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Pear Formula for Respiratory Care

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Abstract This study unveils the possibility of a pear-based formula for reparatory care. Pears contain abundant polyphenols and triterpenoids in their fruit and skin, which confer antoxidative and anti-inflammatory effects on the prevention of chronic diseases. Pears have been used in several traditional remedies for reliving respiratory syndromes and constipation for over 2000 years in China. However, the use of pears for daily care is not easily accessible for most people. A pear-based sachet is a convenient approach to the beneficial ingredients of pears. The pear-based formula here enhanced wound healing rate by 27% and significantly improved the expression of allergy-, lung disease-, and DNA repair-related genes in lung cells. Moreover, the formula could increase the phagocytic activity of macrophages by 43%. In short, pear-based formula might provide the comprehensive respiratory care as evidenced by improvement in the wound healing efficiency of lung cells, the phagocytic activity of macrophages, down-regulation of the expression of respiratory disease-associated genes, and up-regulation the expression of DNA mismatch repair genes in cellular models.

Keywords: *pear, antioxidation, phagocytosis, mismatch DNA repair, wound healing*

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1. Introduction

Pears (*Pyrus spp.*), belonging to the Rosaceae family, are commonly edible fruits around the world; according to the place of origin, pears can generally be categorized into oriental or occidental ones [1]. In China, pears have been utilized in the traditional herbal remedies as relieving agents for cough, constipation, or alcoholism for over 2000 years [2]. The compositions of pears mainly contain ~71% of insoluble fiber, ~29% of soluble fiber, sugars, vitamins, organic acids, minerals, polyphenols, triterpenoids, and so on [3,4]. In addition to vitamins, polyphenols and triterpenoids in pears have also been identified as superior anti-oxidative and anti-inflammatory agents [5]. In particular, chlorogenic acid and arbutin are the two major polyphenolic compounds in pears [6]. Polyphenols, secondary plant metabolites, act as powerful reducing agents for scavenging free radicals, so that it is believed that they are beneficial for disease prevention (e.g., cancer, cardiovascular diseases, and diabetes) in light of alleviation of oxidative damage to protein, DNA, or lipid [7]. Notably, chlorogenic acid is the most abundant polyphenolic compound in the skin of some pears [8]. Chlorogenic acid is able to inhibit the expression of several inflammatory proteins (e.g., interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and cyclooxygenase (COX-2) [9,10]. Arbutin can suppress melanogenes through the down-regulation of tyrosinase expression in melanocytes [11]. Furthermore, arbutin may affect the expression of inflammatory-related genes (such as iNOS, IL-1β, and TNF-α), ROS production, and nuclear factor of activated T cells cytoplasmic 1 (NFATc1) [12,13]. Aside from polyphenols, triterpenoids (e.g., oleanolic and ursolic acids) also play an important role in anti-oxidation and anti-inflammation in pears in association with the prevention of chronic diseases (e.g., cardiovascular diseases) and cancer [2,14]. Oleanolic and ursolic acids are the most studied triterpenoid compounds in pears considering their predominant suppression effects on NF-κB and IL-6-induced STAT3 signal transduction pathways [15,16,17].

According to the report of World Health Organization (WHO), 91% of the world's population lives in the environment with the unqualified air quality and harmful air pollution leads to 4.2 million deaths every year [18]. Air pollutants primarily stem from industrial sources, automobile traffic, natural disasters (e.g., forest fires), household sources, or cigarette smoking [19]. Especially, particulate matter (PM) has recently drawn considerable attention since ~30% of respiratory diseases (e.g., chronic obstructive pulmonary disease (COPD), asthma) may result from high levels of ambient PM [20]. Alveolar

macrophages (AMs) and airway epithelial cells in human body provide the fundamental protection from air pollutants [18]. AMs remove the air particles in the lung by phagocytosis and secret proinflammatory cytokines (e.g., TNF-α, IL-1), granulocyte/macrophage colony-stimulating factor (GM-CSF) to the circulatory system followed by the systemic inflammatory response [21]. Human bronchial epithelial cells (HBECs), building a compact tissue barrier, block air pollutants on the tissue surface and dispose of them by mucociliary clearance in the low respiratory tract [22]. In addition, HBECs may also produce proinflammatory cytokines (e.g., IL-1, IL-8, pollutants [23]. In this study, we attempted to explore a pear-based formula, called Lung Care, for possible respiratory care. The use of snow pears in this formula is from Lishan (Taiwan). The formula can be made into a sachet form, which is handy for most people to consume the beneficial components of pears. The pear-based formula demonstrates the following advantages: i) enhancement of the wound healing rate and the phagocytic activity of macrophages; ii) improvement in antioxidative capability; iii) suppression of the respiratory disease-associated genes; and, iv) the increase of the expression of DNA mismatch repair genes. In brief, this research reveals some scientific evidences of a pear-based formula for potent respiratory care.

