Ind. J. Pharm. Edu. Res., 2026; 60(1):421-430.
https://www.ijper.org

Original Article

Bilobetin Shows Immunomodulatory and Chemopreventive Activities in Benzo(a)pyrene-Induced Lung Cancer in Mice Model

Zhenjie Xu[1,#], Lihuan Zhu[2,#], Shentao Wang[3,*]

[1]Department of Laboratory Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou Guangdong, CHINA.
[2]Department of Respiratory and Critical Care Medicine, Ezhou Central Hospital, Ezhou Hubei, CHINA.
[3]Department of Pathology and Pathophysiology, College of Basic Medicine Qilu Medical University, Zibo Shandong, CHINA.
[#]These authors have contributed equally to this work.

ABSTRACT

Background: Lung cancer, a malignancy resulting from the uncontrolled growth of cells in pulmonary tissues, remains to be a primary cause of cancer-associated deaths worldwide, requiring a thorough understanding of its complex pathophysiology to develop effective treatment techniques. **Objectives:** The present work was aimed at analyzing the anticancer properties of the bilobetin against benzo(a)pyrene (B(a)P)-induced lung cancer in mice. **Materials and Methods:** Lung cancer was induced in mice with oral treatment of B(a)P for four consecutive weeks (from second to sixth week). Bilobetin (25 mg/kg) was provided to the mice one week prior to and throughout the 16 weeks via oral gavage. The concentrations of phase-I and -II detoxifying enzymes, immunoglubulins (Igs), and xenobiotic dysfunction markers were assessed in the experimental mice. The concentrations of tumor biomarkers, pro-inflammatory cytokines, and other tumor-related molecular markers were assessed using respective diagnostic kits. The inflammatory cells and phagocytic and avidity indexes were also evaluated in the experimental mice. **Results:** The present results evidenced that the treatment of 25 mg/kg of bilobetin significantly regulated the concentrations of phase-I and -II enzymes, Igs levels, and reduced the xenobiotic dysfunction markers in B(a)P-induced mice. Moreover, the bilobetin considerably decreased the tumor biomarker levels, inflammatory cytokine levels, and increased the inflammatory cells in B(a)P-treated mice. The concentrations of PCNA, NF-κB, CYP1A1, and NRF-2 was considerably reduced by the bilobetin treatment in the B(a)P-treated mice. **Conclusion:** Our results demonstrate that bilobetin treatment exhibits immunomodulatory and chemopreventive properties in lung cancer in mice. Consequently, it was evident that bilobetin treatment possesses immunomodulatory and chemopreventive properties against lung cancer and may enhance chemotherapy approaches for lung cancer.

Keywords: Cytochrome P450, Immunoglobulins, Glutathione-S-transferase, Bilobetin, Lung cancer.

Correspondence:
Dr. Shentao Wang
Department of Pathology and Pathophysiology, College of Basic Medicine Qilu Medical University, Zibo Shandong-255300, CHINA.
Email: shentao-wang@outlook.com

Received: 27-05-2025;
Revised: 14-07-2025;
Accepted: 02-09-2025.

INTRODUCTION

Lung cancer, a pervasive and devastating malignancy, has steadily increased in incidence and mortality in each year worldwide, establishing itself as a major cause of cancer-associated mortalities globally. The definition of lung cancer centers on the uncontrolled growth of malignant cells within the lung tissues, leading to the formation of tumors that can metastasize and invade other parts of the body.[1] Lung cancer is defined into two subtypes: Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC), each exhibiting distinct clinical behaviors and requiring tailored treatment strategies. NSCLC accounts for 85% of all incidences. Despite advancements in therapeutic interventions, the prognosis for lung cancer remains poor, with a mere 10% to 20% five-year survival rate in numerous countries.[2] The global burden of lung cancer is disproportionately distributed, with higher incidence and mortality rates observed in developed nations, particularly among individuals aged 65 to 84 years.[3] The rise in lung cancer cases is particularly pronounced in developing countries, where approximately half of all diagnoses now occur. The burdens associated with lung cancer extend beyond mortality, encompassing significant morbidity, reduced quality of life, and substantial economic costs for patients, families, and healthcare

DOI: 10.5530/ijper.20262240

systems. Lung cancer accounts for approximately one in five cancer deaths worldwide.[4]

The etiology of lung cancer is multifactorial, involving a complex interplay of genetic predisposition, environmental exposures, and lifestyle factors. Tobacco smoking stands out as the predominant risk factor, implicated in approximately 80-90% of lung cancer incidences. Other established causes include exposure to radon, asbestos, and other occupational carcinogens, as well as air pollution and certain genetic mutations. The association between lung infections, such as tuberculosis, and lung cancer has garnered increasing attention, suggesting a potential link between chronic inflammation and carcinogenesis.[5,6] The pathogenesis of lung cancer involves several molecular and cellular events that disrupt normal lung tissue homeostasis. These alterations result in uncontrolled cell growth, escape of apoptosis, and metastasis. Genetic and epigenetic alterations are hallmarks of lung cancer, with frequent mutations noted in genes like EGFR, KRAS, TP53, and ALK. These mutations can drive tumor development and progression, and they also serve as therapeutic targets for personalized medicine approaches.[7] Furthermore, lung cancer is highly heterogeneous, arising from different sites in the bronchial tree, leading to varying symptoms depending on the anatomic location.[8]

Lung cancer continues to pose a substantial worldwide health issue despite progress in diagnosis and therapies. The complex nature of lung cancer necessitates a multifaceted approach, integrating conventional therapies such as surgery, radiotherapy, chemotherapy, and targeted therapy.[9] Treatment decisions are based on cancer type and stage, but responses to treatment strategies are poor, except for localized tumors. The limitations and challenges associated with current lung cancer treatments underscore the urgent need for innovative therapies that can overcome resistance mechanisms, improve treatment effectiveness, and improve patient outcomes.[10] Plant-derived bioactive compounds have garnered increasing attention as potential therapeutic agents for lung cancer, owing to their diverse pharmacological activities and favorable safety profiles. Many plant-derived compounds are being studied for their anticancer properties, offering a rich source of potential therapeutic agents. It has been shown that plant-derived compounds can inhibit the growth of lung cancer cells.[11] Bilobetin is a natural bioactive bioflavonoid compound widely occur in the Ginkgo biloba and some other gymnosperm plants. Several previous studies has already mentioned the various biological activities of bilobetin, including antioxidant, antimicrobial, and anti-inflammatory effects.[12,13] Furthermore, it has been also found that bilobetin showed anticancer activity,[14] ameliorated insulin resistance in rat model,[15] and protected the cisplatin-induced testicular toxicity.[16] However, there are no much evidences for the anticancer activity of bilobetin against lung cancer. Therefore, this work was focused at analyzing the anticancer properties of the bilobetin against benzo(a)pyrene (B(a)P)-induced lung cancer in mice.

MATERIALS AND METHODS

Chemicals

B(a)P and bilobetin were acquired commercially from Sigma Aldrich, St. Louis, USA. The biomarker levels were assessed with commercial diagnostic kits sourced from My Bio Source and Abcam, USA, respectively. All additional reagents acquired were of the utmost experimental grade quality.

Experimental mice

Healthy male Swiss-albino mice were acquired from institutional animal house and were treated humanely as per the Institutional Animal Ethical Committee guidelines, as well as adhering to ARRIVE guidelines. Mice were housed in infection-free and dry polypropylene confines, sustained under a 12-hr light and 12-hr dark cycles, with a humidity of 25±2°C.

