cells and inflammatory mediators, in which redox-sensitive signaling pathway plays a primary role [15]. The exposure of respiratory system to CS leads to an increased production of reactive oxygen species (ROS) by NADPH oxidase [16]-[18]. The ROS, in turn, triggers the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) [19][20], via the mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways. TNF-α, together with other chemokines, facilitates the recruitment of effector cells, including macrophages, neutrophils and lymphocytes, to eliminate foreign particles mainly by phagocytosis [21]. The effector cells, after being exposed to CS, lead to further and excessive production of inflammatory substances/enzymes that cause an increase in mucosal secretion and destructions of collagen and elastin, with the resultant decline in respiratory function of the lungs [22].

*Lily bulb* (the flower bulb of *Lilium brownie* F. E. Brown var. *viridulum* Baker, *Lilium lancifolium* Thunb. and *Lilium pumilium* DC.), *Pyrus pyrifolia* N. (a member of the Rosaceae family and Pomaceae tribe), *Siraitia grosvenorii* (a herbaceous perennial vine of the Cucurbitaceae family), and *Apricot kernel* (the dried and mature seed of *Prunus armeniaca* L. and *Prunus ameniaca* L. var. Ansu Maxim.) are listed in the Chinese Pharmacopoeia (2010) as medicinal plants. According to the theory of traditional Chinese medicine, these herbs produce “Yin-nourishing” and “Lung-moistening” effects, and are therefore commonly prescribed for the treatment of bronchitis, pneumonia and cough in the practice of traditional Chinese medicine [23]-[29]. Their beneficial effects on respiratory system were also demonstrated in recent pharmacological studies. Oral administration of the aqueous extracts of *Lily bulb* and *Siraitia grosvenorii* suppressed the SO2-induced coughing in mice, as evidenced by the reduced number and delayed onset of SO2-induced coughing, and improved tracheobronchial expectorant action [28][30]. The cough relieving and phlegm expelling activities could also be observed in *Apri-
cot kernel-pretreated mice through which hydrocyanic acid, a metabolite of amygdalin in Apricot kernel, reflexively stimulated the respiratory center in the brain of the pretreated animals [29]. Moreover, Lily bulb was found to exhibit anti-inflammatory activity on lipopolysaccharide-stimulated Raw 264.7 mouse macrophages, presumably by down-regulating the NF-κB and extracellular signal-regulated kinases (ERK)/c-Jun N-terminal kinases (JNK) signaling pathways [23].

Lily bulb Nectar (LBN), which is comprised of Lily bulb, Pyrus, Siraitia grosvenorii and Apricot kernel, is a health supplement intended to improve the wellness of the respiratory system in humans. In the present study, we investigated the effect of LBN on tracheobronchial expectorant action by phenol red secretion test in mice. The anti-tussive and anti-inflammatory activities were also examined in the mouse model of SO2-induced coughing and CS-induced inflammation in the respiratory tract.

2. Materials and Methods

2.1. Chemicals

Ammonium chloride (NH₄Cl) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Cigarettes (Camel; filters, Japan Tobacco Inc.) were purchased from local distributors. Concentrated sulfuric acid (H₂SO₄) was purchased from BDH Reagents & Chemicals (Poole Dorset, UK). Phenol red and sodium bisulfite (NaHSO₃) were purchased from Sigma (St. Louis, MO). Dextromethorphan hydrobromide (DH) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). ELISA kits for immunoglobulin G (IgG), interleukin-8 and secretory immunoglobulin A (sIgA) were purchased from Cusabio Biotech Co., Ltd. (Suffolk, UK), whereas ELISA kits for interleukin-6 (IL-6) and TNF-α were purchased from Life Technologies (Grand Island, NY).

2.2. LBN Preparation

LBN is a syrup preparation comprising water extracts of Lily bulb, Pyrus pyrifolia N., Siraitia grosvenorii, and Apricot kernel. The commercial product was manufactured and supplied by Infinitus (China) Company Ltd., Guangzhou, China.

