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Beta-elemene alleviates cigarette smoke-triggered inflammation, apoptosis, and oxidative stress in human bronchial epithelial cells, and refrains the PI3K/AKT/mTOR signaling pathway

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Abstract
Background: Chronic obstructive pulmonary disease (COPD) is a grievous disease that adversely affects human health and life. β-elemene is a type of sesquiterpenoid extracted from *Curcuma wenyujin* (Zingiberaceae) and displays effects on suppressing tumor growth. However, the regulatory impact of β-elemene in COPD development is not reported.
Objective: This study explored the functioning of β-elemene in the progression of COPD.
Material and Methods: The cell survival rate was confirmed through Cell Counting Kit-8 (CCK-8) assay. The cell apoptosis was evaluated through flow cytometry. The protein expressions were examined through western blot. The levels of malondialdehyde (MDA), superoxide dismutase (SOD) and reactive oxygen species (ROS) were examined through the corresponding commercial kits. The levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β were inspected through Enzyme-Linked Immunosorbent Assay (ELISA).
Results: The study demonstrated that β-elemene exaggerated cell viability and reduced cell apoptosis in BEAS-2B human bronchial epithelial cell line stimulated by cigarette smoke extract (CSE). Oxidative stress was heightened after 5% CSE induction, but this impact was counteracted by β-elemene treatment. In addition, enhancive inflammation induced by cigarette smoke was attenuated by β-elemene treatment. Finally, our results indicated that the triggered the phosphatidylinositol 3-kinase-protein kinase B-mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway mediated by cigarette smoke was refrained by β-elemene treatment.
Conclusion: It was concluded that β-elemene reduced cigarette smoke-triggered inflammation, apoptosis, and oxidative stress in human bronchial epithelial cell line, and refrained PI3K/AKT/mTOR signaling pathway. This study proposed that β-elemene could act as a hopeful drug for the treatment of COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is featured by progressive airway obstruction and chronic inflammation, thereby resulting into emphysema and chronic bronchiolitis.[1] If no timely treatment is provided, this disease can generate irreversible damage to breathing and even lead to patient's death.[2] COPD is estimated to produce over 3 million deaths annually, and projected to result into about 8 million deaths by 2030.[3,4] Inflammation in COPD immensely promotes the occurrence of comorbidities, including lung cancer.[5] Smoking influences the immune system, and it is one most important inducement in COPD.[6,7] Multiple negative effects of cigarette smoke on airways include immunologic aspects and second-hand exposure.[8,9] Although some treatments were utilized in treating COPD patients,[10,11] these were not very ideal. Therefore, it is indispensable to understand further the pathogenesis of COPD, seek useful drugs, and produce novel therapeutic approaches.

β-elemene, a type of sesquiterpenoid, is extracted from *Curcuma wenyujin*,[12] and is proved to retard tumor growth. For example, in non-small cell lung cancer, β-elemene evokes the focal adhesion kinase–steroid receptor coactivator (FAK-Src) pathway to restrain cell migration and invasion.[13] Moreover, in colorectal cancer, β-elemene modulates the *reactive oxygen species*–adenosine monophosphate-activated protein kinase–mechanistic target of rapamycin (ROS/AMPK/mTOR) pathway to trigger cell apoptosis and autophagy.[14] Besides, β-elemene affects the FAK/claudin-1 pathway in gastric cancer to refrain peritoneal metastasis.[15] Importantly, it was shown that β-elemene affected anti-inflammation and anti-oxidative stress. For instance, β-elemene retards the Janus kinase 2–signal transducer and activator of transcription 3–nuclear factor *kappa B* (JAK2/STAT3/NF-κB) pathway to mitigate hyperglycemia-evoked cardiac inflammation and remodeling.[16] β-elemene weakens ROS generation and inflammation in atherosclerosis.[17] Furthermore, β-elemene modulates nitric oxide (NO) level and relieves oxidative stress to improve atherosclerosis.[18] However, the regulatory effects of β-elemene in the development of COPD were kept vague.