Experimental groups

The experimental mice were distributed into four experimental groups with six mice in each (n=6). Group I: Control mice administered corn oil orally as vehicle for 16 weeks; Group II: Mice administered an oral dose of 50 mg/kg of B(a)P biweekly for 4 consecutive weeks (from second to sixth week) to induce lung cancer; Group III: Mice received a continuous oral treatment of 25 mg/kg of bilobetin one week before to and throughout 16 weeks, following a schedule similar to Group II; Group IV: Mice received oral treatment with 25 mg/kg of bilobetin alone for 16weeks without the B(a)P induction. Following the termination of treatments, the mice from all groups were sacrificed under anesthesia using cervical dislocation, and samples were obtained for future investigations.

Analysis of immune complexes

The avidity index was examined using the technique described by Chia *et al.*[17] The Muniz-Junqueira *et al.*[18] Approach was employed to assess phagocytic index. The Nitroblue Tetrazolium (NBT) reduction test and Soluble Immune Complexes (SIC) were evaluated assessed using the methodologies of Choi *et al.*[19] and Chen *et al.*,[20] respectively. The immune cell counts such as neutrophils, leucocytes, lymphocytes, and absolute neutrophil and lymphocytes were performed based on the previous technique by Boyum *et al.*[21]

Analysis of phase-I and -II detoxifying enzymes

The concentrations of cyt-b5, cyt-P450, and NADPH-cyt-c-reductase were assessed using the methodologies of Omura and Sato,[22] and Wharton and Tzagoloff,[23] respectively. The Glutathione-S-Transferase (GST), Uridine 5'-Diphospho-Glucuronosyltransferase (UDP-GT), and Quinone

Reductase (QR) were evaluated using the methodologies established by Habig *et al.*,[24] Luquita *et al.*,[25] and Benson *et al.*,[26] respectively.

Analysis of immunoglobulin (Ig) levels

The levels of Ig-A, Ig-M, and Ig-G in the serum was examined using the commercial test kits. The experiments were conducted in triplicate as per the manufacturer's specifications (Elabscience, USA).

Analysis of xenobiotic dysfunction and biochemical markers

The levels of Gamma Glutamyltransferase (GGT), 5′-Nucleotidase (5′-NT), Aryl Hydrocarbon Hydroxylase (AHH), Adenosine Deaminase (ADA), and Lactate Dehydrogenase (LDH) in liver tissue homogenates were evaluated utilizing diagnostic kits (MyBioSource, USA). The Cytochrome P450 1A1 (CYP1A1), Nuclear Factor Erythroid 2 related Factor-2 (NRF-2), Nuclear Factor-κB (NF-κB), and Proliferating Cell Nuclear Antigen (PCNA) in the lung tissue homogenates were investigated using the commercial test kits (MyBioSource, USA). The studies were performed in triplicate according to the manufacturer's specifications.

Analysis of Cytokeratin Fragment-19 (CYFRA 21-1) and Carcinoembryonic Antigen (CEA) levels

The serum CYFRA 21-1 and CEA concentrations in the experimental mice were assessed using commercially available diagnostic kits from My Bio Source, USA. All tests were conducted with three replicates as per the manufacturer's specifications.

Analysis of inflammatory cytokines

The serum IL-6, IL-1β, and TNF-α in the experimental animals were investigated using diagnostic kits (Abcam, USA). All tests were conducted in triplicates in accordance to the manufacturer's protocols.

Statistical analysis

Statistical assays were done using GraphPad software. Data were depicted as the mean ± SD from triplicates. The disparities among treatment groups were examined using one-way ANOVA with Tukey's *post hoc* assay. A p-value <0.05 was fixed to define the significance.

RESULTS

Effect of bilobetin on the phase-I and phase-II detoxifying enzymes in experimental mice

The current study noted increased cyt-b5, cyt-P450, and NADPH-cyt-c-reductase concentrations and diminished GST, UDP-GT, QR concentrations in the serum samples of the B(a)P-treated mice with lung cancer compared to other groups. Conversely, the bilobetin treatment at 25 mg/kg concentration markedly diminished the cyt-b5, cyt-P450, and NADPH-cyt-c-reductase levels while elevating the GST, UDP-GT, QR concentrations in the B(a)P-induced mice, as illustrated in Figure 1. Notably, the 25 mg/kg of bilobetin alone treatment had a negligible impact on the levels of these detoxifying enzymes in the mice. These findings indicate that administering bilobetin to the B(a)P-induced mice may be beneficial in detoxifying the carcinogen in the B(a)P-induced mice.

Effect of bilobetin on the Igs and xenobiotic dysfunction markers in the experimental mice

Figure 2 illustrates the effects of bilobetin treatment on the levels of Igs and xenobiotic dysfunction markers in the experimental mice. A reduction in IgG and IgM and an elevation in IgA concentrations were noted in the B(a)P-exposed mice than the control. Furthermore, increased concentrations of GGT, 5′-NT, AHH, and LDH was found in B(a)P-treated mice. Fascinatingly, the treatment of 25 mg/kg of bilobetin significantly regulated the Igs concentrations and diminished the5′-NT, AHH, GGT, and LDH concentrations in the B(a)P-treated mice. Furthermore, the levels of these Igs and xenobiotic dysfunction markers in mice remained mainly unaffected by bilobetin (25 mg/kg) treatment alone.

Effect of bilobetin on immune cells, avidity and phagocytic index, and SIC in experimental mice

Figure 3 illustrates the effects of bilobetin on the immune cells and immune complexes in the experimental animals. The B(a)P-treated mice exhibited reduced levels of inflammatory cells, including lymphocytes, neutrophils, leucocytes, and absolute neutrophils and lymphocytes when compared with control. In addition, the B(a)P-exposed mice also demonstrated reduced avidity and phagocytic index, NBT reduction, and enhanced SIC in comparison to the control mice. Interestingly, these alterations were well regulated by the bilobetin treatment. The concentrations of immune cells were elevated in B(a)P-exposed mice following the administration of 25 mg/kg of bilobetin. A notable elevation in the avidity and phagocytic indexes, NBT reduction, and a decrease in SIC was also detected in the bilobetin-treated mice (Figure 3). Furthermore, 25 mg/kg of bilobetin alone treatment did not significantly alter the levels of these parameters in the mice. The data indicated that bilobetin exhibited considerable immunomodulatory effects on the B(a)P-exposed mice.

Effect of bilobetin on tumor biomarkers and inflammatory cytokines in experimental mice

The tumor biomarkers and inflammatory cytokine concentrations in the experimental mice was evaluated with the results depicted in Figure 4. As indicated in Figure 4, the B(a)P-induced mice depicted a substantial augmentation in IL-1β, IL-6, and TNF-α concentrations in comparison to the control. Furthermore, the