2. Material and Methods

2.1. Materials

Lung Care drink [OXYCORE Drink, Melaleuca (China); main ingredients (> 6.7%): snow pear extract, pear juice, Chinese jujube extract liquid, fructose, water; minor ingredients (< 0.8%): lilium extract, *Auricularia auricula-judae* extract, *Tremella fuciformis* extract, broccoli sprout powder, *Polygonatum odoratum* extract, green tea extract, perilla seed extract, citric acid monohydrate, spices], BEAS-2B (ATCC, CRL9609), BEAS-2B growth medium (BEBM, Lonza), phosphate buffered saline (PBS, Gibco), THP-1 (ATCC, TIB202), THP-1 growth medium [RPMI-1640 (Gibco) with 10% fetal bovine serum (FBS, Gibco), 0.05 mM 2-mercaptoethanol, 100 unit/mL penicillin, and 100 μg/mL streptomycine], phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich), fluorescent microparticles (1% w/w, diameter: 1.7-2.2 μm, Spherotech), RNA extraction kit (Genaid Biotech), flow cytometry (Beckman Coulter), microscopy (ZEISS), nCounter® platform (NanoString Technologies).

2.2. Wound Healing Assay

1.5×10^5 BEAS-2B cells were seeded into each well of 24-well plates and incubated at 37°C and 5% CO_2 for 24 hours. Subsequently, we used a 200 μL tip to create a ~0.5 μm cell free gap and removed the old media. Adding 0.5% or 1% Lung Care drink in the media to the corresponding wells, the cells were then incubated for 16 hours. Finally, the results were recorded by a microscopy.

2.3. Measurement of Phagocytic Activity of Macrophages

5×10^5 THP-1 cells in media with 500 nM PMA were dispersed into each well of 6- well plates and incubated the cells at 37°C and 5% CO_2 for 48 hours. Afterwards, the old media were replaced with fresh media. Following 48 hours incubation, the cells were treated with 0.25% and 0.5% Lung Care drinks for 24 hours. Subsequently, 0.5% fluorescent microparticles were added to the cultures wells followed by 4 hours co-incubation of the cells and the microparticles. Lastly, the media were drawn off, and the cells were washed by PBS three times and loaded into a flow cytometry (Ex: 485 nm; Em: 515-545 nm).

2.4. Analysis of mRNA Expression

1.5×10^5 BEAS-2B cells in 2 mL of the media with 0.25% Lung Care, 200 ng/mL LPS, or 200 ng/mL LPS and 0.25% of Lung Care were placed in each well of 6-well plates and incubated for 24 hours. Subsequently, the BEAS-2B cells were collected to a tube and total RNA were acquired by the RNA extraction kit. The concentration of total RNA were adjusted to 75 ng/μL as templates for the analyses of superoxide dismutase 2 (SOD2), IL-6, CD40, epidermal growth factor (ERBB2), non-catalytic region of tyrosine kinase adaptor protein 1(NCK1), dynein light chain LC8-type 2 (DYNLL2), G protein-coupled receptor kinase 5 (GRK5), retinol dehydrogenase 10 (RDH10), uracil DNA glycosylase (UNG), 8-oxoguanine DNA glycosylase (OGG1), mutS homolog 2 (MSH2), mutL homolog 2 (MLH2), and MSH6 genes through a nCounter® platform. The operation was following the nCounter protocol.

2.5. Statistical Analysis

All the experimental results were statistically analyzed by unpaired and two-tailed Student's t-test though the Excel software; $p < 0.05$ represented significant difference.

3. Results and Discussion

3.1. Lung Cell Repair

Figure 1 shows the wound healing results of BEAS-2B cells after treatment with 0.5% and 1% of Lung Care drinks. The mean wound healing rate of the cells treated with 1% of Lung Care drink was ~27% higher than that of the control group, but 0.5% of Lung Care drink did not contribute much improvement. The improvements in the wound healing rates showed a dose-dependent tendency. As a result, Lung Care drink might enhance the capabilities of the cell migration and proliferation of BEAS-2B cells and have the potential to improve the repair efficiency of lung cells.

3.2. Phagocytosis in Macrophages

To mimic the phagocytic activity of macrophages in humans, we used the fluorescent microparticles

(1.7-2.2 μm in diameters) to investigate the phagocytosis of macrophages for PM2.5 removal. It has been reported that high levels of PM2.5 (over 38.98 μg/m3) may increase the susceptibility of COPD in non-smonking individuals [25,26]. THP-1 macrophage cells were treated with 0.25% and 0.5% of Lung Care drinks and 0.5% fluorescent microparticles, and the phagocytic activities were identified by the fluorescence intensity signals in the THP- 1 cells (Figure 2). 0.25% and 0.5% of Lung Care drinks could increase the fluorescent intensity signals by 43.7 % and 47%, respectively, as compared with the result of the control group. In accordance with the results, Lung Care significantly ameliorated the phagocytic activity of macrophages, which implies that Lung Care drink may somewhat improve the capability of the respiratory protection from air pollution.