In this work, it is manifested that β-elemene alleviated cigarette smoke-triggered inflammation, apoptosis, and oxidative stress in human bronchial epithelial cells, and refrained the phosphatidylinositol 3-kinase–protein kinase B–mTOR (PI3K/AKT/mTOR) signaling pathway. This work could offer useful insights in the treatment of COPD.

Materials and Methods

Cell culture and treatment

Human bronchial epithelial cell line (BEAS-2B) was acquired from iCell Bioscience (Shanghai, China). In one incubator (37°C, 5% CO_2), BEAS-2B cells were cultivated with Dulbecco's Modified Eagle Medium (DMEM; Gibco, Miami, FL, USA), including 10% fetal bovine serum (FBS; Gibco).

β-elemene (5, 10, and 20 μg/mL; Apexbio, Boston, MA, USA) was utilized to treat BEAS-2B cells.

Treatment of cigarette smoke extract (CSE)

The culture medium of BEAS-2B cells was added with CSE to induce COPD cell model. Through one modified syringe-driven apparatus, one cigarette (Marlboro; Longyan Tobacco Industrial Co. Ltd., Fujian, China) was combusted to generate smoke, and smoke was pumped into DMEM (5 mL) to produce 100% CSE solution, which was filtered using aseptic 0.22-μm filter, and diluted into 5% CSE solution for incubating BEAS-2B cells. The cell survival rate of 50% was confirmed to induce COPD cell model successfully.

Cell counting kit-8 (CCK-8) assay

BEAS-2B cells (1000 cells/well) was set in a 96-well plate. Next, CCK-8 solution (10 μL; Dojindo Laboratories, Kumamoto, Japan) was mixed in each well for 2 h. Eventually, the cell survival rate was notarized through spectrophotometer (Thermo Fisher Scientific, MA, USA).

Flow cytometry

The fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit (BD Biosciences, Franklin Lakes, USA) was used. After washing, BEAS-2B cells were resuspended. Staining (5-μL FITC Annexin V and 10-μL propidium iodide) was performed for BEAS-2B cells in the dark. Eventually, cell apoptosis was inspected through flow cytometer (FACSCalibur™; BD Biosciences, San Jose, CA, USA) with the FlowJo software (Tree Star, Inc., Ashland, USA).

Western blot analysis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE, 10%) was utilized for separating proteins extracted from BEAS-2B cells. Then, proteins were moved to polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). After sealing, membranes were placed with primary antibodies for 12 h. The primary antibodies were as follows: Bcl-2-associated X (Bax; 1/1000; ab32503), B-cell lymphoma-2 (BCL-2; 1/2000; ab182858), cleaved caspase-3 (1/500; ab32042), phosphorylated phosphatidylinositol 3-kinase (p-PI3K; 1/1000; ab235266), PI3K (1/1000; ab191606), phospho-protein kinase B (p-Akt; 1/1000; ab38449), Akt (1/500; ab8805), phosphorylated (p)-mTOR (1/1000; ab109268), mTOR (1/1000; ab32028), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1/500; ab8245). Next, membranes were placed with secondary antibodies (1/2000; ab7090; Abcam, Shanghai, China) for 2 h. The chemiluminescence detection kit (Thermo Fisher Scientific) was used for examining protein blots.

Detection of malondialdehyde (MDA), superoxide dismutase (SOD), and ROS

MDA (ab118970; Abcam), SOD (ab65354), and ROS (ab186027) commercial kits were used for examination.

Enzyme-linked Immunosorbent Serological Assay (ELISA)

Tumor necrosis factor-α (TNF-α; ab181421; Abcam), interleukin-6 (IL-6; ab178013), and IL-1β (ab214025) commercial ELISA kits were used for measurements.

Statistical analysis

Data were displayed as mean ± standard deviation (SD). The GraphPad Prism Software 9 (GraphPad Software, USA) was used for statistical analysis. Differences in groups were evaluated by using one-way analysis of variance (ANOVA). Data followed the normal distribution; $P < 0.05$ was deemed as statistically significant.