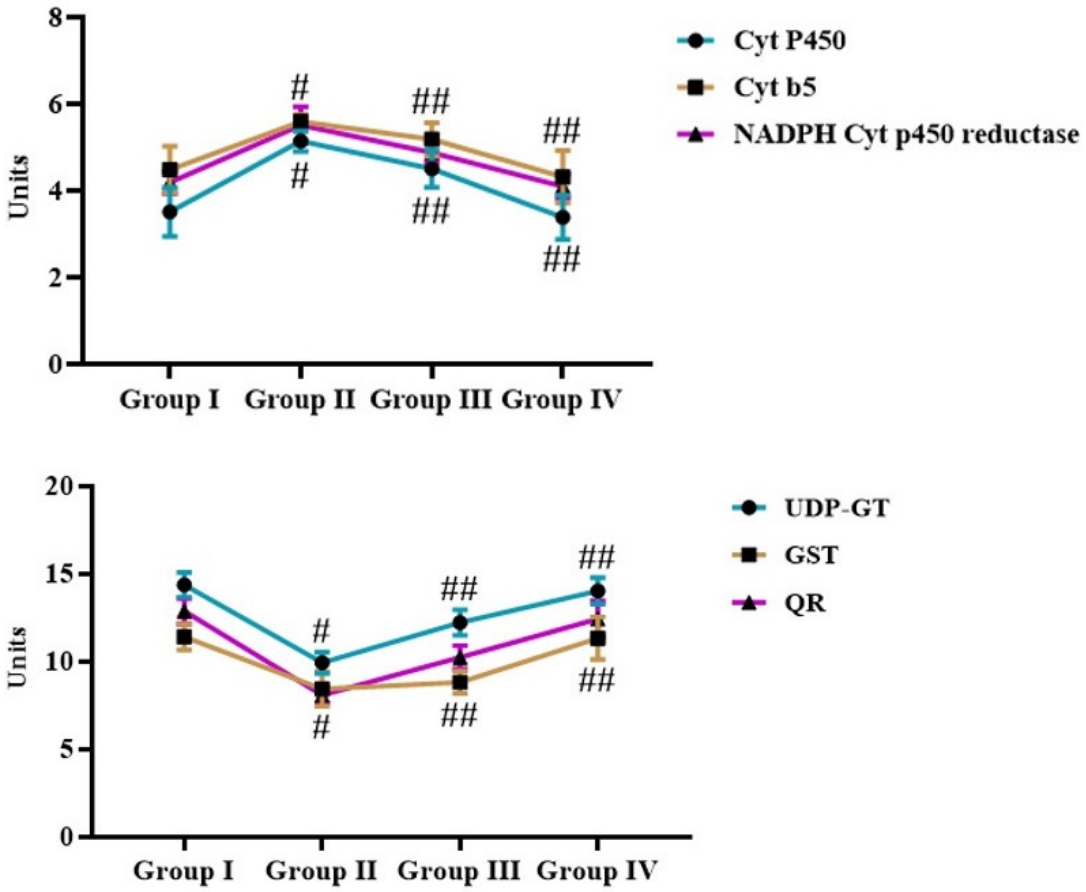

Figure 1: Effect of bilobetin on the phase-I and -II detoxifying enzymes in the experimental mice. The data are expressed as a mean±SD of three replicates. The data are statistically evaluated using one-way ANOVA, subsequently applying Tukey's *post hoc* assay to evaluate the differences between treatment groups. Note: '#' denotes significance at $p < 0.01$ compared to the control group, whereas '##' denotes significance at $p < 0.05$ compared to the B(a)P-induced group.

augmented CEA, CYFRA 21-1, and ADA levels were also noted in the B(a)P-treated mice. Captivatingly, the administration of bilobetin (25 mg/kg) significantly diminished the inflammatory cytokine levels and also subsequently reduced the tumor biomarker concentrations in the B(a)P-induced mice. In addition, the administration of 25 mg/kg of bilobetin alone had no pronounced effect on the levels of these parameters in experimental mice.

Effect of bilobetin on CYP1A1, PCNA, NF-κB, and NRF-2 levels in experimental mice

The quantities of CYP1A1, NF-κB, PCNA, and NRF-2 in the lung tissues of the experimental mice were evaluated, and the findings are depicted in Figure 5. The elevated CYP1A1, PCNA, NF-κB, and NRF-2 concentration were seen in the lung tissues of B(a)P-treated mice in comparison to the control. However, the 25 mg/kg of bilobetin markedly diminished the levels of CYP1A1, PCNA, NF-κB, and NRF-2 in the lung tissues of B(a)P-treated mice with lung cancer (Figure 5). In addition, mice treated with bilobetin (25 mg/kg) alone showed no significant changes in the levels of these biomarkers.

DISCUSSION

Lung cancer, a malignancy arising from the uncontrolled growth of cells within the pulmonary tissues, remains a pivotal cause of cancer-associated deaths worldwide, requiring a comprehensive understanding of its intricate pathophysiology to devise effective therapeutic strategies. The process of carcinogenesis, which involves a series of genetic and epigenetic alterations, can now be approached at the molecular level, leading to advances in prevention, diagnosis, and treatment. Understanding the molecular basis of lung cancer is essential for advancing diagnostic, staging, therapeutic, and preventive strategies.[27] The absence of uniform pathomechanism in NSCLC leads to the absence of a standardized treatment approach. Molecular analysis has revealed that lung cancer exhibits marked heterogeneity, both histologically and molecularly, with distinct subtypes displaying unique genetic and signaling pathway aberrations that drive tumor initiation, progression, and metastasis. The problem stems from the extensive variety of driver mutations, and consequently, the multitude of cancer cell genotypes that regulate the carcinogenic process in the lungs.[28]

Phase-I and -II enzymes play a pivotal role in the metabolism of xenobiotics, including pro-carcinogens like B(a)P, a ubiquitous environmental pollutant implicated in the progression of lung cancer. Phase I enzymes, such as cyt-P450s, catalyze oxidation, reduction, and hydrolysis reactions, often converting pro-carcinogens into more reactive intermediates. Cyt-b5 and NADPH-cytochrome c reductase are critical components of the cyt-P450 enzyme system, playing essential roles in electron transfer and enzymatic activity.[29] The balance between phase-I activation and -II detoxification determines the ultimate fate of these compounds, influencing their carcinogenic potential. In contrast, phase-II enzymes, including GSTs, UDP-GT, and QR, facilitate the conjugation of these reactive intermediates with endogenous molecules rendering them water-soluble and excretable. The coordinated actions of these enzymes dictate the cellular response to benzopyrene exposure and impact the predisposition to lung cancer development.[30] The analysis of cyt-b5, cyt-P450, and NADPH-cytochrome c reductase activities provides valuable insights into the phase I metabolic capacity of lung tissues, reflecting the ability to activate pro-carcinogens into carcinogenic metabolites. Assessing GST, UDP-GT, and QR activities offers insights into the phase II detoxification capacity of lung tissues, reflecting the ability to neutralize reactive metabolites and prevent DNA injury.[31] Analyzing these enzymes in the benzopyrene-exposed mice can offer useful knowledge about the metabolic pathways and effectiveness of detoxification mechanisms.[32] The present findings revealed the increased cyt-b5, cyt-P450, and NADPH-cyt-c-reductase concentrations and diminished GST, UDP-GT, QR concentrations in the B(a)P-treated mice with lung cancer. Interestingly, the bilobetin treatment successfully reduced the cyt-b5, cyt-P450, and NADPH-cyt-c-reductase levels while elevating the GST, UDP-GT,