2.3. Animal Care

Male adult Balb/c mice were used for the assessment of expectorant and anti-tussive activities, whereas male adult ICR mice were used for examining the CS-induced pulmonary immune response. Animals (~8 weeks of age; 25 - 30 g) were randomly assigned to 4 groups, respectively, with 5 - 10 animals in each. LBN, at daily doses of 2.46, 8.20, 24.6 g/kg (with human equivalent dose being 8.20 g/kg), was administered intragastrically 5 days per week for 4 weeks (i.e., a total of 20 doses). Control animals received the vehicle (water) only. All experimental procedures were approved by the Research Practice Committee (Hong Kong University of Science and Technology) (Animal Protocol Approval No. 2013064; Approved Date: 12 November 2013; Experiment Duration: 3 years).

2.4. Measurement of Expectorant Activity

The tracheobronchial expectorant activity was measured by phenol red secretion test. NH₄Cl, which was used as a positive control, was administered intragastrically at 1 g/kg for 3 consecutive days. Twenty-four h after the last dosing with vehicle/LBN/NH₄Cl, phenol red [suspended in 0.9% saline (w/v)] was administered intraperitoneally, at a dose of 500 mg/kg. The mice were sacrificed 30 min after phenol red injection and the tracheas were carefully excised. All connective tissues and traces of blood were removed from the excised trachea. Half-centimeter of the trachea was cut out, and was then incubated in 0.5 mL 0.9% saline (w/v) with 100 µL 1 M NaOH for 30 min with thorough mixing by vortexing. After the incubation, the absorbance at 550 nm of the solution was measured. The amount of phenol red eliminated through tracheobronchial secretion was calculated using a calibration curve of phenol red [31]-[35].

2.5. Anti-Tussive Activity

Coughing was induced by SO₂ and the frequency of coughing was recorded [36] [37]. The experimental set-up is shown in Figure 1. Briefly, the mouse was put into chamber B and 0.2 mL concentrated H₂SO₄ was added
Figure 1. Experimental set-up of anti-tussive assay.

into chamber A which contained 500 mg/mL NaHSO₃ in distilled water. The generated SO₂ was flushed into chamber A by compressed air at a flow rate of 5 L/min. The mouse in chamber B was then exposed to SO₂ for 30 s and withdrawn from the chamber thereafter. One minute after the withdrawal, the number of coughing of the mouse was counted until up to 5 min post-exposure. The concentration of SO₂ was determined by spectrophotometric method and the average concentration of SO₂ used in this experiment was found to be 207 ± 23.3 mg/m³. DH was administered 30 min prior to the SO₂ exposure to serve as a positive control for the suppression of SO₂-induced tussive activity.

2.6. CS Challenge

Camel cigarettes (containing 10 mg tar and 0.8 mg nicotine per cigarette) were used to produce CS. A cage with 5 mice was placed into a container (57 × 41.8 × 34.8 cm) connecting to a circulation pump (see Figure 2). The container was closed tightly immediately after the cigarette was ignited. Mice were exposed to CS flowing through the container for 6 min (one cigarette burning time was equal to one session). For the experiment with differential cell counts, mice were exposed to CS during the last 11 days of LBN administration, wherein mice were exposed to CS ten sessions per day, five days per week. For the experiment with measurements of cytokines and immunoglobulins, mice were exposed to CS during the last 4 days of LBN administration, wherein mice were exposed to CS ten sessions per day.

2.7. Collection of Bronchoalveolar Lavage Fluid (BALF)

Twenty-four h following the last exposure to CS, the BALF was collected from ketamine-anesthetized mice by cannulating the upper part of the trachea using 27-G syringe. The lavaging procedure was done once with 1 mL phosphate-buffered saline-type A (PBS-A) for measurements of cytokines and immunoglobulins and twice with a total of 1.6 mL PBS-A for differential cell counts.

2.8. Differential Cell Counts

The collected BALF in PBS-A samples were centrifuged at 1000× g for 10 min at room temperature. The total number of leukocytes was counted using a hemocytometer. Differential cell counts were determined by staining the cells on a glass slide with Giemsa-Wright stain. More than one hundred cells per slide were counted and the percentages of macrophages, neutrophils and lymphocytes with respect to the total number of cells were estimated [38]-[40].