Results

β-elemene aggrandized cell viability and reduced cell apoptosis in BEAS-2B cells stimulated by cigarette smoke

First, the chemical formula of β-elemene was exhibited, and different concentrations (5, 10, and 20 μg/mL) of β-elemene had no cytotoxicity (Figure 1A). The cell survival rate was lessened after 5% CSE treatment (from 100 to 49; $P < 0.001$), but this impact was rescued after β-elemene treatment (5, 10, and 20 μg/mL) (from 49 to 71, 82, and 90, respectively; $P < 0.001$) (Figure 1B). Besides, cell apoptosis increased with 5% CSE induction (from 6.18 to 36.13; $P < 0.001$), but this change was counterbalanced by different concentrations of β-elemene treatment (5, 10, and 20 μg/mL) (from 36.13 to 25.78, 21.13, and 15.02, respectively; $P < 0.01$) (Figures 1C-D). Bax and cleaved caspase-3 protein expressions decreased and that of BCL-2 increased after 5% CSE stimulation ($P < 0.001$), but these effects were reversed after β-elemene treatment ($P < 0.05$) (Figure 1E). To sum up, β-elemene aggrandized cell viability and reduced cell apoptosis in BEAS-2B cells stimulated by cigarette smoke.

β-elemene alleviates cigarette smoke-evoked oxidative stress

Next, the regulatory impacts of β-elemene on oxidative stress were explored. Levels of MDA and ROS were aggrandized and that of SOD was decreased after 5% CSE induction ($P < 0.001$); however, these changes were counteracted after β-elemene treatment ($P < 0.001$) (Figures 2A-C). Taken together, β-elemene alleviates cigarette smoke-evoked oxidative stress.