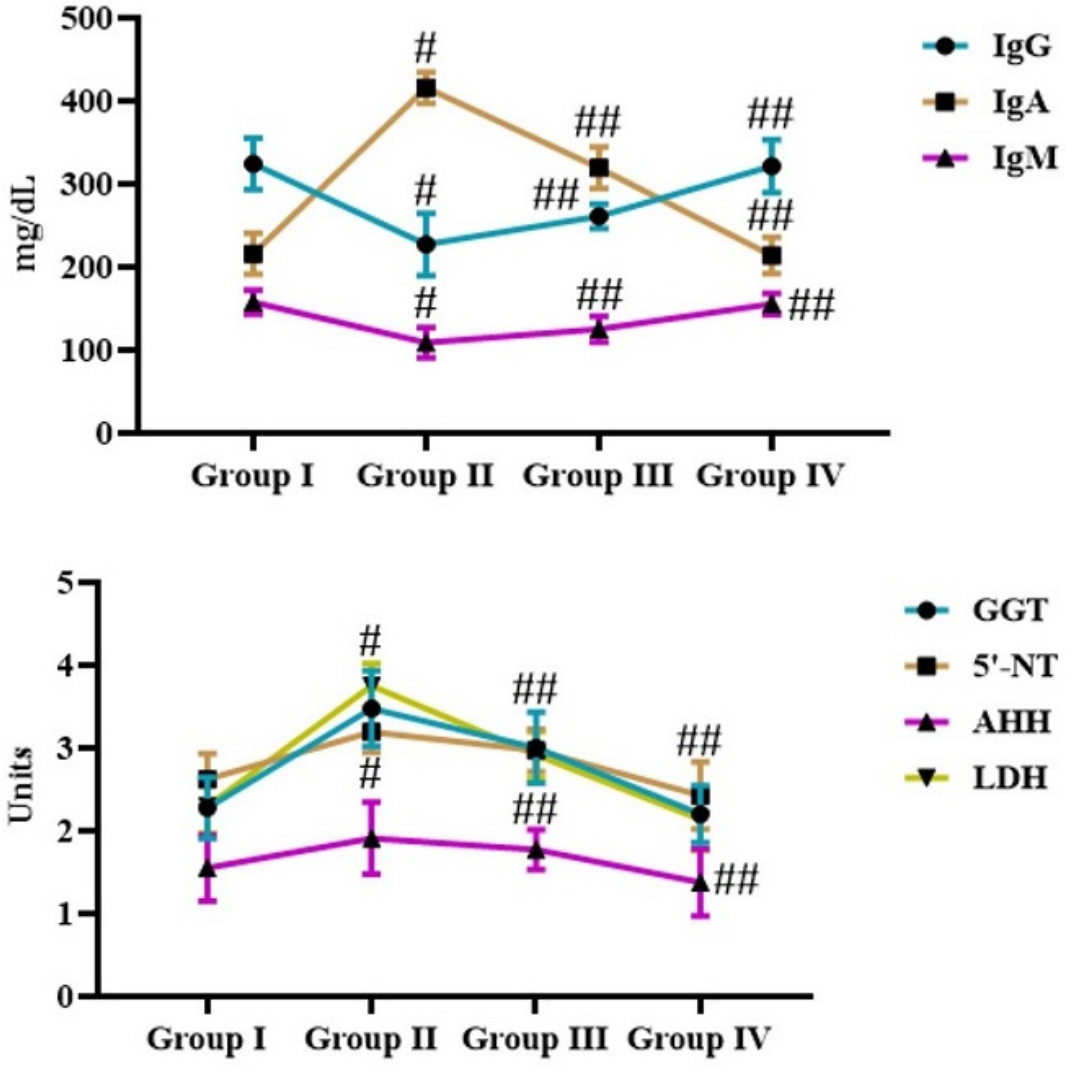

Figure 2: Effect of bilobetin on the Igs and xenobiotic dysfunction markers in the experimental mice. The data are expressed as a mean±SD of three replicates. The data are statistically evaluated using one-way ANOVA, subsequently applying Tukey's *post hoc* assay to evaluate the differences between treatment groups. Note: '#' denotes significance at $p < 0.01$ compared to the control group, whereas '##' denotes significance at $p < 0.05$ compared to the B(a)P-induced group.

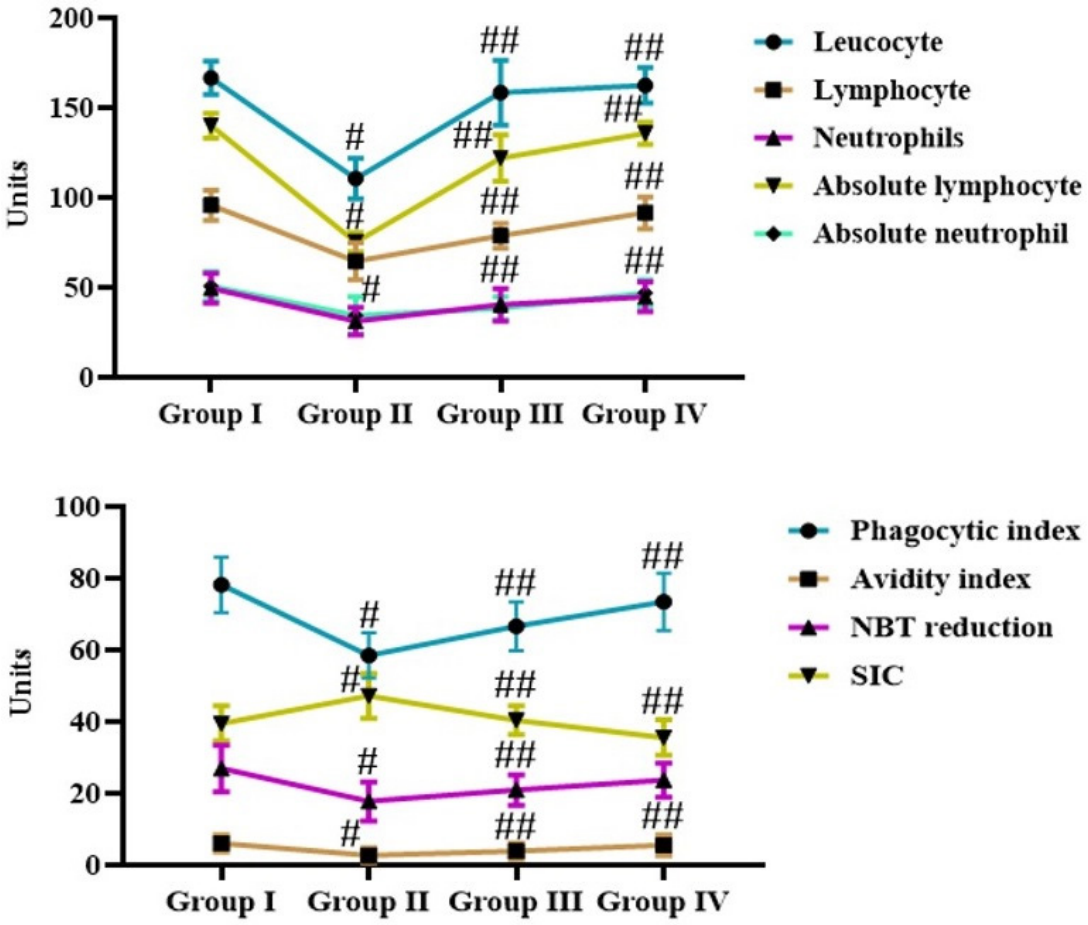

Figure 3: Effect of bilobetin on the immune cells, avidity and phagocytic index, and SIC in the experimental mice. The data are expressed as a mean±SD of three replicates. The data are statistically evaluated using one-way ANOVA, subsequently applying Tukey's *post hoc* assay to evaluate the differences between treatment groups. Note: '#' denotes significance at $p < 0.01$ compared to the control group, whereas '##' denotes significance at $p < 0.05$ compared to the B(a)P-induced group.

QR concentrations in the B(a)P-treated mice, which evidences the beneficial effects of bilobetin in detoxifying or metabolizing the carcinogen in the mice model.