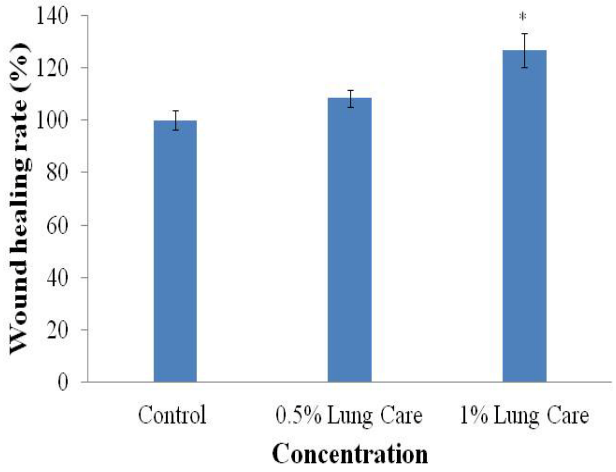

Figure 1. Analysis of wound healing efficiency for the BEAS-2B cells after treatment with 0.5% and 1% Lung Care drinks. (n = 3, mean ± S.D.; *, $p < 0.05$)

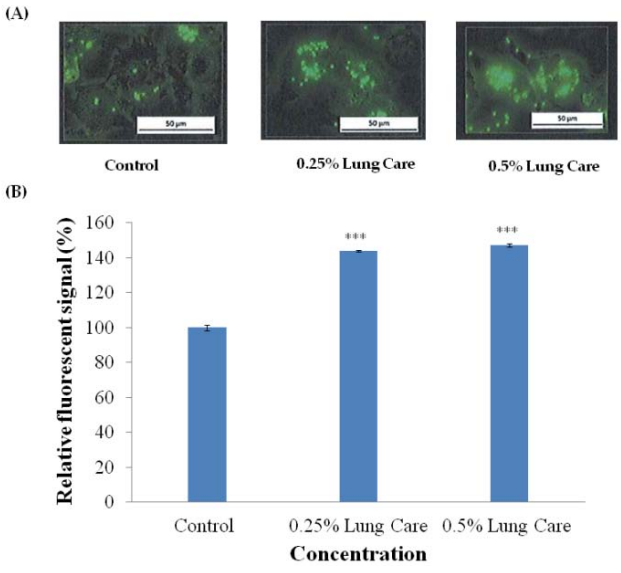

Figure 2. Analysis of phagocytosis in macrophages – THP-1 cells after treatment with 0.25% and 0.5% of Lung Care drinks. (A) Fluorescence images. (B) Histogram result. (n = 3, mean ± S.D.; ***, $p < 0.001$)

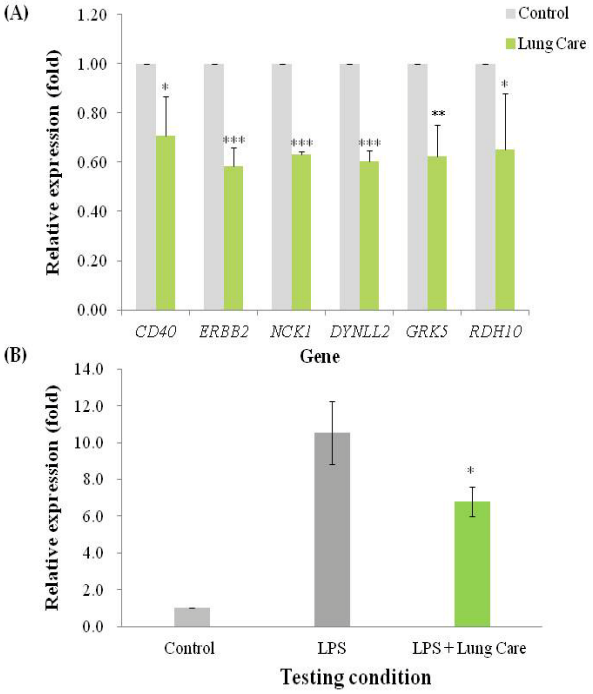

Figure 3. Gene analysis. (A) The expression of allergy-/cancer-related genes for BEAS-2B cells after treatment with 0.25% of Lung Care drink. (B) The expression of IL-6 gene for the BEAS-2B cells after treatment with 200 ng/mL LPS or 200 ng/mL LPS and 0.5% of Lung Care drink (the significant meaning in comparison with the LPS group). (n = 3, mean ± S.D.; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