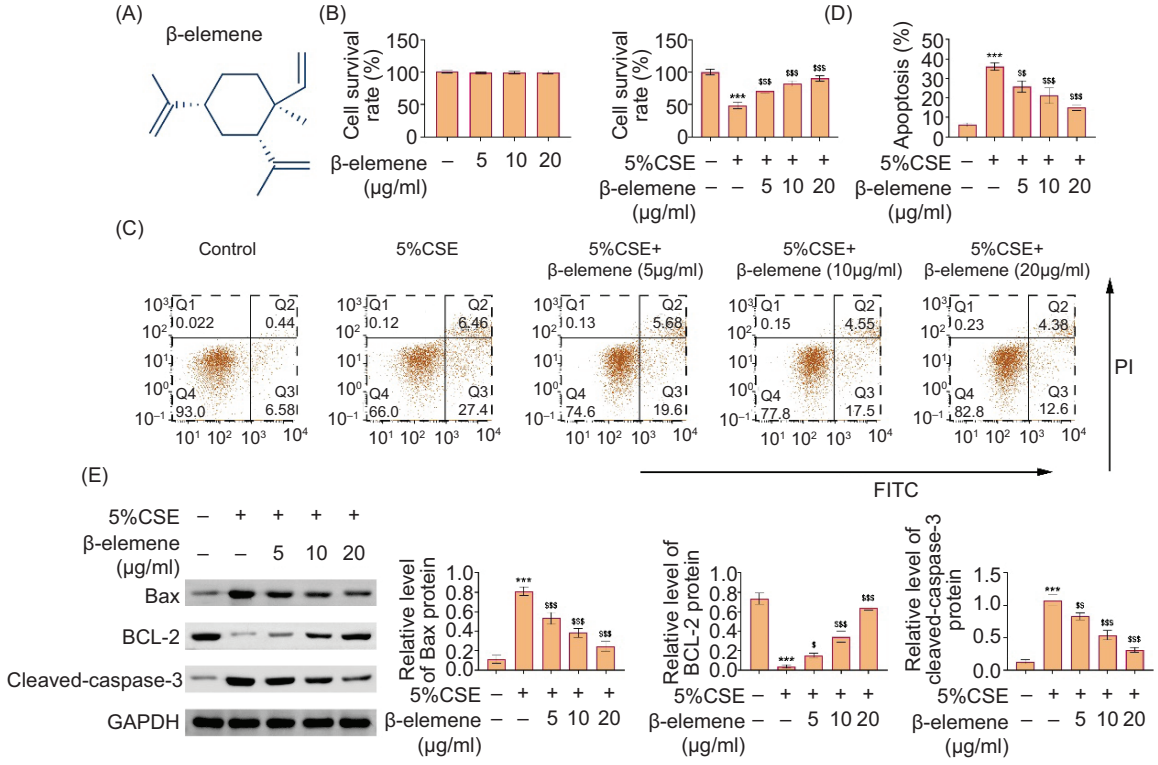

Figure 1 β-elemene aggrandized cell viability and reduced cell apoptosis in BEAS-2B cells stimulated by cigarette smoke. (A) The chemical formula of β-elemene was displayed, and the cytotoxicity of β-elemene (5, 10, and 20 μg/mL) was proved through CCK-8 assay. (B) The cell survival rate was confirmed through CCK-8 assay in control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. (C-D) The cell apoptosis was evaluated through flow cytometry in control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. (E) The protein expressions of Bax, BCL-2, and cleaved caspase 3 were measured through Western blot analysis in control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. ***P < 0.001 versus the control group; $P < 0.05, $$P < 0.01, $$$P < 0.001 versus the 5% CSE group.

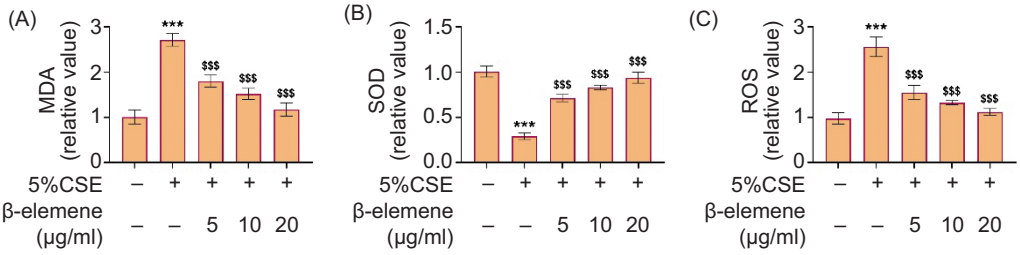

Figure 2 β-elemene can alleviate cigarette smoke-evoked oxidative stress. Groups were separated as follows: control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. (A) The MDA level was assessed through MDA kit. (B) The SOD level was tested through SOD kit. (C) The ROS level was examined through ROS kit. ***P < 0.001 versus the control group; $$$P < 0.001 versus the 5% CSE group.

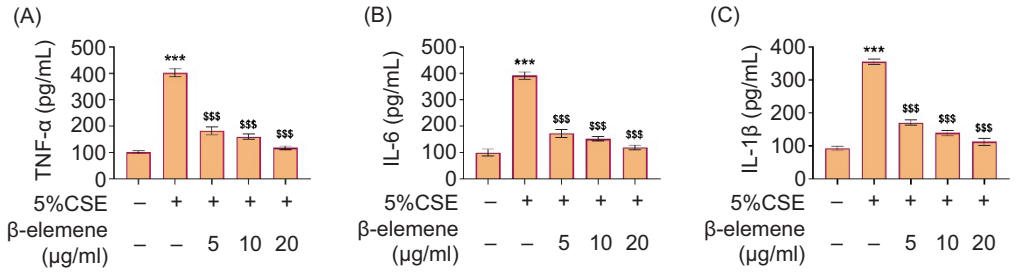

Figure 3 β-elemene attenuated cigarette smoke-induced inflammation. Groups were separated as follows: control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. (A–C) The levels of TNF-α, IL-6, and IL-1β were inspected through ELISA. ***P < 0.001 versus the control group; $$$P < 0.001 versus the 5% CSE group.