Igs, crucial components of the adaptive immune system, play multifaceted roles in the progression of lung cancer, ranging from tumor promotion to immune surveillance. Dysregulation of Ig production and function can significantly impact the tumor microenvironment and influence the clinical course of lung cancer. IgG, the most abundant antibody isotype in the circulation, mediates a variety of effector functions, which can target and abolish tumor cells.[33] IgM, the first antibody generated during an immune reaction, is especially potential in activating the complement cascade, leading to direct lysis of cancer cells and enhanced antigen presentation. IgM can also neutralize tumor-associated antigens and prevent their interaction with growth factor receptors, thereby inhibiting tumor cell proliferation and angiogenesis. IgA, predominantly found in mucosal secretions, plays an essential role in maintaining immune homeostasis at the lung mucosal surface, which is the primary site of lung cancer development.[34] IgA can neutralize pathogens and prevent their entry into the underlying tissues, thereby reducing chronic inflammation, a major risk factor for lung cancer. IgA can also regulate antibody-dependent cytotoxicity and phagocytosis, thereby eliminating cancer cells at the mucosal surface. Dysregulation of IgA production and transport can compromise mucosal immunity and promote lung cancer development.[35] The analysis of IgG, IgM, and IgA can provide valuable model system for elucidating the mechanisms by which these Igs contribute to lung cancer pathogenesis. Understanding the mechanisms by which these Igs influence tumor immunity and the tumor microenvironment is crucial for developing effective techniques to treat lung cancer.[36] In this work, the decreased IgG and IgM and elevated IgA was seen in the B(a)P mice. Interestingly, the bilobetin treatment considerably regulated the Igs concentrations in the B(a)P mice.

Xenobiotic metabolism, the process by which the body detoxifies foreign substances, plays an essential role in cancer initiation, especially in organs like the liver and lung that are constantly exposed to environmental toxins. Dysfunction in xenobiotic metabolism can result in the accumulation of carcinogenic compounds, heightening the risk of lung cancer. Understanding the molecular mechanisms of carcinogenesis is essential for

developing effective prevention, diagnosis, and treatment strategies. Biomarkers that are post-translationally modified can serve as prognostic markers, aiding in the evaluation of clinical outcomes in malignancies.[37] GGT, 5'-NT, AHH, ADA, and LDH are key enzymes involved in xenobiotic metabolism and cellular function, and their altered levels can indicate metabolic dysfunction and cellular damage. GGT is an enzyme involved in GSH metabolism, which is crucial for detoxification and antioxidant defense. Elevated GGT levels often indicate liver damage and oxidative stress, which can be associated with increased cancer risk. 5'-NT is involved in nucleotide metabolism and its activity is often elevated in cancer cells due to increased cell proliferation and metabolic activity. AHH is a phase I metabolic enzyme responsible for the activation of procarcinogens like B(a)P into their reactive forms, thus promoting DNA damage and tumor initiation.[38] ADA is involved in purine metabolism and immune function, and its altered levels can indicate immune dysregulation and inflammation, which are key components of the tumor microenvironment. LDH plays an essential role in anaerobic glycolysis, a metabolic pathway often upregulated in tumor cells to support rapid growth. Analyzing these enzymes in the context of B(a)P-exposed mice provides a valuable tool to understand the complex interplay between xenobiotic metabolism, cellular stress, and tumorigenesis.[39] In this work, we seen that B(a)P-treated mice demonstrated increased GGT, 5′-NT, AHH, ADA, and LDH concentrations in comparison to the control. However, the bilobetin treatment significantly diminished the GGT, 5′-NT, AHH, ADA, and LDH in the B(a)P-treated mice.

CYFRA 21-1 and CEA have emerged as significant tumor biomarkers in lung cancer, offering potential insights into disease pathophysiology and clinical management. CYFRA 21-1, a soluble fragment of cytokeratin 19, is released into the bloodstream during epithelial cell turnover and is frequently elevated in various malignancies, including lung cancer. CEA, a glycoprotein participated in cell adhesion, is overexpressed in numerous tumors, including lung cancer, and is connected with tumor growth, metastasis, and angiogenesis.[40] Examining the pathophysiology of lung cancer, CYFRA 21-1's involvement stems from its role as a product of cytokeratin 19, a key component of the cytoskeleton in epithelial cells; its increased presence in serum indicates heightened epithelial cell turnover and tumor burden. The carcinogenicity of B(a)P, a ubiquitous environmental pollutant, initiates a cascade of molecular events leading to DNA damage, genomic instability, and ultimately, tumor formation. The analysis of CYFRA 21-1 in B(a)P mice can reveal the dynamics of epithelial cell damage and proliferation during the different stages of tumor development.[41] The significance of CEA in lung cancer pathophysiology is linked to its function in cell adhesion and its influence on tumor cell interactions with the surrounding microenvironment; overexpression of CEA promotes tumor cell adhesion to endothelial cells, facilitating metastasis and angiogenesis, which are critical processes in tumor progression. Monitoring CEA levels in B(a)P mice can provide insights into

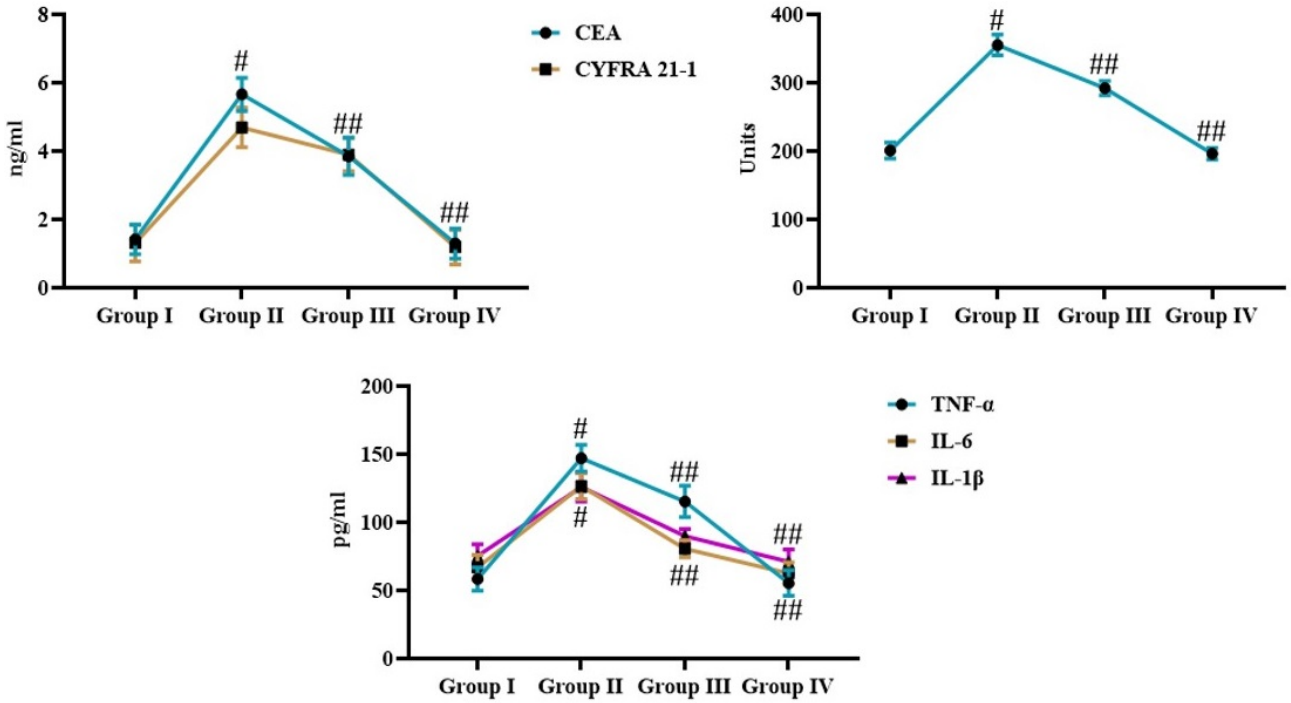

Figure 4: Effect of bilobetin on the tumor biomarkers and inflammatory cytokines in the experimental mice. The data are expressed as a mean±SD of three replicates. The data are statistically evaluated using one-way ANOVA, subsequently applying Tukey's *post hoc* assay to evaluate the differences between treatment groups. Note: '#' denotes significance at $p < 0.01$ compared to the control group, whereas '##' denotes significance at $p < 0.05$ compared to the B(a)P-induced group.