2.9. Measurements of Cytokines and Immunoglobulins in BALF

For measurements of cytokines and immunoglobulins, BALF samples were collected and centrifuged at 2150× g
Figure 2. Experimental set-up for CS challenge.

for 10 min at 4°C. The supernatants were used for analyses. Levels of IL-6, TNF-α, IL-8, IgG and sIgA in BALF were measured using ELISA kits [38]-[45].

3. Results

Long-term treatment with LBN at daily doses of 2.46 to 24.6 g/kg did not produce any detectable changes in body weight in mice, with or without exposure to CS, when compared with the respective untreated control (data not shown).

Whereas NH₄Cl caused an increase in expectorant activity by 105% in mice, long-term LBN treatment, at a daily dose of 24.6 g/kg, significantly increased the expectorant activity by 30%, when compared with the untreated control (Figure 3).

The exposure to SO₂ caused coughing response in mice, with 196 coughs from 1 - 5 min post-exposure (Figure 4). Treatment with DH, at a dose of 10 mg/kg, significantly suppressed the SO₂-induced coughing by 33%. Long-term treatment with LBN caused a dose-dependent suppression in SO₂-induced coughing, with the number of coughs being significantly reduced by 42% at a daily dose of 24.6 g/kg (Figure 4).

While long-term LBN treatment did not produce any detectable changes in total cell count, macrophage number and lymphocyte number in the BALF of mice, CS exposure for 11 days caused significant decreases in total cell count (26%) and macrophage number (10%) as well as an increase in lymphocyte number (by 10-fold), when compared with the non-CS exposed control (Figure 5). Long-term LBN treatment restored the CS-induced decreases in total cell count (by 100%) at a daily dose of 8.20 g/kg and in the number of macrophage (by 23%) at a daily dose of 24.6 g/kg. The CS-induced increase in lymphocyte number was suppressed by LBN treatment (by 45%) at a daily dose of 24.6 g/kg. However, neither LBN treatment nor CS exposure produced any detectable changes in neutrophil number in the BALF of mice.

Long-term LBN treatment did not cause any detectable changes in IL-6, IL-8, sIgA, IgG and TNFα levels in the BALF of mice (Figure 6), whereas 4-day CS exposure increased the levels of IL-6 (25%), IL-8 (1.79 fold), sIgA (1.33 fold), IgG (26%) and TNF-α (22.7%). Long-term LBN treatment at a daily dose of 2.46 g/kg completely inhibited the CS-induced increase in IL-6 level. The CS-induced increase in sIgA level was largely suppressed by LBN treatment by 85%. LBN treatment also inhibited the CS-induced increase in IgG (94%) and TNF-α (125%) levels at daily doses of 8.20 and 24.6 g/kg, respectively. However, LBN treatment did not produce any detectable changes in the CS-induced increase in IL-8 level (Figure 6).

The tracheobronchial expectorant activity was assessed as described in materials and methods. Data were expressed in percent control with respect to vehicle-treated control [amount of expectorant of control (µg/mL) = 2.07 ± 0.15]. Values given are mean ± SEM, with n = 5 to 10.

The extent of SO₂-induced tussive activity was measured as described in materials and methods. Data were expressed in percent control with respect to vehicle-treated control [number of coughing of control = 196.45 ± 14.61]. Values given are mean ± SEM, with n = 5 to 10.

BALF was collected and cells were quantified as described in materials and methods. Data were expressed in percent control with respect to vehicle-treated control [percent cell count of control = 84107.14 ± 3572.64 (total cell count); 236.67 ± 20.36 (macrophages) 2.62 ± 0.38; (lymphocytes) and 4.23 ± 1.19 (neutrophils)]. Values given are mean ± SEM, with n = 5 to 10.
The effects of long-term LBN treatment on the expectorant activity in Balb/c mice. *Significantly different from vehicle control (p < 0.05).

Figure 3. The Effects of long-term LBN treatment on SO2-induced tussive activity in Balb/c mice. *Significantly different from vehicle control (p < 0.05).