β-elemene attenuated cigarette smoke-induced inflammation

The influence of β-elemene on inflammation was investigated through ELISA. The levels of TNF-α, IL-6, and IL-1β elated after 5% CSE stimulation (P < 0.001), but these impacts decreased after β-elemene treatment (P < 0.001) (Figures 3A-C). In general, β-elemene attenuated cigarette smoke-induced inflammation.

β-elemene refrained the PI3K/AKT/mTOR pathway

The protein expressions of p-PI3K/PI3K, p-AKT/AKT, and p-mTOR/m-TOR elevated after 5% CSE induction (P < 0.001), but these phenomena attenuated after β-elemene treatment (P < 0.001) (Figure 4). Briefly, β-elemene refrained the PI3K/AKT/mTOR pathway.

Discussion

Results of the study demonstrated that β-elemene exaggerated cell viability and reduced cell apoptosis in BEAS-2B cells stimulated by cigarette smoke. β-elemene alleviated cigarette smoke-evoked oxidative stress and attenuated cigarette smoke-induced inflammation. In addition, β-elemene refrained the PI3K/AKT/mTOR pathway.

β-elemene displays its effects of suppressing tumor growth, inflammation, and oxidative stress.[13-19] However, the regulatory effects of β-elemene in COPD development are not reported. COPD is accompanied by airway damage and repair, and apoptosis in airway epithelial cells takes part into these processes to modulate COPD development.[20] Similarly, in this study, it was illustrated that β-elemene aggrandized cell viability and reduced cell apoptosis in BEAS-2B cells stimulated by cigarette smoke.

Oxidative stress and inflammation are pivotal processes in the development of COPD.[21] Many researchers have focused on the modulation of oxidative stress and inflammation in COPD progression. For example, alantolactone restrains inflammation and oxidative stress in COPD.[22] In addition, surfactant protein D suppresses lung inflammation in COPD.[23] Astaxanthin mitigates oxidative stress and inflammation triggered by cigarette smoking in COPD.[24] Furthermore, the LINC00987/let-7b-5p/SIRT1 axis affects oxidative stress, autophagy, and inflammation evoked by lipopolysaccharides (LPS) in COPD.[25] Similar to these findings, this work revealed that oxidative stress was heightened after 5% CSE induction, but this impact was neutralized after β-elemene treatment. Besides, the augmentative inflammation induced by cigarette smoke is attenuated after β-elemene treatment.

The PI3K/AKT/mTOR pathway was verified as a key pathway in progression of COPD. For example, puerarin evokes the PI3K/AKT/mTOR pathway to suppress mitochondrial autophagy and cell apoptosis in CSE-triggered human bronchial epithelial cells.[26] Moreover, S-allylmercapto-N-acetylcysteine (ASSNAC) retards the PI3K/Akt/mTOR pathway to improve elastase-stimulated COPD.[27] Endothelial alpha-enolase (ENO1) modulates the PI3K/Akt/mTOR pathway to relieve pulmonary hypertension.[28] Importantly, it was clarified that β-elemene blocks the PI3K/AKT/mTOR pathway in non-small cell lung cancer