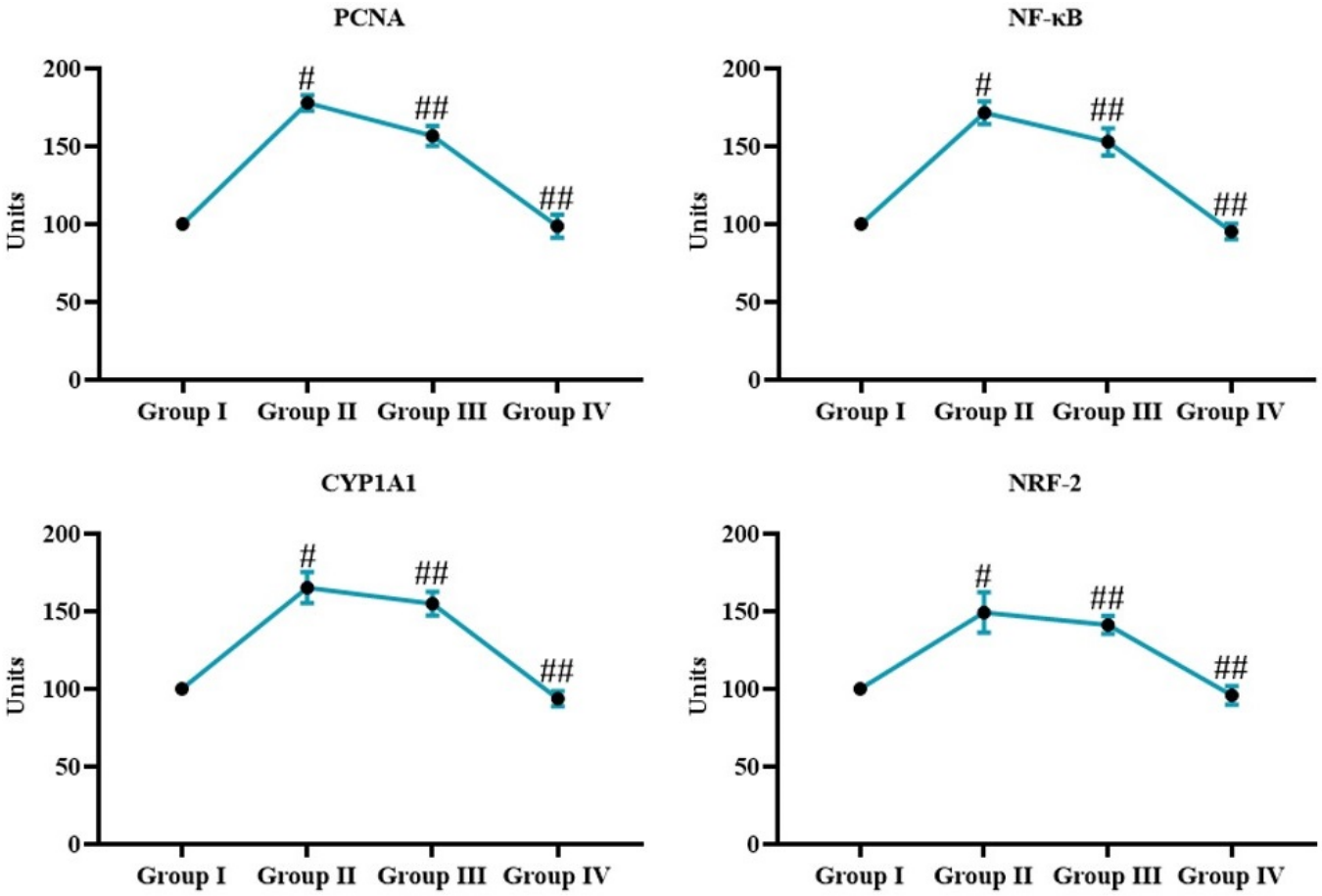

Figure 5: Effect of bilobetin on the CYP1A1, PCNA, NF-κB, and NRF-2 levels in the experimental mice. The data are expressed as a mean±SD of three replicates. The data are statistically evaluated using one-way ANOVA, subsequently applying Tukey's *post hoc* assay to evaluate the differences between treatment groups. Note: '#' denotes significance at $p < 0.01$ compared to the control group, whereas '##' denotes significance at $p < 0.05$ compared to the B(a)P-induced group.

the metastatic potential and angiogenic activity of lung tumors.[42] In this work, the results demonstrated the increased CEA and CYFRA 21-1 concentrations in the B(a)P-treated mice. However, the bilobetin treatment effectively diminished these tumor biomarkers in the B(a)P-treated mice.

The several biomarkers including CYP1A1, PCNA, NF-κB, and NRF-2 represent a complex interplay of proteins intricately participated in the onset of lung cancer, especially in the context of exposure to carcinogenic compounds such as B(a)P. CYP1A1, a member of the cyt-P450 superfamily of enzymes, plays a pivotal role in the metabolic activation of pro-carcinogens like B(a)P. This metabolic activation results in the development of reactive intermediates that can bind to DNA, forming DNA adducts and initiating mutations that drive carcinogenesis.[43] PCNA, a protein essential for DNA replication and repair, serves as an indicator of cell growth and is frequently overexpressed in cancer cells, reflecting their uncontrolled growth. NF-κB, a transcription factor participated in inflammation, immunity, and cell survival, is often constitutively activated in lung cancer, enhancing tumor development and therapy resistance. NRF-2, a transcription factor regulating antioxidant gene expression, is crucial for cellular defense against oxidative stress and is often dysregulated in lung cancer, influencing tumor development and suppression based on the context.[44] The intricate involvement of NF-κB in cellular functions underscores the importance of its tightly regulated activation and termination, but dysregulation of this pathway is participated in several diseases, including cancer. Activation of NF-κB can be triggered by diverse stimuli, including inflammatory cytokines, growth factors, and genotoxic stress, resulting in the transcription of genes participated in cell survival, growth, and inflammation. Abnormal NF-κB activation has been noted in numerous cancers, including lung cancer, where it promotes tumor growth, angiogenesis, and metastasis.[45] Analysis of these markers in B(a)P-induced mice provides valuable insights into the mechanisms underlying lung cancer. Evaluating CYP1A1 expression and activity can reveal the extent of metabolic activation of B(a)P and the generation of carcinogenic metabolites. Assessing PCNA levels can indicate the proliferative capacity of lung cancer cells and the effectiveness of therapeutic interventions. Examining NF-κB activation status can elucidate its role in inflammation, immune evasion, and resistance to therapy. Investigating NRF-2 expression and activity can provide insights into the cellular response to oxidative stress and the potential for

therapeutic targeting of this pathway.[46] In this work, we found the elevated levels of CYP1A1, PCNA, NF-κB, and NRF-2 were seen in the lung tissues of B(a)P mice. Captivatingly, the treatment of 25 mg/kg of bilobetin markedly diminished the CYP1A1, PCNA, NF-κB, and NRF-2 concentrations in the B(a)P-induced mice.

CONCLUSION

Our results demonstrate that bilobetin treatment exhibits immunomodulatory and chemopreventive properties in B(a)P-induced lung cancer in mice. The chemopreventive activity of bilobetin in the context of B(a)P-induced cancer growth is linked to its immunomodulatory properties and its capacity to modulate phase-I and -II detoxifying enzymes. Moreover, bilobetin treatment considerably reduced xenobiotic dysfunction markers, regulated the Igs levels, and reduced tumor biomarkers in the B(a)P-treated mice. Consequently, it was evident that bilobetin treatment possesses immunomodulatory and chemopreventive properties against lung cancer and may enhance chemotherapy approaches for this malignancy. Future studies should investigate the molecular mechanisms underlying bilobetin's immunomodulatory and chemopreventive effects. Additionally, clinical trials are warranted to explore bilobetin's efficacy and safety in human lung cancer patients.