Figure 4. The effects of long-term LBN treatment on SO2-induced tussive activity in Balb/c mice. *Significantly different from vehicle control (p < 0.05).

The amount of pro-inflammatory cytokines and immunoglobulins were measured as described in materials and methods. Data were expressed in percent control with respect to non-CS vehicle control [control values = 10.02 ± 0.91 pg/mg protein (IL-6); 16295.73 ± 2623.85 pg/mg protein (IL-8); 18.52 ± 1.09 pg/mg protein (TNF-α); 10233.67 ± 493.26 (IgG) and 204.14 ± 15.24 ng/mg protein (sIgA)]. Values given are mean ± SEM, with n = 5 to 10.

4. Discussions

Results obtained from the present study showed that long-term treatment with LBN enhanced the expectorant activity in mice, as assessed by tracheobronchial clearance of the intraperitoneally-injected phenol red. The expectorant action is characterized by increased secretion and hydration of sputum for more efficient sputum re-
moval via mucociliary transport in the respiratory tract [46] [47]. Long-term LBN treatment was also found to produce anti-tussive activity against SO2-induced coughing in mice. While coughing secures the removal of mucus, noxious substances and infectious particles from the respiratory tract, persistent coughing, which affects over 40% of non-smokers in the United States and Europe, presents psychological and social burdens, as well as contributes to the spread of airborne microbes [48]-[51]. The cough-relieving activity of LBN, therefore, has clinical implications in suppressing the hypersensitivity of coughing provoked by mildly irritating or innocuous stimuli [52].

The beneficial effect of LBN on the respiratory tract was also evidenced by its action on CS-induced lung inflammation in mice. CS exposure caused significant increases in pro-inflammatory cytokines (IL-8, IL-6 and TNF-α) and the subsequent recruitment of lymphocytes in mouse respiratory tract, indicative of a localized inflammation in the lungs. Long-term LBN treatment reduced the CS-induced elevations in pro-inflammatory cytokines and the associated lymphocyte infiltration, suggesting a reduction in the degree of CS-induced lung inflammation in mouse respiratory tract. Inflammation is a defensive mechanism that protects tissues against pathogenic invasion [53]. Nevertheless, depending on the extent and characteristic of inflammation, it may also result in the functional impairment in relevant tissues/organs, presumably through macrophage-/leukocytes-released ROS and the associated protease activities [54] [55]. In this regard, while the CS exposure caused significant decreases in total and macrophage cell counts in BALF, possibly due to oxidative stress-induced cell death...
Figure 6. The effects of long-term LBN treatment with 4-day CS exposure on cytokines and immunoglobulins in BALF of ICR mice. *Significantly different from non-CS vehicle control; †significantly different from CS-exposed vehicle control (p < 0.05).

in mouse respiratory tract during inflammation [54], the anti-inflammatory action of LBN was paralleled by its ability to suppress such decreases, suggests the potential beneficial effect of LBN on innate immunity in CS-
exposed animals. In addition, a marked increase in lymphocyte number in BALF of CS-exposed mice was likely related to the increases in levels of IgG and sIgA, which are the two most abundant types of antibodies in the mucosal lining of respiratory tract. These antibodies, when acting in concert with effector cells, can eliminate foreign particles from mouse respiratory tract, possibly through the induction of both antibody-dependent cell-mediated cytotoxicity and intracellular antibody-mediated proteolysis of foreign particles [56]. The reduced extent of the CS-induced IgG and sIgA secretion in LBN-treated CS-exposed mice was likely an effect secondary to the anti-inflammatory activity of LBN. The findings, therefore, implicate the potential use of LBN for the prevention of CS-induced inflammation in the respiratory tract in humans.

5. Conclusion

In conclusion, long-term LBN treatment produced expectorant and anti-tussive activities in mouse respiratory tract. It also suppressed the CS-induced lung inflammatory response in mice. The mechanisms by which LBN produces these effects remain to be investigated. Taken together, LBN may serve a health supplement for safeguarding the function of the respiratory system, particularly in the respiratory tract.

References