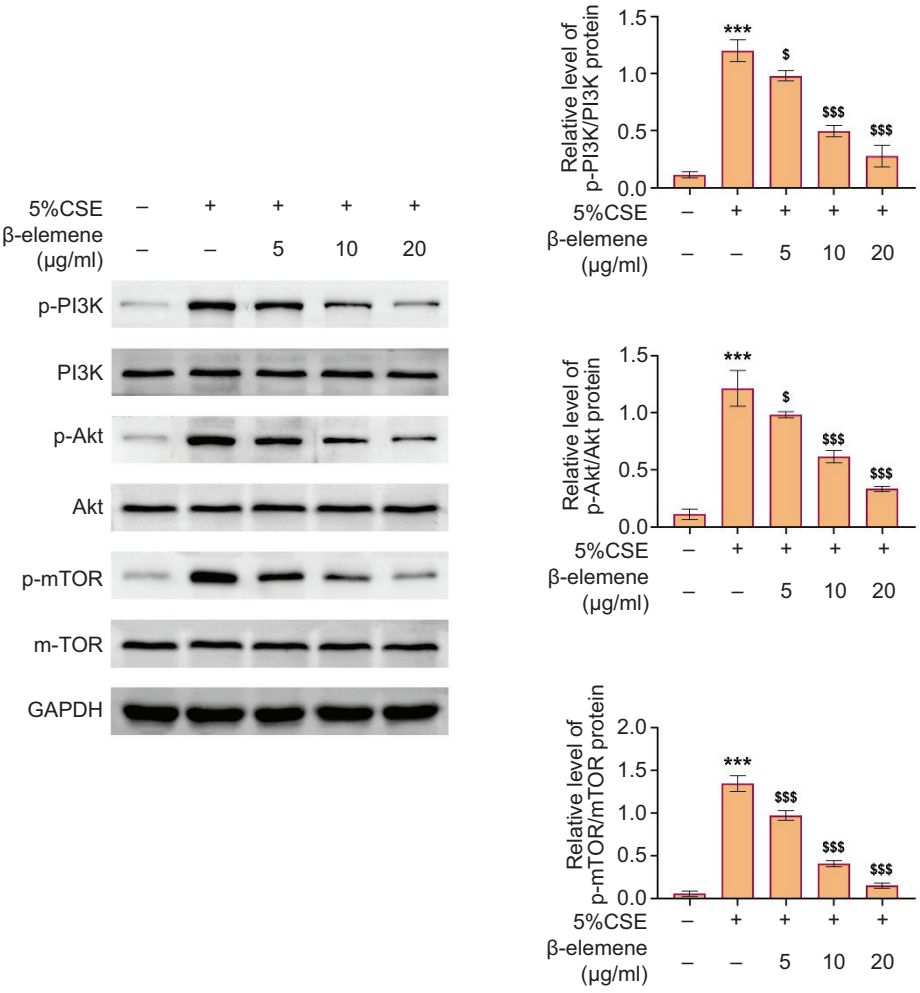

Figure 4 β-elemene refrained the PI3K/AKT/mTOR pathway. Groups were separated as follows: control, 5% CSE, 5% CSE+β-elemene (5 μg/mL), 5% CSE+β-elemene (10 μg/mL), and 5% CSE+β-elemene (20 μg/mL) groups. The protein expressions of p-PI3K, PI3K, p-AKT, AKT, m-TOR, and p-mTOR were determined through Western blot analysis. ***P < 0.001 versus the control group; $P < 0.05, $$$P < 0.001 versus the 5% CSE group.

and renal-cell carcinoma.[29,30] However, the regulatory function of β-elemene in the regulation of the PI3K/AKT/mTOR pathway in COPD progression remains unclear. Similarly, our data indicated that the triggered PI3K/AKT/mTOR pathway mediated by cigarette smoke was refrained after β-elemene treatment.

Conclusion

The study was the first to manifest that β-elemene alleviates cigarette smoke-evoked inflammation, apoptosis, and oxidative stress in human bronchial epithelial cells, and refrains the PI3K/AKT/mTOR signaling pathway. Our results provide novel clinical perspectives of β-elemene for treating COPD. However, this work also has some limitations, such as the scarcity of animal model, clinical investigations, and exploration of other cellular phenotypes. In the future, a thorough exploration of β-elemene in the progression of COPD progression must be endorsed.

Competing Interests

The authors stated that there was no conflict of interest to disclose.

Data Availability

All data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Yan Li and Li Zhang designed and carried the study. Both authors supervised data collection, analyzed and interpreted the data, and prepared the manuscript for publication. Both reviewed draft of the manuscript, and read and approved the final manuscript.

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