ACKNOWLEDGEMENT

This work was supported by College of Basic Medicine Qilu Medical University, Zibo Shandong, 255300, China.

ABBREVIATIONS

B(a)P: Benzo(a)Pyrene; **Igs:** Immunoglubulins; **SIC:** Soluble Immune Complexes; **GST:** Glutathione-S-Transferase; **5′-NT:** 5′-Nucleotidase; **CYP1A1:** Cytochrome P450 1A1; **NF-κB:** Nuclear Factor-κB; **UDP-GT:** Uridine 5'-Diphospho-Glucuronosyltransferase; **AHH:** Aryl Hydrocarbon Hydroxylase; **ADA:** Adenosine Deaminase; **CYFRA 21-1:** Cytokeratin Fragment-19; **IL:** Interleukin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by College of Basic Medicine Qilu Medical University, Zibo Shandong, 255300, China.

SUMMARY

Lung cancer, a widespread and lethal type of cancer, has consistently increasing in incidence and mortality annually worldwide. Bilobetin is a naturally occurring bioactive biflavonoid prevalent in Ginkgo biloba and certain other gymnosperms. This study's results indicate that bilobetin therapy possesses immunomodulatory and chemopreventive effects in B(a)P-induced lung cancer in mice. Thus, it was clear that bilobetin treatment has immunomodulatory and chemopreventive effects against lung cancer and may augment chemotherapy strategies for this disease.

REFERENCES

1. Kratzer TB, Bandi P, Freedman ND, Smith RA, Travis WD, Jemal A, *et al.* Lung cancer statistics, 2023. Cancer. 2024; 130(8): 1330-48. doi: 10.1002/cncr.35128, PMID 38279776.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023; 73(1): 17-48. doi: 10.3322/caac.21763, PMID 36633525.
3. Henley SJ, Thomas CC, Lewis DR, Ward EM, Islami F, Wu M, *et al.* Annual report to the nation on the status of cancer, part II: Progress toward Healthy People 2020 objectives for 4 common cancers. Cancer. 2020; 126(10): 2250-66. doi: 10.1002/cncr.32801, PMID 32162329.
4. Smolarz B, Łukasiewicz H, Samulak D, Piekarska E, Kołaciński R, Romanowicz H. Lung cancer-epidemiology, pathogenesis, treatment and molecular aspect (review of literature). Int J Mol Sci. 2025; 26(5): 2049. doi: 10.3390/ijms26052049, PMID 40076671.
5. Rina A, Maffeo D, Minnai F, Esposito M, Palmieri M, Serio VB, *et al.* The genetic analysis and clinical therapy in lung cancer: current advances and future directions. Cancers. 2024; 16(16): 2882. doi: 10.3390/cancers16162882, PMID 39199653.
6. Gasparri R, Sabalic A, Spaggiari L. The early diagnosis of lung cancer: critical gaps in the discovery of biomarkers. J Clin Med. 2023; 12(23): 7244. doi: 10.3390/jcm12237244, PMID 38068296.
7. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, *et al.* Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists. J Thorac Oncol. 2018; 13(3): 323-58. doi: 10.1016/j.jtho.2017.12.001, PMID 29396253.
8. Imyanitov EN, Preobrazhenskaya EV, Orlov SV. Current status of molecular diagnostics for lung cancer. Explor Target Antitumor Ther. 2024; 5(3): 742-65. doi: 10.37349/etat.2024.00244, PMID 38966170.
9. Petrella F, Rizzo S, Attili I, Passaro A, Zilli T, Martucci F, *et al.* Stage III non-small-cell lung cancer: an overview of treatment options. Curr Oncol. 2023; 30(3): 3160-75. doi: 10.3390/curroncol30030239, PMID 36975452.
10. Chaft JE, Rimner A, Weder W, Azzoli CG, Kris MG, Cascone T. Evolution of systemic therapy for Stages I-III non-metastatic non-small-cell lung cancer. Nat Rev Clin Oncol. 2021; 18(9): 547-57. doi: 10.1038/s41571-021-00501-4, PMID 33911215.
11. Choudhary N, Bawari S, Burcher JT, Sinha D, Tewari D, Bishayee A. Targeting cell signaling pathways in lung cancer by bioactive phytocompounds. Cancers. 2023; 15(15): 3980. doi: 10.3390/cancers15153980, PMID 37568796.
12. Patel DK. Biological importance of a biflavonoid 'Bilobetin' in the medicine: medicinal importance, pharmacological activities and analytical aspects. Infect Disord Drug Targets. 2022; 22(5): e210322202490. doi: 10.2174/1871526522666220321152036, PMID 35319397.
13. Zhang C, Zhang W, Zhu S, Hu C, Che S, Wang M, *et al.* Bilobetin attenuates Staphylococcus aureus virulence by targeting von Willebrand factor-binding protein and staphylocoagulase. World J Microbiol Biotechnol. 2023; 39(12): 358. doi: 10.1007/s11274-023-03812-z.
14. Zhang C, Sui Y, Liu S, Yang M. Bioinformatic analysis and computer-aided study to investigate the potential application of a bioflavonoid compound Bilobetin in liver cancer treatment. Front Biosci (Landmark Ed). 2023; 28(12): 360. doi: 10.31083/j.fbl2812360, PMID 38179744.
15. Kou XH, Zhu MF, Chen D, Lu Y, Song HZ, Ye JL, *et al.* Bilobetin ameliorates insulin resistance by PKA-mediated phosphorylation of PPARα in rats fed a high-fat diet. Br J Pharmacol. 2012; 165(8): 2692-706. doi: 10.1111/j.1476-5381.2011.01727.x, PMID 22091731.
16. Negm WA, El-Kadem AH, Hussein IA, Alqahtani MJ. The mechanistic perspective of Bilobetin protective effects against cisplatin-induced testicular toxicity: role of Nrf-2/Keap-1 signaling, inflammation, and apoptosis. Biomedicines. 2022; 10(5): 1134. doi: 10.3390/biomedicines10051134, PMID 35625871.
17. Chia D, Nasu H, Yamagata J, Barnett EV. Avidity indices of anti-IgG antibodies in diseases. Arthritis Rheum. 1981; 24(6): 803-8. doi: 10.1002/art.1780240608, PMID 6454418.
18. Muniz-Junqueira MI, Peçanha LM, da Silva-Filho VL, de Almeida Cardoso MC, Tosta CE. Novel microtechnique for assessment of postnatal maturation of the phagocytic function of neutrophils and monocytes. Clin Diagn Lab Immunol. 2003; 10(6): 1096-102. doi: 10.1128/cdli.10.6.1096-1102.2003, PMID 14607873.
19. Choi HS, Kim JW, Cha YN, Kim C. A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. J Immunoassay Immunochem. 2006; 27(1): 31-44. doi: 10.1080/15321810500403722, PMID 16450867.