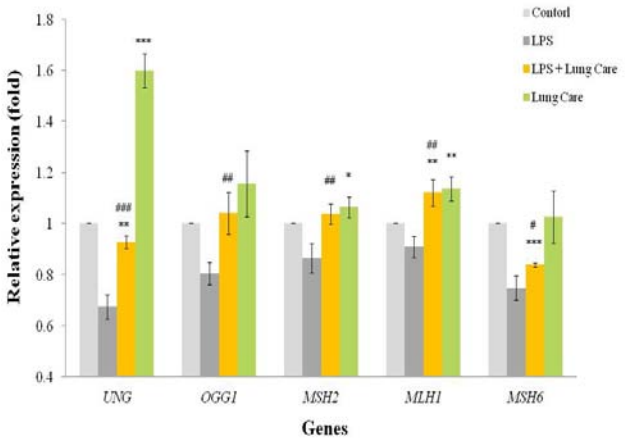

Figure 4. Gene analysis of DNA mismatch repair genes. BEAS-2B cells were treated with 200 ng/mL LPS or 200 ng/mL LPS and 0.25% of Lung Care drink (asterisk symbol means the comparison with the control; number sign means the comparison the LPS). (n = 3, mean ± S.D.; */#, $p < 0.05$; **/##, $p < 0.01$; ***/###, $p < 0.001$)

3.3. Gene Analysis

We further conducted gene analysis regarding respiratory diseases (e.g., allergy) and lung cancer. Figure 3A shows the significant improvement in down-regulation of the mRNA expression levels of *CD40*, *ERBB2*, *NCK1*, *DYNLL2*, *GRK5*, and *RDH10* genes. It is imperative for the CD40-CD40 ligand interaction to

modulate T cell-mediated activation of allergen-deprived bronchial hyperresponsiveness (asthma) [27]. The up-regulation of ERBB2 signaling networks (e.g., MAPK signal transduction) is involved in the asthma pathogenesis [28,29]. On the other hand, the down-regulation of NCK1 eliminates pro-inflammatory cytokine expression which is and macrophage infiltration [30]. Hence, the reduction of the expression of the three genes may profoundly ameliorate the onsets of asthma and allergy. Interestingly, we also discovered that the pear formula could influence some vital genes for cancer formation or cancer patient survival rate. GRK5 affects cancer cell cycle progression through the modulation of p53 phosphorylation and degradation, and its abnormality may give rise to the development of non-small-cell lung cancer [31]. RDH10 regulates the synthesis of retinoic acid (RA) and the RA signaling, for example, RDH10 mutation commonly occurs in malignant non-small-cell lung cancer [32]. With respect to the cancer treatment, single nucleotide polymorphisms of DYNLL2 affect the response of chemotherapy in lung cancer patients [33]. Although the exact anti-cancer benefits of the pear-based formula require further investigation, we, more and less, have confirmed that the formula might possibly interfere with the cancer metabolism in this study. Also, we looked into the representative cytokine genes under the circumstance of an additional stimulation. Figure 3B unveils that the Lung Care significantly reduced IL-6 expression by 3.7 folds as compared to the LPS group. Some scientific reports revealed that PM2.5 organic extract prompted the increase of IL-6 expression in BEAS-2B cells and aggravated the chronic inflammatory response, frequently observed in COPD [34] As such, the formula can be used to alleviate the inflammatory phenomenon and probably achieve certain prevention benefits for respiratory diseases. On top of that, it is no doubt that cancer formation is subject to the accumulation of oxidative damage to DNA, so appropriate DNA mismatch repair (DMR) and base excision repair (BER) systems are quite important to stop the onset of cancer. The DNA repairs contributed by *UNG*, *OGG1*, *MSH2*, *MLH2*, and *MSH6* genes heavily influence the development of lung cancer [35,36,37]. Lung Care could enhance these genes expression levels in both original and LPS-induced BEAS-2B cells (Figure 4). The high expression of these genes sustains better efficiencies of DMR and BER for rectifying oxidative stress-associated DNA damage.

4. Conclusion

In conclusion, this research demonstrates a possibility of pear-based formula for respiratory care. The cell experiment results show that the pear-based formula facilitated the increase of the wound healing rate and galvanized the phagocytosis in macrophages for removing PM2.5-like microparticels. The gene analysis indicates that the formula could down-regulate the inflammatory response and lung cancer-correlated genes, and up-regulate DNA mismatch repair genes in connection with the risk of lung cancer. As a result, the pear formula may consolidate the anti-oxidative capability by mitigating the corresponding inflammatory response to allergy and improve the detoxification system and DNA mismatch correction. Thus, we believe that the pear extract is a good candidate for the development of respiratory protection drinks for daily care use.

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