20. Chen K, Nishi H, Travers R, Tsuboi N, Martinod K, Wagner DD, *et al.* Endocytosis of soluble immune complexes leads to their clearance by FcγRIIIB but induces neutrophil extracellular traps via FcγRIIA *in vivo*. Blood. 2012; 120(22): 4421-31. doi: 10.1182/blood-2011-12-401133, PMID 22955924.
21. Boyum A. Separation of white blood cells. Nature. 1964; 204: 793-4. doi: 10.1038/204793a0, PMID 14235685.
22. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem. 1964; 239: 2370-39: 2370-8, PMID 14209971.
23. Wharton DC, Tzagoloff A. Cytochrome oxidase from beet heart mitochondria. Methods Enzymol. 6th ed. 1967;X:245-50.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249(22): 7130-24: 7130-9. doi: 10.1016/S0021-9258(19)42083-8, PMID 4436300.
25. Luquita MG, Sánchez Pozzi EJ, Catania VA, Mottino AD. Analysis of p-nitrophenol glucuronidation in hepatic microsomes from lactating rats. Biochem Pharmacol. 1994; 47(7): 1179-47: 1179-85. doi: 10.1016/0006-2952(94)90390-5, PMID 8161347.
26. Benson AM, Hunkeler MJ, Talalay P. Increase of NAD(P) H: quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc Natl Acad Sci U S A. 1980; 77(9): 5216-20. doi: 10.1073/pnas.77.9.5216, PMID 6933553.
27. Garg P, Singhal S, Kulkarni P, Horne D, Malhotra J, Salgia R, *et al.* Advances in non-small cell lung cancer: current insights and future directions. J Clin Med. 2024; 13(14): 4189. doi: 10.3390/jcm13144189, PMID 39064229.
28. Šutić M, Vukić A, Baranašić J, Försti A, Džubur F, Samaržija M, *et al.* Diagnostic, predictive, and prognostic biomarkers in non-small cell lung cancer (NSCLC) management. J Pers Med. 2021; 11(11): 1102. doi: 10.3390/jpm11111102, PMID 34834454.
29. Mota P, Silva HC, Soares MJ, Pego A, Loureiro M, Cordeiro CR, *et al.* Genetic polymorphisms of phase I and phase II metabolic enzymes as modulators of lung cancer susceptibility. J Cancer Res Clin Oncol. 2015; 141(5): 851-60. doi: 10.1007/s00432-014-1868-z, PMID 25388590.
30. Moorthy B, Chu C, Carlin DJ. Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicol Sci. 2015; 145(1): 5-15. doi: 10.1093/toxsci/kfv040, PMID 25911656.
31. Du X, Li D, Wang G, Fan Y, Li N, Chai L, *et al.* Chemoprotective effect of atorvastatin against benzo[a]pyrene-induced lung cancer via the inhibition of oxidative stress and inflammatory parameters. Ann Transl Med. 2021; 9(4): 355. doi: 10.21037/atm-20-7770, PMID 33708982.
32. Morozkina SN, Nhung Vu TH, Generalova YE, Snetkov PP, Uspenskaya MV. Mangiferin as new potential anti-cancer agent and mangiferin-integrated polymer systems-A novel research direction. Biomolecules. 2021; 11(1): 79. doi: 10.3390/biom11010079, PMID 33435313.
33. Cui M, Huang J, Zhang S, Liu Q, Liao Q, Qiu X. Immunoglobulin expression in cancer cells and its critical roles in tumorigenesis. Front Immunol. 2021; 12: 613530. doi: 10.3389/fimmu.2021.613530, PMID 33841396.
34. Pan J, Yu L, Wu Q, Lin X, Liu S, Hu S, *et al.* Integration of IgA and IgG autoantigens improves performance of biomarker panels for early diagnosis of lung cancer. Mol Cell Proteomics. 2020; 19(3): 490-500. doi: 10.1074/mcp.RA119.001905, PMID 31924693.
35. Kdimati S, Mullins CS, Linnebacher M. Cancer-cell-derived IgG and its potential role in tumor development. Int J Mol Sci. 2021; 22(21): 11597. doi: 10.3390/ijms222111597, PMID 34769026.
36. Zhao J, Peng H, Gao J, Nong A, Hua H, Yang S, *et al.* Current insights into the expression and functions of tumor-derived immunoglobulins. Cell Death Discov. 2021; 7(1): 148. doi: 10.1038/s41420-021-00550-9, PMID 34226529.
37. Thum T, Erpenbeck VJ, Moeller J, Hohlfeld JM, Krug N, Borlak J. Expression of xenobiotic metabolizing enzymes in different lung compartments of smokers and nonsmokers. Environ Health Perspect. 2006; 114(11): 1655-61. doi: 10.1289/ehp.8861, PMID 17107849.
38. Khan A, Alhumaydhi FA, Alwashmi AS, Allemailem KS, Alsahli MA, Alrumaihi FA, *et al.* Diallyl sulfide-mediated modulation of the fatty acid synthase (FASN) leads to cancer cell death in BaP-induced lung carcinogenesis in Swiss mice. J Inflamm Res. 2020; 13: 1075-87. doi: 10.2147/JIR.S284279, PMID 33324084.
39. Alrumaihi F. Exploring the chemopreventive potential of Artemisia annua methanolic extract in colorectal cancer induced by azoxymethane in mice. Pharmaceuticals (Basel). 2024; 18(1): 34. doi: 10.3390/ph18010034, PMID 39861099.
40. He Y, Cui Y, Chang D, Wang T. Postoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I pulmonary adenocarcinoma. Oncotarget. 2017; 8(42): 73115-22. doi: 10.18632/oncotarget.17611, PMID 29069854.
41. Tang J, Ge QM, Huang R, Shu HY, Su T, Wu JL, *et al.* Clinical significance of CYFRA21-1, AFP, CA-153, CEA, and CA-199 in the diagnosis of lung cancer ocular metastasis in hypertension population. Front Cardiovasc Med. 2021; 8: 670594. doi: 10.3389/fcvm.2021.670594, PMID 34595214.
42. Wang J, Jiang W, Zhang T, Liu L, Bi N, Wang X, *et al.* Increased CYFRA 21-1, CEA and NSE are prognostic of poor outcome for locally advanced squamous cell carcinoma in lung: A nomogram and recursive partitioning risk stratification analysis. Transl Oncol. 2018; 11(4): 999-1006. doi: 10.1016/j.tranon.2018.05.008, PMID 29958123.
43. Arlt VM, Krais AM, Godschalk RW, Riffo-Vasquez Y, Mrizova I, Roufosse CA, *et al.* Pulmonary inflammation impacts on CYP1A1-mediated respiratory tract DNA damage induced by the carcinogenic air pollutant benzo[a]pyrene. Toxicol Sci. 2015; 146(2): 213-25. doi: 10.1093/toxsci/kfv086, PMID 25911668.
44. Zimta AA, Cenariu D, Irimie A, Magdo L, Nabavi SM, Atanasov AG, *et al.* The role of Nrf2 activity in cancer development and progression. Cancers. 2019; 11(11): 1755. doi: 10.3390/cancers11111755, PMID 31717324.
45. Bukowska B, Duchnowicz P. Molecular mechanisms of action of selected substances involved in the reduction of benzo[a]pyrene-induced oxidative stress. Molecules. 2022; 27(4): 1379. doi: 10.3390/molecules27041379, PMID 35209168.
46. Bodduluru LN, Kasala ER, Barua CC, Karnam KC, Dahiya V, Ellutla M. Antiproliferative and antioxidant potential of hesperetin against benzo[a]pyrene-induced lung carcinogenesis in Swiss albino mice. Chem Biol Interact. 2015; 242: 345-52. doi: 10.1016/j.cbi.2015.10.020, PMID 26546711.

Cite this article: Xu Z, Zhu L, Wang H. Bilobetin Shows Immunomodulatory and Chemopreventive Activities in Benzo(a)pyrene-Induced Lung Cancer in Mice Model. Indian J of Pharmaceutical Education and Research. 2025;60(1):421-30.